

INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

IMPACTO LOCAL E SISTÉMICO DA INDUÇÃO DE PERIODONTITE EM MODELOS ANIMAIS: REVISÃO SISTEMÁTICA E META-ANÁLISE

Trabalho submetido por
Catarina Carmo Santos
para a obtenção do grau de Mestre em Medicina Dentária

junho de 2023

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Resumo

Contexto: A periodontite é uma doença inflamatória crónica dos tecidos de suporte do dente, iniciada por uma disbiose da microbiota oral num hospedeiro suscetível. Diversos modelos de periodontite têm sido utilizados nas últimas décadas, embora permaneça incerteza sobre quais os mais adequados para a indução da periodontite. O nosso objetivo foi resumir e comparar a perda óssea alveolar de diferentes protocolos de periodontite induzida em modelos animais e as suas respetivas repercussões locais e sistémicas.

Materiais e Métodos: PubMed, Embase e Web of Science foram pesquisadas até abril de 2023. Modelos animais saudáveis com indução de periodontite que relatasse perda óssea alveolar com um grupo de controlo foram elegíveis. Os estudos foram agrupados de acordo com o método de indução. O risco de enviesamento foi avaliado através do *Systematic Review Center for Laboratory animal Experimentation* (SYRCLE). As estimativas dos subgrupos foram efetuadas através de uma meta-análise de rácio de médias (ROM).

Resultados: Após seleção de 16311 artigos, foram incluídos 450. Todos os estudos utilizaram uma ou mais técnicas diferentes para induzir periodontite e compararam-nas com um grupo de controlo. Globalmente, o modelo de periodontite induzida por ligadura (n=290; effect=2.39; 95% CI: 2.21-2.58; I²=99.4%) e a combinação de ligadura e inoculação periodontal com lipopolissacáridos (LPS) bacterianos (n=10; effect=2.46; 95% CI: 2.11-2.86; I²=99.5%) foram os métodos com maior perda de osso alveolar, seguidos pela indução de LPS (n=38; effect=2.01; 95% CI: 1.67-2.42; I²=99.6%), indução por ligadura com inoculação oral de bactérias associadas à periodontite (n=34; effect=2.11; 95% CI: 1.79-2.49; I²=99.5%), inoculação bacteriana (n=57; effect=1.60; 95% CI: 1.36-1.89; I²=99.0%) e gavagem oral (n=30; effect=1.28; 95% CI: 1.20-1.37; I²=97.5%).

Conclusões: No geral, os modelos de periodontite induzida por ligadura e a combinação de ligadura com LPS foram os modelos que melhor conseguiram mimetizar a periodontite e mostrar maior perda de osso alveolar.

Palavras-chave: Indução Periodontal; Modelo Animal; Perda óssea alveolar; Revisão Sistemática.

Abstract

Background: Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth, initiated by dysbiosis of the oral microbiota in a susceptible host. A variety of periodontitis induced models have been established over the past few decades, although there is much uncertainty about which models are suitable for the induction of periodontitis. Therefore, we aimed to summarise and compare the alveolar bone loss (ABL) of different protocols of periodontitis induction in animal models and their respective local and systemic effects.

Material & Methods: PubMed, Embase and Web of Science were searched up to April 2023. Systemically healthy animal models of experimentally induced periodontitis with a control group reporting ABL were eligible for inclusion in this systematic review. Studies were subgrouped according to the disease induction method. Risk of bias (ROB) was appraised using the Systematic Review Center for Laboratory animal Experimentation (SYRCLE). Pooled estimates were made using a through ratio of means (ROM) random-effects meta-analysis.

Results: After screening 16311 articles, 450 were included. All studies performed one or more different techniques to induce periodontitis and compared them with a control group. Overall, the ligature-induced periodontitis model (n=290; effect=2.39; 95% CI: 2.21-2.58; $I^2=99.4\%$) and the combination of ligature and periodontal inoculation with bacterial lipopolysaccharides (LPS) (n=10; effect=2.46; 95% CI: 2.11-2.86; $I^2=99.5\%$) were the methods with the highest ABL, followed by the LPS induction (n=38; effect=2.01; 95% CI: 1.67-2.42; $I^2=99.6\%$), the ligature-induction with oral inoculation of periodontitis-associated bacteria (n=34; effect=2.11; 95% CI: 1.79-2.49; $I^2=99.5\%$), the bacterial inoculation (n=57; effect=1.60; 95% CI: 1.36-1.89; $I^2=99.0\%$) and oral gavage (n=30; effect=1.28; 95% CI=1.20-1.37; $I^2=97.5\%$).

Conclusions: Overall, ligature-induced periodontitis models and the combination of ligature with LPS were the models that best performed in mimicking periodontitis and with higher ABL.

Keywords: Periodontitis Induction; Animal Model; Alveolar Bone Loss; Systematic Review.

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List of Abbreviation

AAP – American Academy of Periodontology

ABL – Alveolar Bone Loss

BOP – Bleeding on Probing

CAL - Clinical Attachment Loss

CI - Confidence intervals

DM – Diabetes Mellitus

EFP – European Federation of Periodontology

ICAMs – Intercellular Adhesion Molecules

IL-1 – Interleukin 1

IL-1 β – Interleukin 1 Beta

IL-6 – Interleukin 6

IL-8 – Interleukin 8

LPS – Lipopolysaccharides

MEDLINE – Medical Literature Analysis and Retrieval System Online

MeSH – Medical Subject Heading

NA – Not Applicable

NR – Not Reported

OPG – Osteoprotegerin

PD – Periodontal Disease

PG – Prostaglandin

PMNs – Polymorphonuclear Neutrophils

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RANK – Receptor-Activating Factor-kB

RANKL – Receptor-Activating Factor-kB Ligand

ROB – Risk of Bias

ROM – Ratio of Means

SD– Standard Deviation

SE– Standard Error

SYRCLE – Systematic Review Center for Laboratory Animal Experimentation

TNF – Tumor Necrosis Factor

TNF- α – Tumor Necrosis Factor Alpha

TRAP + – Tartrate-resistant acid phosphatase positive cells

WHO – World Health Organization

I. INTRODUCTION

1. PERIODONTAL HEALTH

The periodontium, also known as the "attachment apparatus" or "tooth supporting tissues", is a complex and dynamic set of tissues that line and support the tooth (1). Consists of mineralised tissues, with the root cementum and alveolar bone, and soft connective tissues, with the gingiva and periodontal ligament. Of these tissues, only the gingiva is clinically visible on intraoral examination in healthy periodontal conditions (2,3). The main function of the periodontium is to attach the tooth to the bone tissue to ensure the integrity of the masticatory mucosal surface of the oral cavity (1).

In 1946, the World Health Organization (WHO) defined health as a "state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (4). However, until recently, the definition of periodontal health remained highly controversial. In its earliest form, periodontal health was defined as no histological evidence of periodontal inflammation and no evidence of anatomical changes in the periodontium (5). However, for most adults, this concept does not apply because the periodontium, like any other human tissue, is affected by changes caused by intrinsic and extrinsic factors (5). Recently, the 2017 European Federation of Periodontology (EFP)/American Academy of Periodontology (AAP) World Workshop defined periodontal health as "a state free of inflammatory periodontal disease (PD) that allows an individual to function normally and suffer no consequences (mental or physical) as a result of past disease", summarised in two main clinical situations: 1) intact periodontium, either pristine or clinically well preserved; and 2) reduced periodontium, with stable PD or in remission/control (5). Therefore, periodontal health should be defined as a significant reduction or absence of clinical signs and symptoms of inflammation associated with gingivitis or periodontitis (5).

The main parameter for monitoring periodontal health or inflammation is based on the percentage of bleeding on probing (BOP) with light pressure (0.25 Newtons) (5). This clinical parameter is measured during or after periodontal probing of a periodontal sulcus/pocket (6).

2. PERIODONTITIS

PD is a chronic, non-transmissible, polymicrobial inflammatory condition caused by a dysbiotic, host-mediated and plaque that targets the periodontium, and can progress from gingivitis to periodontitis if not prevented (7–9).

Gingivitis is a local inflammatory response often triggered by the accumulation of dental biofilme (10). It is usually painless and presents with swelling, bleeding, and lack of periodontal clinical attachment loss (CAL), these subtle clinical changes mean that most patients are unaware of it (10). However, this inflammatory condition is reversible by removing the dental biofilm (10). If left untreated, persistent gum inflammation combined with poor oral hygiene can lead to periodontitis and the formation of gaps between the gums and the tooth root, known as periodontal pockets (10,11).

Periodontitis is defined as chronic bacterial inflammation of the supporting tissues of the tooth, specifically in alveolar bone and periodontal ligament, dependent on the oral microbiome biofilm and the host immune response, resulting in periodontal CAL (9,12–14). However, periodontitis is triggered by an imbalance between host defenses and subgingival microflora in susceptible individuals, leading to the formation of pathological periodontal pockets (11). The triggering of a systemic immuno-inflammatory response does not depend only on periodontopathogenic microorganisms, but on an exacerbation of the inflammatory state leading to the destruction of the underlying tissues, resulting from the disturbance of the homeostasis between the subgingival microflora and the host defense in susceptible individuals (9,15). The onset and progression of periodontitis are also influenced by genetic and environmental risk factors (16).

2.1 Clinical Manifestations

Between the tooth surface and the epithelial surface is a space called the gingival sulcus, which is filled with gingival crevicular fluid (17). This is considered to be an osmotic capillary transudate containing several biochemical components, namely: antibodies, leucocytes, oral microbioma, enzymes, cytokines and tissue degradation products (17).

Plaque accumulation is inevitable, especially where brushing forces are not effective (18). While biofilm and bacteria negatively affect the junctional epithelium by invading it, rupturing the fibers of the periodontal ligament, and destroying the underlying connective tissue, some bacteria are able to spread to the subgingival level (Figure 1) (17,19). Consequently, the subclinical inflammatory infiltrate found in the junctional epithelium in a physiological state is destabilised by the presence of subgingival bacterial subproducts, which triggers the release of host inflammatory mediators (19). This homeostatic disturbance is accompanied by an increase in gingival inflammation, which causes a change from osmotic crevicular fluid transudate to inflammatory exudate, leading to a deepening of the gingival sulcus and, consequently, the formation of pathological periodontal pockets (Figure 1) (19,20).

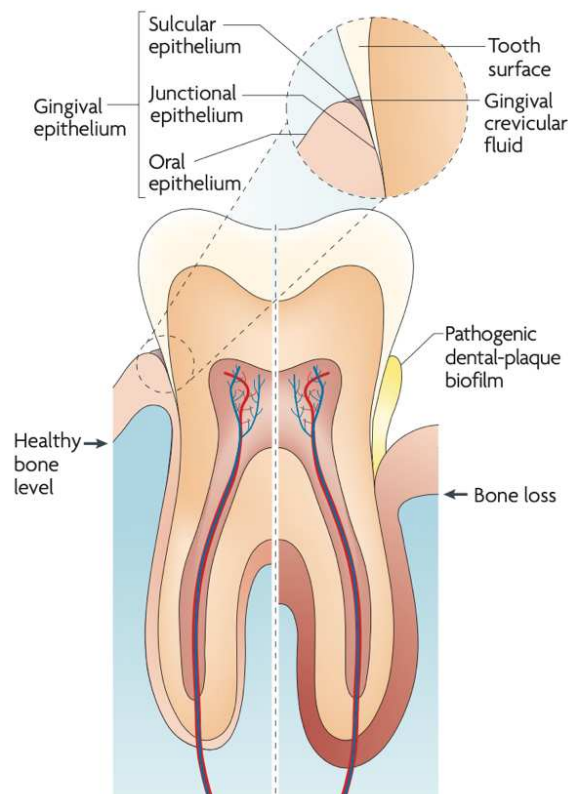


Figure 1 | *The local effects of periodontitis.*

Left side - periodontal health, where the supporting tissues of the tooth roots (connective tissue and alveolar bone) are present, covered by oral epithelium and properly connected to the tooth surface by the specialised junctional epithelium. The space between the epithelial surface and the tooth, which is filled with gingival crevicular fluid and lined with sulcular epithelium, is called the gingival sulcus. Right side - the effects of periodontitis, where the biofilm of dental plaque accumulates on the surface of the tooth, promotes the destruction of the junctional epithelium, breaks the fibers of the periodontal ligament, and consequently causes the destruction of the supporting tissues (connective tissue and alveolar bone), resulting in tooth loss (7).

If left unchecked, exacerbation of periodontitis can lead to swelling and bleeding during tooth brushing, persistent foul odour (halitosis), reduced chewing efficiency, gingival recession, root furcation involvement, as well as painful tooth mobility and ultimately tooth loss (7,21). Despite the destruction of the periodontium, this disease can develop as a "silent disease" without the patient being aware of the signs and symptoms (21,22).

In addition to its local effects, periodontitis can negatively affect the systemic health, increasing the risk of several systemic diseases, such as cardiovascular disease, namely atherosclerosis and infectious endocarditis (23,24), diabetes mellitus (DM) (25,26), obesity (27), chronic kidney disease (28), rheumatoid arthritis (29), adverse pregnancy outcomes (30) and cancer (31). Current evidence has shown an association between periodontitis and polycystic ovarian syndrome (32) and an increased risk of Covid-19 complications (33). Recently, an umbrella review systematised all the scientific evidence in this area (34).

2.2 Etiopathogenic Mechanisms

The periodontium is strongly associated with a diverse microbiota community present at the subgingival level, where approximately 500 species have been identified to date (35).

The junctional epithelium is known to have a small mucus layer, as the cells present are only connected by a few desmosomes and occasional gap junctions, resulting in large intracellular spaces with high porosity (7). Due to the intimate relationship between the periodontium and the dental biofilm, periodontal defence is controlled by the high expression of mediators of the host's innate immune system (7).

In an attempt to maintain periodontal homeostasis, a barrier is formed between the periodontium and the dental plaque biofilm by the passage of polymorphonuclear neutrophils (PMNs) from the highly vascularised gingival tissue into the gingival sulcus and other immune cells (which recognise pathogenic and commensal bacteria) through the coordinated expression of E-selectin, intercellular adhesion molecules (ICAMs) and interleukin-8 (IL-8) (7).

However, if the biofilm is not removed, it provides favourable conditions for the development of periodontalpathogenic bacterial species (36). These can produce chemical signals and trigger an inflammatory process while altering the available nutrients (36).

A group of anaerobic gram-negative species known as the red complex (*Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*) has been shown to be strongly associated with disease sites and to negatively modulate the host innate immune response (7,37). However, periodontopathogenic microorganisms are also found in periodontally healthy individuals. In a more recent paradigm, it is known that other complexes are involved in the pathophysiology of periodontitis, resulting from polymicrobial synergy and dysbiosis, which subsequently leads to biofilm imbalance associated with periodontal homeostasis (7,37).

Thus, to disrupt periodontal hemostasis, periodontopathogenic bacteria require commensal bacteria and the susceptibility of host's immune mechanisms. This process promotes the transition from a healthy to a pathological state, i.e., there is a transition from a symbiotic microbial community (mainly facultative gram-positive bacterial species) to a dysbiotic microbial community (mainly anaerobic gram-negative bacterial species), triggering the release of inflammatory cytokines (7,36,37).

Another molecular mechanism contributing to periodontal tissue damage is the dysregulation between pro- and anti-inflammatory cytokines (38). Elevated levels of pro-inflammatory cytokines have been strongly correlated with alveolar bone loss (ABL), a known feature of periodontitis (7). This mechanism results from an increase in the ratio of receptor-activating factor-kB ligand (RANKL)/osteoprotegerin (OPG), which allows RANKL to bind to receptor-activating factor-kB (RANK) on osteoclast precursors, causing them to differentiate into active, osteoclast-like cells. macrophages that secrete enzymes that degrade bone (7).

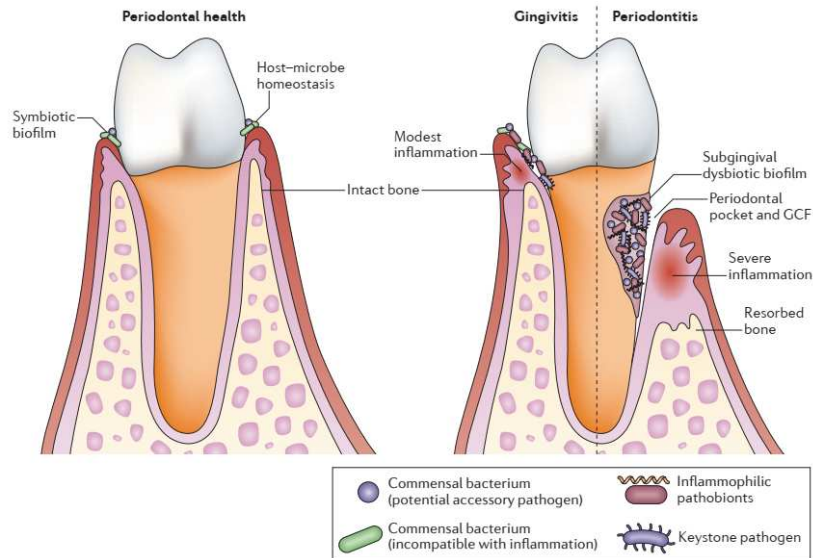


Figure 2 | ***Etiopathogenesis of periodontitis.***

In healthy periodontal tissue (left), the subgingival commensal biofilm triggers several hosts innate immune responses, establishing a state of homeostasis between the microbiota and the host. However, if the accumulation of supragingival plaque is constant, moderate gingival inflammation - gingivitis - develops (middle). In susceptible hosts, if the accumulation of bacterial plaque is not removed, some commensal bacteria (accessory pathogens) can provoke a more aggressive inflammatory process and consequently a microbial change. This dysbiotic change can reduce the host immune response and lead to pathological periodontal pocket formation and bone resorption (right) (37).

2.3 Causal and Risk Factors

The main aetiological factor of periodontitis is the accumulation of dental plaque, mainly in the subgingival region, largely promoted by poor oral hygiene habits (21). However, dysbiosis of the gingival microbiota alone cannot trigger periodontitis, i.e., it is the intersection with other risk factors that alters the susceptibility and resistance of an individual's immune system and lead to the development of PD (37).

It is important to recognise that risk factors are associated with a disease, but do not necessarily cause the disease (39).

Several risk factors for periodontitis have been proposed, divided into: 1) modifiable, as environmental and behavioural; and 2) non-modifiable, intrinsic to the individual (39). Examples of modifiable risk factors include smoking, poorly controlled diabetes, obesity, osteoporosis, excessive alcohol consumption, a diet low in calcium and low levels of vitamin D, and stress (16,40,41). These factors can be controlled by the patient or the healthcare professional (42). On the other hand, non-modifiable risk factors, which

include age, sex, genetics, and ethnicity, cannot be changed but can contribute to the dysregulation of the host immune system and increase the risk of periodontitis onset and progression and are, therefore, considered of paramount importance in identifying individuals at higher risk for PD (42).

None of these factors alone can cause PD, but the interactions between them lead to the development of periodontitis (39).

2.4 Prevalence

Periodontitis is a highly prevalent disease worldwide (43), contributing to the global burden of chronic disease (12). Previous studies have shown that severe periodontitis is considered the sixth most prevalent disease in the world (44). Its prevalence is estimated to affect more than 50% of the adult population worldwide, considering that it has been significant and increasing over the last three decades (45).

According to the work of Trindade et al. (2023), the overall prevalence of periodontitis in the last decade was 62%, while its most severe stage affected around 23.6% of the population studied (43). Compared with estimates made between 1990 and 2010, these results showed an alarming increase in the prevalence of periodontitis (43).

Moreover, PD has an impact on quality of life (46), nutrition (47), aesthetic confidence and general well-being, considering that patients are more prone to chewing dysfunction, tooth mobility and even tooth loss (48), especially with increased severity of PD (46). In addition to its pronounced social and economic impact (49,50), PD is strongly associated with systemic disease and inflammatory burden (51,52).

Additionally, the global prevalence of PD is expected to increase in the coming years due to the growth of the elderly population (44).

The current estimates of periodontitis are a critical factor for research and development of public health strategies that maximise their effectiveness, thus policy changes that address the global periodontitis landscape are of utmost urgency (43,45).

3. EXPERIMENTAL ANIMAL MODELS

As awareness of the global burden of PD has increased, the study of this disease has become of paramount importance to the scientific community, since a better understanding of its pathogenesis allows for improved treatment with PD (45). Several experimental animal models have been proposed to evaluate both the pathogenesis and treatment options (53).

The animal models used to study human diseases have limitations inherent in the very name of the term "model", i.e., they resemble something that is being studied and, therefore, never capture all human characteristics (54,55). However, their benefits outweigh the harms, as they demonstrate cause-and-effect relationships, establish the safety and efficacy of new therapeutics, and mimic interactions between the microbiome, immune response, and host tissues (55). The use of animal models has the following main advantages: 1) their ease of handling, for example we can control diet and environmental temperature; 2) the biological similarity to humans; 3) the fact that animals are susceptible to many of the same health problems as humans; 4) the omission of demanding ethical considerations such as those required in human studies; 5) the fact that it allows the longitudinal study of the progression of the disease in the different tissues under analysis (53,56,57).

However, the major limitation of all animal models is that there is no single model that represents all the characteristics of PD, so the selection of a particular model is based on its ability to test a specific hypothesis (53,54). All characteristics that are similar to those of humans, such as biological, anatomical and organic properties, are taken into account in order to draw good conclusions (54).

3.1 Animal species

A variety of animal species could be used to model periodontitis, but rats, dogs, primates, pigs, rabbits, hamsters, and ferrets are the most commonly used (58). Depending on the animal, PD may occur naturally, or it may have to be induced experimentally (58).

Disadvantages of small animal models, such as rats, mice, rabbits and hamsters, include the limited similarity of their dentition to that of humans, the fact that they do not have the same oral microbiota as humans, the fact that some strains are naturally resistant to periodontitis, and the difficulty of performing certain experiments, for example due to the limited availability of tissue for analysis (58,59). However, small animal models are the most widely used due to their lower cost compared to large animals (54,58). Other major advantages are that they allow significant data to be obtained on various interactions between soft and hard tissues and, finally, they allow the possible elimination of the need to use larger species prior to human experiments (58).

On the other hand, large animals, such as monkeys, dogs, sheep, and miniature pigs, have similar dental and oral microbiota characteristics to humans, in addition to being phylogenetically closer (58). However, they have greater ethical restrictions, higher costs and require specialised facilities and personnel (58–60). Another drawback is the reduced availability of animals, which should be reserved for the final stages of validation of new treatments before clinical use in humans (58–60).

3.1.1 Rodents

This group includes mice, rats, and hamsters (58). Rodents have only one incisor and 3 molars in each quadrant (58,59).

These animal models have been shown to be useful for assessing microbial-host interactions (58,59). In addition, they have been shown to be suitable for studying soft-hard tissue dynamics relevant to identifying pathways of inflammatory regulation, resolution, and healing processes in periodontal tissues (58).

Mice can spontaneously develop periodontitis from their own bacteria, but only when their ability to control commensal bacteria is compromised by genetic defects (58).

On the other hand, rats are also widely used in studies of PD, but they have the disadvantage of body size as well as tooth size, making it more difficult to apply experimental protocols (58). Finally, PD does not occur spontaneously in hamsters, but can be induced experimentally (60).

3.1.2 Rabbits

The pathogenic microorganisms found in the rabbit oral cavity have been shown to be similar to those found in human PD, including *Arcanobacterium haemolyticum*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Streptococcus milleri* group, *Peptostreptococcus micros* and *Actinomyces israelii* (59,61).

It is known that periodontitis in the rabbit is caused by local factors, plaque, and calculus accumulation, leading to inflammatory exacerbation which leads to ABL (61). Furthermore, the physiology and pathology of periodontal tissues is similar to that of humans in terms of pro- and anti-inflammatory mechanisms (58). Therefore, they provide an excellent model to study the impact of inflammation on healing patterns and regeneration in PD (58).

3.1.3 Non-human primates

Non-human primates resemble humans given the similarities in terms of oral structures and in accumulation of plaque, calculus, and oral microbial pathogens (e.g., *P. gingivalis*) (59), which makes them phylogenetically similar to humans (60). In particular, species of rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*) and baboon (*Papio anubis*) are prone to spontaneous PD (59).

Although periodontitis is similar to the human disease, the cost and requirements of rearing these animals limits their use in periodontal studies (61). In addition, these animals are susceptible to infectious diseases such as tuberculosis, making them a less practical model (61).

3.1.4 Dogs

In dogs, subgingival plaque accumulation is associated with bacteria similar to those found in humans, such as *P. gingivalis* and *F. nucleatum* (59,61). There are also many similarities between the human and canine dentition, however there are limitations such as the lack of occlusal contact, the lack of lateral movement and the presence of open

contacts between teeth (61). Another difference between dogs and humans is the frequent lack of gingival sulcus and crevicular fluid in dogs (58).

The prevalence and severity of periodontal diseases, including gingivitis and periodontitis, increase with age, more rapidly than in humans, but with the same aetiological factors, often resulting in tooth loss, as in humans (59,61). Therefore, dogs are a good model to study naturally occurring gingivitis and periodontitis (59).

The Beagle is one of the most commonly used dogs in research due to its size and cooperation (58).

3.1.5 Miniature pigs

Minipigs share several similarities with humans, both in terms of disease development and in terms of the anatomy and physiology of the oral and maxillofacial structures (59,61).

Minnesota pigs have been widely used in various scientific studies (59). However, they have some disadvantages, such as high cost, breeding problems and the fact that there are few studies to support their use (59). Typically, after 6 months, the minipigs develop gingivitis, translated by gingival inflammation, BOP, accumulation of bacterial plaque and tartar (59). At 16 months, gingival inflammation progresses to periodontal inflammation with histopathology similar to that reported in humans (59,61).

3.2 Methods of periodontitis induction

There are several methodologies used to induce periodontitis in animal models, as described by Acqua et al. (2022) (57). These include, but are not limited to, ligature placement, oral gavage, inoculation with periodontopathogenic bacteria and injection of lipopolysaccharide (LPS), or a mixture of these (57). These techniques function as critical steps in the experimental process.

Each of these methods is described in more detail in the following paragraphs.

3.2.1 Ligature

The ligature induction model of periodontitis was first described in 1966 and remains one of the most widely used models for studying PD in various animals (62–64). The technical procedure involves the placement of a suture, most commonly made of silk, cotton or nylon, around the tooth or in its interproximal region (63). The insertion of the ligature acts as a niche for bacterial colonisation by triggering a local immunoinflammatory response in the host (63,64).

It aims to facilitate the development of a dysbiotic oral microbiota and induce periodontal tissue damage similar to that observed in human periodontitis, including disruption of supporting tissues through microulceration of the sulcular epithelium, facilitating connective tissue invasion, apical migration of the junctional epithelium, influx of inflammatory cells and ABL (53,62–65).

Placement of ligatures in large animals, such as non-human primates, does not involve mechanical trauma (54). However, in small animals, we cannot exclude the possibility of mechanical trauma from ligatures due to their small body size (54). Therefore, the relevance of this model has been questioned when used in small animals with the justification that the mechanical injury of the ligature could exacerbate periodontal destruction (54).

It has some advantages, such as the knowledge of the moment of onset of PD and the predictable sequence of events ending in ABL, and the fact that the removal of ligatures allows the study of both the resolution of inflammation and the healing response, as it is easy to localize the inflamed gingival tissue (66).

In an attempt to induce PD more rapidly, many authors have chosen to apply dressings soaked in periodontopathogenic bacteria or to combine the dressing model with a method of oral inoculation, either by bacterial injection, or by an isolated bacterial component, e.g., LPS (54,67). The bacteria most commonly used in these methods is *P. gingivalis*, one of the main microorganisms associated with PD (54).

3.2.2 Oral gavage

Oral gavage induced PD consists of the oral administration of a predefined amount of human bacterial strains in a viscous suspension, usually 10⁹ colony forming units in 2% carboxymethylcellulose (54,56).

This model essentially uses *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, *T. forsythia*, *P. intermedia* and *F. nucleatum*, as well as a combination of different bacterial strains, since the pathogenicity of these species in the human oral microbiota has been confirmed (54,56,68). This model has also been used with the microorganism *Porphyromonas gulae*, a periodontopathogen found in animals and similar to *P. gingivalis* in humans (53,54).

However, the bacterial strain often needs to be administered several times, which is not always feasible because the experimental compound may be available in limited quantities or at high cost (54,66).

3.2.3 Bacterial inoculation

The bacterial inoculation model involves the localised microinjection of bacteria or an isolated bacterial component, such as LPS (67). This method is characterised by increased expression of pro-inflammatory cytokines, apical migration of the junctional epithelium and activation of osteoclastogenesis, leading to bone resorption (67).

This model is useful for analysing pathogenic differences between different periodontal bacteria, as mono-infection with a known bacterium allows a high degree of experimental control over the pathogenic stimulus (67).

3.2.3.1 LPS

This oral inoculation method uses a defined amount of suspended bacterial LPS, which is subsequently injected into the gingival tissue around the teeth, inducing an immune-inflammatory response in the host (54,56).

LPS is a component of the cell wall of most subgingival gram-negative organisms that are considered important periodontal pathogens (54,56,69). These molecules induce a significant inflammatory stimulus, with infiltration of polymorphonuclear leukocytes, oedema, vascular dilation in the inflamed periodontal tissue, production of inflammatory mediators such as interleukin 1 (IL-1), interleukin 6 (IL-6) and IL-8, tumor necrosis factor (TNF) or prostaglandins (PG), and, finally, osteoclast activation (54,56,69).

LPS injection is therefore an appropriate model to analyse how the host innate immune response to the bacterial component leads to the induction of osteoclastogenesis and ABL (56).

In 2015, it was confirmed that there were more than 2,000 articles in the field of periodontology using animal models (58). Furthermore, the variability of models raises the question of which is the most appropriate and which best mimics human local and systemic effects, considering the objective of a given study (53,70). In this sense, Acqua et al. (2022) published a systematic review on the local and systemic effects according to the different periodontitis induction models in mice (57). However, only seven articles were included, and various limitations are inherent in this systematic review (57).

4. AIMS

The aim of the present study is a systematic review of periodontitis induction protocols in animal models and their respective local and systemic effects.

II. MATERIAL AND METHODS

1. PROTOCOL

This systematic review of animal studies is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (detailed in supplementary information 1) (71).

We developed a protocol to answer the PECO question that follows: " What are the local and systemic effects of induction of periodontitis in animal models?" accompanied by the following pillars:

- P (Population): Animal model of periodontitis induction
- E (Exposure): Induction of periodontitis method
- C (Comparison): Healthy periodontal animal model
- O (Outcome): Local and systemic effects of periodontitis induction.

2. ELEGIBILITY CRITERIA

To be included, studies should have induced periodontitis in an animal model and included a healthy control group of the same species. Exclusion criteria were as follows: 1) studies of induction of periodontitis in humans; 2) studies in which experimental induction of periodontitis was not confirmed; 3) studies in which the protocols of interest were not available for analysis after contact with the original authors. There were no language or date restrictions.

3. RESEARCH SOURCES AND RESEARCH STRATEGY

The electronic search was conducted independently by two reviewers (C.S. and V.M.) in MEDLINE using PubMed (<https://pubmed.ncbi.nlm.nih.gov>), Embase

(<https://www.embase.com>) via Elsevier (<https://www.elsevier.com>) and Web of Science - WOS (<https://webofknowledge.com>) for articles published up to April 2023.

Searches were also conducted in specialty journals, namely Journal of Periodontology; Journal of Clinical Periodontology; Journal of Periodontal Research; Periodontology 2000; Journal of Periodontal & Implant Science; The International Journal of Periodontics & Restorative Dentistry; The Journal of American Dental Association; Journal of Oral Pathology & Medicine; Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology.

The search strategy uses MeSH terms combined by Boolean operators "OR" and "AND" adapted for each database: “(Periodontitis OR Periodontitides OR Pericementitis OR Pericementitides OR Disease, Periodontal OR Diseases, Periodontal OR Periodontal Disease OR Parodontosis OR Parodontoses OR Pyorrhea Alveolaris OR Alveolar Bone Losses OR Alveolar Process Atrophy OR Alveolar Process Atrophies OR Alveolar Resorption OR Alveolar Resorptions OR Resorption, Alveolar OR Resorptions, Alveolar OR Bone Loss, Periodontal OR Bone Losses, Periodontal OR Periodontal Bone Loss OR Periodontal Resorption OR Periodontal Resorptions OR Resorption, Periodontal OR Alveolar Bone Atrophy OR Alveolar Bone Atrophies OR Bone Atrophies, Alveolar OR Bone Atrophy, Alveolar OR Bone Loss, Alveolar OR interradicular bone loss OR interradicular bone) AND (Periodontal Disease Induction, Experimental Periodontitis OR Experimental Model OR Model, Experimental OR Models, Experimental OR Experimental Models OR Animal Model OR Animal Models OR Model, Animal OR Laboratory Animal Models OR Animal Model, Laboratory OR Animal Models, Laboratory OR Laboratory Animal Model OR Model, Laboratory Animal OR Models, Laboratory Animal OR Experimental Animal Models OR Animal Model, Experimental OR Animal Models, Experimental OR Experimental Animal Model OR Model, Experimental Animal OR Models, Experimental Animal OR Disease Models, Animal OR Animal Disease Model OR Animal Disease Models OR Disease Model, Animal)”.

4. SELECTION PROCESS

The selection process was carried out independently by two reviewers (C.S. and V.M.) in two steps: step 1, reviewing the titles and abstracts of all identified references, applying the inclusion process, and excluding non-relevant studies; and, step 2, applying exclusion criteria to the other studies mentioned above, based on the reading of the full text (blinded process), and detail the reasons for exclusion. Disagreement at any stage was resolved through discussion and mutual decision with a third reviewer (J.B.). All duplicate items have been removed manually.

5. DATA COLLECTION PROCESS

The articles were evaluated as a whole independently by two reviewers (C.S. and V.M.). However, when two or more studies had overlapping samples and used similar methodologies, the most complete study was included. A data collection table was created *a priori*, including author information, year of publication, number and type of animal used, periodontitis induced method, local effects, and systemic effects. When studies had missing information relevant to this systematic review the corresponding author was contacted via email to obtain it with a response deadline of 5 working days. Data available in graphs were retrieved using both the ImageJ program (<https://imagej.net/ij/index.html>) and WebPlotDigitizer (<https://automeris.io/WebPlotDigitizer/>).

6. QUALITY ASSESSMENT

The methodological quality of the articles included in this review was assessed by two investigators using the Systematic Review Center for Laboratory animal Experimentation (SYRCLE) tool for animal studies to assess the risk of bias (ROB) (72). The following items were assessed: sequence generation, baseline characteristics, allocation concealment, random housing, blinding, random outcome assessment, blind outcome assessor, incomplete outcome data, selective outcome reporting and other sources of bias.

For each domain of bias, studies were classified as representing low, unclear, or high ROB. Studies were independently assessed by two investigators, and agreement was reached in a meeting with a third investigator (J.B.).

7. DATA ITEMS AND SYNTHESIS

Standard spreadsheet software (using Google Sheets) was used for data extraction. Categorical variable was described using frequencies and percentages, whereas continuous variables were presented as mean \pm standard deviation (SD). The unit of measurement used in the meta-analysis was not restricted.

Estimates were calculated in R version 3.4.1 (R Studio Team 2018) using a DerSimonian-Laird random-effects model using the 'meta' package (73,74). The meta-analysis was performed to calculate the ABL and systemic changes after periodontitis induced models compared to controls. Ratio of Means (ROM) meta-analysis was used in order to not limit the unit of measurement and to allow easier and more direct interpretation of the results as a percentage (75,76). Results are presented as ROM and with the respective 95% confidence intervals (CI), and p-values less than 0.05 were considered statistically significant. To explore potential sources of heterogeneity, we performed a subgroup analysis by induction method, animal species, female versus male animals, and ligature material and ligature thickness. Meta-regression was used to explore the adjusting effects, particularly confounding variables, on ligature thickness, time of periodontitis induction and number of teeth involved in the periodontitis ligature induction method. Heterogeneity was explored through the I^2 index and Cochran's Q statistic ($p < .1$) and χ^2 test for the overall homogeneity, and substantial heterogeneity was considered when I^2 statistics exceeded 50% (77). For meta-analysis that included at least 10 results, in which the publication bias was analysed (77).

III. RESULTS

1. STUDY SELECTION

The electronic searches retrieved a total of 16,311 records (8,916 from PubMed, 2,715 from Web of Science, 3,130 from EMBASE and 1,550 from Journals). After removing duplicates, 10,299 articles were excluded based on title and abstract evaluation. Of the remaining articles, 1,041 articles were excluded after evaluation of the full text as they did not meet the inclusion criteria, with the reason for exclusion (71). Finally, a total of 450 articles were included in the qualitative and quantitative analyses of this systematic review (Figure 3). Data from two articles were repeated, so these papers were combined into one, as shown: (78,79).

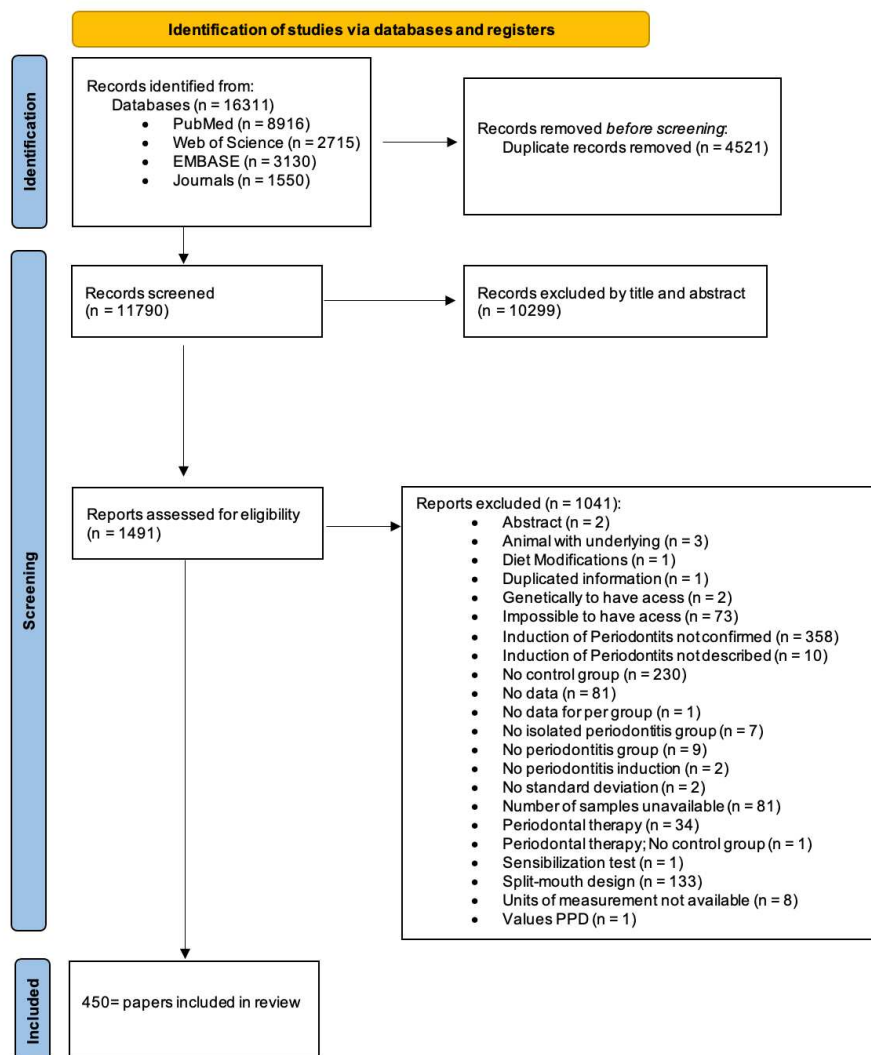


Figure 3 | Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flowchart of studies inclusion

2. STUDY CHARACTERISTICS

Table 1 summarises the characteristics of the articles in chronological order of the included studies. Studies from twenty different countries in Asia, America, Europe, and Australia were included.

Approximately 398 articles used only one method of periodontitis induction, while 52 articles used two or more methods of periodontitis induction. Regarding the gender parameter, about 76.2% (n=343) used only male species. Finally, regarding the duration of periodontitis induction, the minimum duration was two days, and the maximum duration was two hundred and thirty-one days.

Table 1 | *Articles Characteristics*

Author et al. Year	Country	Family; Genus; Species; Strain	N Total (N experimental / N Control)	Sex	Initial Weight (g) / Weeks	Periodontitis Induction Protocol	Number of teeth & teeth type	Induction period (days)
Chang et al. (1994)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	300 / NR	Oral gavage	NA	35
Doxey et al. (1998)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	200-250 / NR	Bacteria inoculation	NR	77
Sobaniec & Sobaniec-Lotowska (2000)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	30 (15/15); 30 (15/15)	Male	38-400 / NR	Ligature (NR Cotton)	1st mandibular molar right	7; 21
Bezerra et al. (2000)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	180-200 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) bilateral	7
Kimura et al. (2000)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	16 (10/6); 16 (10/6)	Male	NR / 7	Ligature (9-0 Silk) & Bacteria inoculation; Ligature (9-0 Silk)	1st maxillary molar (360°) Right	105
Mandalunis et al. (2003)	Argentina	Argentina; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	250 / NR	Ligature (NR Cotton)	1st mandibular molar	31
Jain et al. (2003)	Brazil	Brazil; Leporidae; <i>Oryctolagus cuniculus</i>	12 (6/6)	Male	NR / NR	Ligature & Bacteria inoculation (3-0 Silk)	2nd mandibular premolars bilateral	49
Tani-Ishii et al. (2003)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Female	NR / 7	Bacteria inoculation	NA	30
Achong et al. (2003)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 5	Ligature (NR Floss)	2nd maxillary molar (360°) bilateral	21
Vardar et al. (2004)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	200 / NR	LPS	NA	3
Dumitrescu et al. (2004)	UK	UK; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5); 10 (5/5); 10 (5/5)	Male	225-250 / NR	LPS	NA	3; 7; 10
Nassar et al. (2004)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	NR	100 / NR	Ligature (NR Cotton)	1st mandibular molar bilateral	30
Buduneli et al. (2004)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	205 / NR	LPS	NA	7
Wilensky et al. (2005)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6); 12 (6/6)	Female	NR / 9-10	Oral gavage	NA	NR
Vardar et al. (2005)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	200 / NR	LPS	NA	3
Bentzen et al. (2005)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	28 (14/14); 28 (14/14); 28 (14/14)	Male	337 / NR	Ligature (4-0 Silk); LPS	NA	6; 7; 28

Keles et al. (2005)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	30 (15/15)	Male	150-250 / NR	Ligature (NR Silk)	mandibular molars	40
Garat et al. (2005)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	250 / NR	Ligature (n.4 Cotton)	1st maxillary molar bilateral	2
Buduneli et al. (2005)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	21 (9/12)	Male	205 / NR	LPS	NA	7
Anbinder et al. (2006)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Female	NR / 12	Ligature (NR Cotton)	2nd maxillary molar (360°) bilateral	35
Cetinkaya et al. (2006)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	100 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	60
Xu et al. (2006)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8); 16 (8/8)	Male	280 / NR	Ligature (3-0 Silk)	2nd maxillary molar (360°)	28; 56
Pontes et al. (2006)	Denmark	Danish; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (9/11)	Male	NR / 12	Ligature (4-0 Silk)	2nd maxillary molar (360°) bilateral	42
Zhao et al. (2006)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6)	Male	200-250 / 6	Ligature (0.2mm diameter Wire) & Bacteria inoculation	1st maxillary molar bilateral	30; 60
Srivastava et al. (2006)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	180-210 / 9	Ligature (3-0 Silk)	1st mandibular molar left	56
Vardar-Şengül et al. (2006)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	21 (9/12)	Male	207 / NR	Bacteria inoculation	NA	15
Cetinkaya et al. (2007)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	100 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	60
Li & Amar (2007)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	11 (5/6); 11 (5/6); 11 (5/6); 4 (2/2); 4 (2/2); 4 (2/2)	Male	NR / 12	Ligature (5-0 Silk) & Bacteria inoculation	2nd maxillary molar (360°) left	3; 7; 10
Guimarães et al. (2007)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	90 (45/45); 90 (45/45); 90 (45/45); 90 (45/45); 18 (8/10); 20 (10/10)	Male	NR / NR	Ligature (NR)	1st mandibular molar right	5; 10; 15; 30
Pontes et al. (2007)	Denmark	Denmark; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (8/10)	Male	NR / 5	Ligature (4-0 Silk)	2nd maxillary molar (360°) bilateral	28
Pathirana et al. (2007)	Australia	Australia; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	NR	NR / 7-9	Bacteria inoculation	NA	20
Kirkwood et al. (2007)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (12/6)	Female	250 / NR	LPS	NA	56
Park et al. (2007)	USA	United States; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4)	Male	250 / NR	Bacteria inoculation	NA	56
Tomofuji et al. (2007)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	NR / 8	LPS	NA	56
Vaziri et al. (2007)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Female	110-145 / NR	Ligature (3-0 Silk)	1st mandibular molar right	28

Vardar-Sengul et al. (2008)	Finland	Finland; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	205 / NR	LPS	NA	3
Tomofuji et al. (2008)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 10	Ligature (n.3 Cotton)	1st mandibular molar bilateral	28
Watanabe et al. (2008)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Female	NR / 5	Ligature & LPS (4-0 Silk)	2nd maxillary molar (360°) bilateral	91
Cai et al. (2008)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	180-230 / 8	Ligature (3-0 Nylon)	1st mandibular molar bilateral	7
Ekuni et al. (2008)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 8	LPS	NA	56
Chen et al. (2008)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	6 (3/3)	Male	230 / 10	Bacteria inoculation	NA	9
Vardar-Sengul et al. (2008)	Finland	Finland; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	205 / NR	LPS	NA	3
Peruzzo et al. (2008)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	40 (20/20)	Male	NR / 8	Ligature (NR Cotton)	1st mandibular molar bilateral	30
Tomofuji et al. (2009)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 8	Ligature (n.3 Cotton)	1st mandibular molar bilateral	28
Hassumi et al. (2009)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	250-350 / NR	Ligature	1st mandibular molar bilateral	30
Cantley et al. (2009)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Female	NR / 9	Bacteria inoculation	NA	91
Polak et al. (2009)	Israel	Israel; Muridae; Rattus; <i>Mus musculus</i>	6 (3/3); 6 (3/3); 6 (3/3)	NR	NR / 4-5	Bacteria inoculation	NA	42
Toker et al. (2009)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	300-330 / NR	Ligature (4-0 Silk)	1st mandibular molar right	11
Ekuni et al. (2009)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 16 (8/8)	Male	NR / 8	Ligature (n.3 Cotton)	1st mandibular molar bilateral	28
Ekuni et al. (2009)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 16 (8/8)	Male	NR / 8	Ligature (n.3 Cotton)	1st mandibular molar bilateral	28
Napimoga et al. (2009)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	250-350 / NR	Ligature	1st mandibular molar bilateral	30
Tomofuji et al. (2009)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 8	Ligature (n.3 Cotton)	1st mandibular molar bilateral	28
Kuula et al. (2009)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	18 (10/8)	Male	NR / 15	Bacteria inoculation	NA	33
Bendyk et al. (2009)	Australia	Australia; Muridae; Rattus; <i>Mus musculus</i>	15 (10/5); 15 (10/5)	Female	NR / 7-9	Oral gavage	NA	42
Trombone et al. (2009)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 8	Oral gavage	NA	45

Gürkan et al. (2009)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	254 / 10	LPS	NA	11
Verma et al. (2010)	USA	Florida; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6); 12 (6/6)	Female	NR / 8-9	Bacteria inoculation	NA	84
Fernandes et al. (2010)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	144-170 / NR	Ligature (n.4 Cotton)	2nd maxillary molar (360°)	30
Endo et al. (2010)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 5	Ligature (n.4 Cotton)	1st mandibular molar	28
Ekuni et al. (2010)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	NR / 5	Ligature (NR Cotton)	1st mandibular molar	28
Yu et al. (2010)	NR	NR; Canidae; Canis; <i>Canis Lupus</i>	22 (12/10)	Both	18500 / NR	Ligature (2-0 Silk)	2nd mandibular premolars bilateral	90
Ekuni et al. (2010)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	NR / 5	Ligature (NR Cotton)	1st mandibular molar	28
Spolidorio et al. (2010)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	255-300 / NR	Ligature (n.3 Cotton)	1st mandibular molar bilateral	15
Wong et al. (2010)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	24 (12/12)	Both	NR / 8	Bacteria inoculation	NA	50
Mousavi-Jazi et al. (2010)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	NR / 12	LPS	NA	10
Ku et al. (2010)	Korea	Korea; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	160-180 / 7	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Verma et al. (2010)	USA	Florida; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6); 12 (6/6)	Female	NR / 8-9	Bacteria inoculation	NA	84
Watanabe et al. (2010)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 9	Ligature & LPS (8-0 Silk)	2nd maxillary molar (360°) bilateral	63
Cantley et al. (2011)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	7 (4/3)	Female	NR / 9	Bacteria inoculation	NA	44
Coimbra et al. (2011)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	250-300 / NR	Ligature (n.3 Cotton)	1st mandibular molar bilateral	15
Holzhausen et al. (2011)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10); 20 (10/10)	Male	250-300 / 12-14	Ligature (3-0 Silk)	1st mandibular molar right	7; 14
Maekawa et al. (2011)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10); 20 (10/10)	Male	NR / 8	Bacteria inoculation	NA	28; 120
Keles et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10); 20 (10/10)	Male	200-250 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	10; 40
Sugiyama et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 4	Oral gavage	NA	231
Benatti et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	250-350 / NR	Ligature	1st mandibular molar bilateral	30

Toker et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	270-320 / NR	Ligature (4-0 Silk)	1st mandibular molar right	30
Napimoga et al. (2012)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	20-25 / 6-7	Bacteria inoculation	NA	15
Queiroz-Junior et al. (2012)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 6	Bacteria inoculation	NA	30
Liu et al. (2012)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	180 / NR	Ligature (NR Wire)	1st maxillary molar (360°) Right	56
Semenoff-Segundo et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	NR	232,8 / NR	Ligature (4-0 Silk)	2nd maxillary molar (360°) right	50
Ozdemir et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	300 / NR	Ligature (4-0 Silk)	1st mandibular molar right	11
Demirer et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	300 / NR	Ligature (4-0 Silk)	1st mandibular molar right	11
Xiao et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	NR / 8	Ligature (0.2mm diameter Wire)	1st maxillary molar	56
Branco-de-Almeida et al. (2012)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 8	Ligature (NR Cotton)	1st mandibular molar bilateral	15
Zhao et al. (2012)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	64 (32/32); 64 (32/32); 64 (32/32)	Male	250 / 8	Ligature (4-0 Silk)	2nd maxillary molar (360°) right	7; 14; 28
Colombo et al. (2012)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 8	Ligature (4-0 Silk)	1st mandibular molar bilateral	28
Steffens et al. (2012)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	300-400 / NR	Ligature	1st mandibular molar bilateral	15
Bitto et al. (2012)	Italy	Italy; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	350-380 / 12	Ligature (4-0 Silk)	1st mandibular molar left	14
Napimoga et al. (2013)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	20-25 / 6-7	Oral gavage	NA	20
Araújo et al. (2013)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-220 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
de Araújo Júnior et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-220 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Brito et al. (2013)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8); 16 (8/8); 16 (8/8)	Male	200-250 / 10	Ligature (n.4 Cotton)	1st mandibular molars and bilateral maxillary 2nd molars	7; 14; 28
Wang et al. (2013)	China	China; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	NR / 8	Bacteria inoculation	NA	5
Fu & He (2013)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	40 (20/20); 40 (20/20)	Male	180-220 / 7	Ligature (3-0 Nylon)	2nd maxillary molar (360°) bilateral	21; 42

Su et al. (2013)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 12	Ligature & Bacteria inoculation (3-0 Silk)	1st and 2nd maxillary molars bilateral	56
Kara et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	220-250 / 24	Ligature (3-0 Silk)	1st mandibular molar bilateral	28
Endo et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 8	Ligature (n.3 Cotton)	2nd maxillary molar (360°)	14
Suda et al. (2013)	Japan	Tokyo; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / NR	Oral gavage	NA	15
Saadi-Thiers et al. (2013)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5)	Male	NR / 8-12	Ligature (6-0 Silk) & Bacteria inoculation; Ligature (6-0 Silk)	1st maxillary molar bilateral	15
de Medeiros Vanderlei et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / NR	Ligature (NR Cotton)	1st mandibular molar	10
Deves et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4); 8 (4/4)	NR	NR / NR	Ligature (n.4 Cotton); LPS	1st mandibular molar bilateral	10
Sezer et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	300 / NR	Ligature (4-0 Silk)	1st mandibular molar right	11
Zhang et al. (2013)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	280-300 / 9	Ligature (NR Nylon)	maxillary molars	14
Kara et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	220-250 / 24	Ligature (3-0 Silk)	1st mandibular molar bilateral	28
Tomofuji et al. (2013)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	NR / 8	Ligature (n.3 Cotton)	2nd maxillary molar (360°) bilateral	28
Júnior et al. (2013)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	180-220 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Yang et al. (2013)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	40 (20/20)	Female	200-220 / NR	Ligature & Bacteria inoculation (0.2mm diameter Wire)	1st maxillary molar bilateral	28
Akman et al. (2013)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	220-250 / NR	Ligature (n.3 Cotton)	1st mandibular molar	35
Messora et al. (2013)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	170-250 / NR	Ligature (NR Cotton)	1st mandibular molar	44
Jin et al. (2014)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Female	NR / 8	Bacteria inoculation	NA	28
Lu et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180 / NR	Ligature (0.2mm diameter Nylon)	1st maxillary molar	30
Xu et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Female	230-250 / 17	Ligature (3-0 Silk)	1st and 2nd maxillary molars bilateral	28
Tamaki et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	220-240 / 8	Ligature	2nd maxillary molar (360°) right	20

Lin et al. (2014)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5)	NR	NR / 8-10	Ligature (5-0 Silk) & Bacteria inoculation; Ligature (5-0 Silk)	2nd maxillary molar (360°)	14
Zhang et al. (2014)	USA	Texas; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 11-13	Bacteria inoculation	NA	38
Yang et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Female	NR / 7	Ligature & Bacteria inoculation (0.2mm diameter Wire)	1st maxillary molar bilateral	28
Miyajima et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 7	Ligature (3-0 Nylon)	2nd maxillary molar (360°) bilateral	28
Li et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	6 (3/3)	Male	250,7 / NR	LPS	NA	7
Zhang et al. (2014)	USA	Texas; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 11-13	Bacteria inoculation	NA	38
Araújo et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-220 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Balci et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	270-320 / NR	Ligature (4-0 Silk)	1st mandibular molar right	14
Nassar et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5); 10 (5/5); 10 (5/5)	Male	NR / NR	Ligature (NR Cotton)	1st mandibular molar right	5; 10; 15
Chang et al. (2014)	Taiwan	Taiwan; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	NR	NR / NR	Ligature (3-0 Silk)	1st mandibular molars and 2nd maxillary molars bilateral	8
Toker et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	300-330 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	15
Furlaneto et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	250-300 / 12-16	Ligature (NR Nylon)	2nd maxillary molar (360°) left	11
Spolidorio et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8); 16 (8/8)	Male	250-300 / NR	Ligature (n.3 Cotton)	2nd maxillary molar (360°) bilateral	8; 15
Jin et al. (2014)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Female	NR / 8	Bacteria inoculation	NA	28
Wang et al. (2014)	China	China; Muridae; Rattus; <i>Mus musculus</i>	8 (4/4)	Female	20 / 7	Bacteria inoculation	NA	8
Kim et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / NR	Ligature	1st and 2nd mandibular molars bilateral	30
de Molon et al. (2014)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6)	Female	30 / 8	Ligature (6-0 Nylon); Oral gavage	1st maxillary molar	7; 15; 30
Mendes et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	32 (16/16); 32 (16/16)	Male	200-250 / NR	Ligature (n.4 Cotton)	1st mandibular molars and bilateral maxillary 2nd molars	7; 21

Wei et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (18/6)	Male	NR / 8	Ligature (3-0 Silk)	NR	10
Köseoğlu et al. (2014)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9)	Male	340 / 16	Ligature	1st mandibular molar right	11
Kim et al. (2014)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / NR	Ligature	1st and 2nd mandibular molars bilateral	30
Yağan et al. (2014)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Female	250-300 / NR	Ligature (n.5 Cotton)	2nd maxillary molar (360°) bilateral	21
Wang & Wang (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200 / 6	Ligature (0.2mm diameter Wire)	2nd maxillary molar (360°) bilateral	56
Silva et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	192 / 8	Ligature	1st mandibular molar right	14
Hatipoğlu et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	340 / 16	Ligature (4-0 Silk)	1st mandibular molar right	11
Lam et al. (2015)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	NR / 7-9	Bacteria inoculation	NA	54
Dong et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	180-200 / NR	Ligature (3-0 Silk)	1st mandibular molar	7
Yang et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200 / 7	Ligature	1st mandibular molar bilateral	7
Aral et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	300-350 / NR	Ligature (4-0 Silk)	1st maxillary molar bilateral	21
Moraes et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	362 / NR	Ligature (n.3 Cotton)	1st mandibular molar	28
Li et al. (2015)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-220 / 6-8	Ligature	1st maxillary molar (360°) Right	14
Ni et al. (2015)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	200 / NR	Ligature (3-0 Silk)	1st mandibular molar	7
Fu et al. (2015)	Taiwan	Taiwan; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 6	Ligature (3-0 Silk)	1st mandibular molars and 2nd maxillary molars bilateral	8
Lisboa et al. (2015)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-250 / NR	Ligature (NR Cotton)	1st mandibular molar right	11
Lapérine et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10); 20 (10/10); 20 (10/10); 20 (10/10); 20 (10/10)	Male	NR / 14	Ligature (6-0 Silk); Ligature (6-0 Silk) & Bacteria inoculation	1st maxillary molar	4; 14; 28
Dundar et al. (2016)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	26 (13/13)	Female	135 / NR	Ligature (4-0 Silk)	1st and 2nd mandibular molars bilateral	11
Bhattarai et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 8	Ligature & LPS (n.5 Cotton)	2nd maxillary molar (360°) right	14

Nakajima et al. (2016)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	14 (7/7)	Male	NR / 9	Bacteria inoculation	NA	35
Lee et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4)	Male	180-200 / 6	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	28
Chin et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-250 / 8	Ligature (3-0 Silk)	1st mandibular molar bilateral	8
Ilievski et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	NR / 7	Bacteria inoculation	NA	154
Balli et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	15
Papathanasiou et al. (2016)	Spain	Spain; Muridae; Rattus; <i>Mus musculus</i>	11 (5/6)	Male	NR / 16	Bacteria inoculation	NA	21
Martins et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	200-250 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Park et al. (2016)	Korea	Korea; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 7	Ligature (3-0 Nylon)	Maxillary incisor left	10
Sun et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	32 (16/16)	Male	200-220 / NR	Ligature & Bacteria inoculation (5-0 Nylon)	2nd maxillary molar (360°) bilateral	28
Kose et al. (2016)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10); 18 (9/9)	Male	NR / NR	Ligature (n.3 Cotton)	1st mandibular molar	28; 35
Lu et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	60 (30/30)	Male	180 / NR	Ligature (0.2mm diameter Nylon)	1st maxillary molar (360°) Right	30
Ossola et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	350 / 16	LPS	NA	42
Kose et al. (2016)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10); 18 (9/9)	Male	NR / NR	Ligature (Cotton n.3)	1st mandibular molar	28; 35
Lima et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 12	Ligature (n.3 Cotton)	1st mandibular molar	29
Matsuda et al. (2016)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	NR / 6	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	14
Choi et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-220 / 8	Ligature (4-0 Silk)	1st mandibular molar right	15
Balci et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	390-450 / 10-12	Ligature (4-0 Silk)	1st mandibular molar right	28
Çalışır et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (8/6)	NR	280-320 / NR	Ligature (4-0 Silk)	1st mandibular molar right	15
Bhattarai et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 8	Ligature & LPS (n.5 Cotton)	2nd maxillary molar (360°) right	14
Castro et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	26 (6/20)	Male	250-300 / NR	Ligature (NR Cotton)	1st mandibular molar	15

de Molon et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6)	Female	25 / 8	Ligature (6-0 Nylon); Oral gavage	1st maxillary molar bilateral	45; 60; 61; 62
Bae et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-220 / 8	Ligature (4-0 Silk)	1st mandibular molar right	14
Chiang et al. (2016)	Taiwan	Taiwan; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-250 / 6	Ligature (3-0 Silk)	1st mandibular molars and 2nd maxillary molars bilateral	8
Yang et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	NR / 16	Ligature & Bacteria inoculation (6-0 Silk)	1st maxillary molar	NR
O'Brien-Simpson et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	24 (12/12)	Female	NR / 7-9	Bacteria inoculation	NA	58
Madeira et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	NR	NR / 8	Bacteria inoculation	NA	30
Kang et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	170-190 / 8	Ligature (3-0 Nylon)	maxillary incisor left	10
Wang et al. (2016)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	22 / 4	Bacteria inoculation	NA	56
Campi et al. (2016)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	180-200 / NR	Ligature (n.3 Cotton)	1st mandibular molar right	7
Yu et al. (2016)	China	China; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	NR / 6	Ligature & Bacteria inoculation (5-0 Silk)	2nd maxillary molar (360°) bilateral	10
Kırzioğlu et al. (2016)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	19 (10/9)	Male	NR / 24	Ligature	2nd maxillary molar (360°) bilateral	14
Sousa et al. (2016)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 14-15	Ligature (NR Nylon)	2nd maxillary molar (360°) left	11
Bakır et al. (2016)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (10/8)	Male	225-353 / 16	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	14
Nogueira et al. (2016)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (9/9); 18 (9/9); 18 (9/9); 18 (9/9)	Male	300 / NR	Ligature (NR Cotton)	1st maxillary molar bilateral	6; 8; 12; 20
Akpınar et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	250 / NR	Ligature (4-0 Silk)	1st mandibular molar right	12
Teixeira et al. (2017)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Female	200 / 10	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Kim et al. (2017)	Korea	South Korea; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6)	Male	NR / NR	Ligature (NR)	1st mandibular molar bilateral	3; 20
Yamaguchi et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12); 24 (12/12)	Male	NR / NR	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	7; 14
de O Silva et al. (2017)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	203 / NR	Ligature (NR Cotton)	1st mandibular molar bilateral	14

Lin et al. (2017)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5)	NR	NR / 8-10	Bacteria inoculation; Ligature (7-0 Silk)	NA	14
Yu et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	190-220 / NR	Ligature (4-0 Silk)	1st mandibular molar right	28
Elburki et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	150-250 / 8-10	LPS	NA	21
dos Santos Carvalho et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	152,8 / NR	Ligature (3-0 Nylon)	1st mandibular molar right	21
Yiğit et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (10/8)	Male	200 / NR	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	14
Park et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	9 (4/5)	Male	200-220 / 7	Ligature (4-0 Silk)	1st mandibular molar right	14
França et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	22 (11/11)	Female	166 / NR	Ligature (3-0 Nylon)	1st mandibular molar	20
Kuo et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-230 / 6	Ligature (3-0 Silk)	2nd maxillary and 1st mandibular molars	8
Sun et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	32 (16/16)	Male	200-220 / 7-9	Ligature (3-0 Nylon)	1st mandibular molar bilateral	14
Jiang et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	180-220 / NR	Ligature & LPS (5-0 Silk)	2nd maxillary molar (360°) bilateral	28
Balci et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	390-450 / NR	Ligature (4-0 Silk)	1st mandibular and maxillary molars right	28
Köse et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-220 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Oliveira et al. (2017)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	230-250 / 12	Ligature (NR Cotton)	1st mandibular molar	14
Karakan et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	280 / 24	Ligature (4-0 Silk)	1st mandibular molar right	12
Köse et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-220 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Köse et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-220 / NR	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Trindade-da-Silva et al. (2017)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	20-25 / 6-7	Bacteria inoculation	NA	16
Leite et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	NR	260 / 8	Ligature (n.4 Cotton)	1st mandibular molar bilateral	30
Tanaka et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	NR / 24	Ligature (NR Silk)	2nd maxillary molar (360°) bilateral	10
Pillusky et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 10	Ligature (NR Cotton)	1st mandibular molar right	7

Papadakou et al. (2017)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	36 (18/18)	NR	NR / NR	Ligature (7-0 Silk)	2nd maxillary molar (360°) bilateral	21
Gao et al. (2017)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8); 16 (8/8); 16 (8/8); 16 (8/8)	Male	220 / NR	Ligature (NR Silk) & Bacteria inoculation	2nd maxillary molar (360°) bilateral	14; 28; 56; 84
Yamaguchi et al. (2017)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 11	Oral gavage	NA	21
Iguchi et al. (2017)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	30 (15/15)	Male	NR / 8	Ligature (5-0 Silk)	2nd maxillary molar (360°) right	14
Yang et al. (2017)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	220 / 13	Ligature (3-0 Silk)	1st mandibular molar bilateral	14
Andrade et al. (2017)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	22 (11/11)	Male	218,2 / NR	Ligature	1st mandibular molar bilateral	14
Andrade et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	230,8 / NR	Ligature (NR Cotton)	1st mandibular molar bilateral	14
Pereira et al. (2018)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	40 (20/20)	Male	180-220 / NR	Ligature (3-0 Nylon)	2nd mandibular molar (360°) left	11
Ben et al. (2018)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 10	Oral gavage	NA	15
Marchesan et al. (2018)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5)	Female	NR / NR	Ligature (NR Silk)	Interproximal between 1st and 2nd maxillary molars right	3; 6; 9; 12; 15; 18
Kook et al. (2018)	Korea	South Korea; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	280-300 / 10	LPS	NA	20
Kim et al. (2018)	USA	Los Angeles; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 6	Ligature (6-0 Silk)	2nd maxillary molar (360°)	21
Levi et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	170-300 / NR	Ligature (NR Cotton)	1st mandibular molar	44
Ihn et al. (2018)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 6	Ligature (5-0 Silk)	2nd maxillary molar (360°) left	7
Zhang et al. (2018)	NR	NR; Suidae; Sus; <i>Minipig</i>	6 (3/3); 12 (6/6)	Both	500-550 / 48-72	Ligature (4-0 Silk); Oral gavage	1st mandibular molar bilateral	40; 84
Zhang et al. (2018)	NR	NR; Suidae; Sus; <i>Minipig</i>	6 (3/3); 12 (6/6)	Both	500-550 / 48-72	Ligature (4-0 Silk); Oral gavage	1st mandibular molar bilateral	40; 84
Zambrano et al. (2018)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	NR	NR / NR	LPS	NA	28
de Paiva Gonçalves et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 44-48	Ligature (n.3 Cotton)	1st mandibular molar bilateral	15
Pessoa et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Female	188,6 / NR	Ligature (3-0 Nylon)	1st mandibular molar right	20

Ribeiro et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Female	200-220 / NR	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Wang et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	NR	150-250 / 5-10	Ligature & Bacteria inoculation (4-0 Silk)	2nd maxillary molar (360°) bilateral	28
Toker et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	270-350 / 16	Ligature (4-0 Silk)	1st mandibular and maxillary molars right	30
Takamori et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 9	LPS	NA	10
Furlaneto et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 12-16	Ligature (3-0 Nylon)	2nd maxillary molar (360°)	11
Kim et al. (2018)	USA	Los Angeles; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 6	Ligature (6-0 Silk)	2nd maxillary molar (360°)	21
Prestes et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-250 / NR	Ligature (n.4 Cotton)	1st mandibular molars and 2nd maxillary molars bilateral	14
Zhang et al. (2018)	NR	NR; Suidae; Sus; <i>Minipig</i>	6 (3/3); 12 (6/6)	Both	500-550 / 48-72	Ligature (4-0 Silk); Oral gavage	1st mandibular molar bilateral	40; 84
Gatej et al. (2018)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	NR / 6-8	Bacteria inoculation	NA	44
Wada-Mihara et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4); 8 (4/4)	Male	200-250 / NR	Ligature (5-0 Nylon)	2nd maxillary molar (360°) right	5; 14
Khajuria et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12); 16 (8/8)	Male	200 / 13	Ligature (3-0 Silk) & Bacteria inoculation; LPS	NA	14
Khajuria et al. (2018)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12); 16 (8/8)	Male	200 / 13	Ligature (3-0 Silk) & Bacteria inoculation; LPS	NA	14
Balci et al. (2018)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	230-250 / NR	Ligature (4-0 Silk)	1st mandibular molar right	11
Olchanheski Jr et al. (2018)	Brazil	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-250 / 10	Ligature (n.4 Cotton)	1st mandibular molars and 2nd maxillary molars bilateral	14; 21
Lee et al. (2018)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5)	Female		Bacteria inoculation	NA	28
Zolotukhin et al. (2018)	Ukraine	Ukraine; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-220 / 4-6	Ligature	central incisor	15
Virto et al. (2018)	Spain	Spain; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	NR	NR / NR	Oral gavage	NA	182
Li et al. (2018)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Both	NR / 4	Oral gavage	NA	56
Çalışır et al. (2019)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (8/6)	Male	280-320 / NR	Ligature (4-0 Silk)	1st mandibular and maxillary molars right	15

Li et al. (2019)	China	China; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	20-22 / 7	Bacteria inoculation	NA	5
Pereira et al. (2019)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	20 / 12	Ligature (5-0 Silk)	1st mandibular molar bilateral	56
Wang et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12); 24 (12/12); 24 (12/12)	Male	200 / 10	Ligature (0.2mm diameter Wire)	1st maxillary molar bilateral	14; 28; 42
Gürkan et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	150-250 / NR	Ligature (NR Silk)	1st mandibular molar	15
Yildirim et al. (2019)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	220 / 12	Ligature (3-0 Silk)	1st mandibular molar	21
Ereş et al. (2019)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (6/4)	Female	NR / NR	Ligature (3-0 Silk)	1st and 2nd maxillary molars bilateral	21
Sulijaya et al. (2019)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	15 (8/7)	Male	NR / 9	Ligature (5-0 Silk)	2nd maxillary molar (360°) left	7
Kim et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 7	Ligature (4-0 Silk)	2nd mandibular molar (360°) right	56
Balci et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	230-250 / NR	Ligature (4-0 Silk)	1st mandibular molar right	11
Karatas et al. (2019)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	230-350 / NR	Ligature (4-0 Silk)	1st mandibular molar right	15
Kurt et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	150-250 / 8-10	Ligature (NR Silk)	1st mandibular molar	15
Moro et al. (2019)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10); 20 (10/10); 20 (10/10)	Male	250 / NR	Ligature (NR Cotton)	1st mandibular molar bilateral	7; 14; 21
da Silva et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	187,8 / NR	Ligature (3-0 Nylon)	1st mandibular molar right	20
Han et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 12	Bacteria inoculation	NA	63
Wang et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12); 24 (12/12); 24 (12/12)	Male	200 / 10	Ligature (0.2mm diameter Wire)	1st maxillary molar bilateral	14; 28; 42
Jiang et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6)	Male	250-300 / NR	Ligature (elastic ligature); Ligature (elastic ligature) & LPS	Interproximal between 1st and 2nd maxillary molars right	14
Mahmoud et al. (2019)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8); 16 (8/8); 16 (8/8)	NR	NR / 12	Bacteria inoculation	NA	47; 48; 49
Leira et al. (2019)	Spain	Spain; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	300-350 / 7	LPS	NA	14
Pan et al. (2019)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 6-7	Bacteria inoculation	NA	8
Zhang et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	220-250 / NR	Ligature (0.2mm diameter Wire)	1st maxillary molar (360°) left	56

Han et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 12	Bacteria inoculation	NA	63
Yu et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	200-230 / NR	Ligature (NR orthodontic ligatures)	1st mandibular molar bilateral	42
Vasconcelos et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Female	222,4 / NR	Ligature (3-0 Nylon)	1st mandibular molar bilateral	20
Kuo et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	180-230 / 6	Ligature (3-0 Silk)	2nd maxillary and 1st mandibular molars	7
Ideguchi et al. (2019)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	8 (4/4)	Female	NR / 10	Ligature (6-0 Silk)	2nd maxillary molar (360°) left	14
Kizildag et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	220-250 / 12	LPS	NA	28
Ferreira et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	250-300 / 12	Ligature (4-0 Silk)	2nd maxillary molar (360°)	15
Huang et al. (2019)	USA	Florida; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Female	NR / 8	Bacteria inoculation	NA	42
Gu et al. (2019)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 7	Bacteria inoculation	NA	49
Han et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 12	Bacteria inoculation	NA	63
Li et al. (2019)	China	China; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	20-22 / 7	Bacteria inoculation	NA	5
Oballe et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 15	Ligature (4-0 Silk)	2nd maxillary molar (360°) right	12
Taskan et al. (2019)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	NR / NR	Ligature (4-0 Silk)	1st mandibular molar right	14
Bi et al. (2019)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	300 / 8	LPS	NA	28
Yang et al. (2019)	Korea	South Korea; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	180-200 / 7	Ligature & Bacteria inoculation	2nd maxillary molar (360°) bilateral	NR
Silva et al. (2019)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	230-250 / NR	Ligature (NR Cotton)	1st mandibular molar	14
Ossola et al. (2019)	Argentina	Argentina; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	350 / 16	LPS	NA	42
Huang et al. (2019)	USA	Florida; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Female	NR / 8	Bacteria inoculation	NA	42
Zhang et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Female	200-250 / 8	Ligature (n.3 Cotton)	2nd maxillary molar (360°) right	28
Toraman et al. (2020)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	300 / NR	Ligature (3-0 Silk)	1st mandibular molar right	11

Wang et al. (2020)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	NR	NR / 8	Ligature (NR Silk)	Interproximal between 1st and 2nd maxillary molar & Interproximal between 2nd and 3rd maxillary molar	28
Díaz-Zúñiga et al. (2020)	Chile	Chile; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4); 8 (4/4); 8 (4/4)	Male	NR / NR	LPS	NA	7; 45
Boyer et al. (2020)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 7	Oral gavage	NA	35
Kantarci et al. (2020)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	15 (8/7)	Male	NR / 32	Ligature (7-0 Silk)	2nd maxillary molar (360°) bilateral	28
Casili et al. (2020)	Italy	Italy; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-230 / NR	LPS	NA	14
Mulhall et al. (2020)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 12	Oral gavage	NA	42
Li et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200 / 9	Ligature (3-0 Silk)	2nd maxillary molar (360°)	NR
Ribas et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	250 / 6	Ligature (4-0 Silk)	2nd molars upper and lower	14
Ossola et al. (2020)	Argentina	Buenos Aires; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6); 12 (6/6)	Male	350 / NR	LPS	NA	7; 14; 21
Zhang et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Female	200-250 / 8	Ligature (n.3 Cotton)	2nd maxillary molar (360°) right	28
Lektemur et al. (2020)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	NR	370-420 / NR	Ligature (4-0 Silk)	1st mandibular molar bilateral	30
Li et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200 / 9	Ligature (3-0 Silk)	2nd maxillary molar (360°)	NR
Varela-López et al. (2020)	Spain	Spain; Leporidae; <i>Oryctolagus cuniculus</i>	16 (8/8)	Male	2500 / NR	LPS	NA	40
Rocha et al. (2020)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 6-8	Bacteria inoculation	NA	28
Pei et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	50 (25/25)	Male	NR / 5-7	Ligature & Bacteria inoculation (4-0 Silk)	2nd maxillary molar (360°) right	42
Karatas et al. (2020)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (4/8); 12 (4/8)	Female	230-250 / NR	Ligature (4-0 silk)	1st mandibular molar bilateral	15; 30
Branco-de-Almeida et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	300-400 / NR	Ligature (NR Nylon)	1st mandibular molar bilateral	14
Aytekın et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	14 (7/7)	Male	220-250 / NR	Ligature (3-0 Silk)	1st mandibular molar right	11

Gao et al. (2020)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	NR / 9	Bacteria inoculation	NA	56
Hiyoshi et al. (2020)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	NR	NR / 8	Ligature (5-0 Silk)	2nd maxillary molar (360°)	8
Zhan et al. (2020)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 8	Ligature (n.5 Cotton)	2nd molars maxillary and mandibular bilateral	10
Kim et al. (2020)	China	China; Muridae; Rattus; <i>Mus musculus</i>	40 (20/20)	Male	18 / 5	Ligature (6-0 Silk)	2nd maxillary molar (360°) left	14
Kızıldağ et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	220-250 / 12	LPS	NA	NR
Pereira et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	300-350 / 11	Ligature (n.24 Cotton)	1st mandibular molar	14
Li et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200 / 9	Ligature (3-0 Silk)	2nd maxillary molar (360°)	NR
He et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	200-210 / 8	Ligature & Bacteria inoculation (4-0 Silk)	2nd maxillary molar (360°) bilateral	21
Wu et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	180-200 / 8	Ligature (3-0 Silk)	1st maxillary molar bilateral	28
Kim et al. (2020)	China	China; Muridae; Rattus; <i>Mus musculus</i>	40 (20/20)	Male	18 / 5	Ligature (6-0 Silk)	2nd maxillary molar (360°) left	14
Dionísio et al. (2020)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5)	Male	320 or 400 / 8-12	Ligature (NR Silk)	1st mandibular molar	1; 3; 7; 14
Steffens et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200 / 6-7	Ligature (n.3 Cotton)	1st mandibular molar	28
Lu et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	30 (15/15)	NR	NR / 8	Ligature	2nd maxillary molar (360°) bilateral	28
Kuraji et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6)	Male	NR / 8	Ligature (3-0 Silk)	1st maxillary molar bilateral	1; 3; 7; 14
Azeez et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	250-350 / NR	Ligature & Bacteria inoculation (4-0 Nylon)	mandibular incisor in an “8” shape	14
Alves et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	150,6 / NR	Ligature (3-0 Nylon)	1st mandibular molar right	NR
Kravchenko et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	180-220 / 4-6	Ligature	central incisor	15
Galego et al. (2020)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	174,7 / 10	Ligature (3-0 Nylon)	1st mandibular molar right	20
Vasconcelos et al. (2020)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Female	213,5 / NR	Ligature (3-0 Nylon)	1st mandibular molar bilateral	20
Silva et al. (2020)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	220-300 / 8-10	Ligature (3-0 Silk)	1st mandibular molar bilateral	11

Kim et al. (2020)	China	China; Muridae; Rattus; <i>Mus musculus</i>	40 (20/20)	Male	18 / 5	Ligature (6-0 Silk)	2nd maxillary molar (360°) left	14
Yu et al. (2020)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 5	Ligature & Bacteria inoculation (0.2mm diameter Wire)	1st maxillary molar bilateral	56
Köse et al. (2020)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-220 / 8- 16	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Cafferata et al. (2020)	Chile	Chile; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	NR	20 / 7	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	15
Zhou et al. (2021)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Female	NR / 6	Ligature & Bacteria inoculation (NR Silk)	2nd maxillary molar (360°) bilateral	49
Kou et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	25 / NR	Ligature & LPS (5-0 Silk)	2nd maxillary molar (360°)	14
Mustafa et al. (2021)	Malaysia	Malaysia; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6)	Male	292-360 / 9- 10	Ligature (4-0 silk); Ligature (4-0 silk) & Bacteria inoculation; Bacteria inoculation	2nd maxillary molar (360°) bilateral	7; 14; 30
Li et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	200–250 / 8	Ligature (5-0 Nylon)	1st and 2nd maxillary molars unilateral	14
Yiğit et al. (2021)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (10/8)	Male	200 / NR	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	14
Settem et al. (2021)	USA	Buffalo; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	NR / 7-9	Oral gavage	NA	42
Öngöz et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	220-250 / 8	Ligature (3-0 Silk)	1st mandibular molar bilateral	14
Kose et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	250 / 13-17	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Kurt-Bayrakdar et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	220-250 / 13-17	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Chen et al. (2021)	China	China; Muridae; Rattus; <i>Mus musculus</i>	15 (10/5)	Male	18–22 / 6-8	Ligature (6-0 Silk)	2nd maxillary molar (360°) bilateral	8
Yu et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	20 / 9	Ligature (5-0 Silk)	2nd maxillary molar (360°)	35
Zhou et al. (2021)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Female	NR / 6	Ligature & Bacteria inoculation (NR Silk)	2nd maxillary molar (360°) bilateral	49
Liu et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	26 (13/13)	Male	350 / 8	Ligature (0.25mm diameter Wire)	2nd maxillary molar (360°) bilateral	28
Silva et al. (2021)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10); 20 (10/10)	Male	NR / 12; NR / 57	Ligature (NR Silk)	1st mandibular molar bilateral	14
Vieceli et al. (2021)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	24 (12/12)	Male	22-25 / NR	LPS	NA	28

Tanaka et al. (2021)	USA	USA; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Female	NR / 10	Ligature (5-0 Silk)	2nd maxillary molar (360°) left	5
An et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	18 (12/6); 18 (12/6)	Male	NR / 7-9	Ligature (5-0 Silk); Ligature (5-0 Silk) & Bacteria inoculation	2nd maxillary molar (360°) left	35
Ni & Hua (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	250-300 / 8-10	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	14
Moro et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	200-250 / 6-8	Ligature (NR Cotton)	1st mandibular molar bilateral	7
Yang et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 8	Bacteria inoculation	NA	56
Martínez et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	230-250 / NR	Oral gavage	NA	84
Ren et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	220-250 / 6	Ligature (0.2mm diameter Wire)	2nd maxillary molar (360°) bilateral	14
Shen et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	6 (3/3)	Male	NR / 8	Ligature & LPS (5-0 Silk)	2nd maxillary molar (360°)	7
Kose et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	250 / 13-17	Ligature (3-0 Silk)	1st mandibular molar bilateral	35
Yue et al. (2021)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	24 (12/12)	Male	200-220 / 8	Ligature (NR Wire)	1st maxillary molar bilateral	56
Cao et al. (2021)	China	China; Muridae; Rattus; <i>Mus musculus</i>	18 (9/9)	Female	18-20 / 8	Ligature & Bacteria inoculation (6-0 Silk)	2nd maxillary molar (360°)	14
Wang et al. (2021)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5)	Male	NR / 8	Ligature (5-0 Silk); LPS	1st maxillary molar bilateral	14; 28; 56; 84
Liu et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	26 (13/13)	Male	350 / 8	Ligature (0.25mm diameter Wire)	2nd maxillary molar (360°) bilateral	28
Dogan & Dogan et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	224-258 / 10-12	Ligature (3-0 Silk)	2nd maxillary molar (360°)	14
Terrizzi et al. (2021)	Argentina	Buenos Aires; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	250 / NR	Ligature (NR Cotton)	1st mandibular molar	14
Catunda et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 10-12	Oral gavage	NA	14
Banyatworakul et al. (2021)	Thailand	Thailand; Muridae; Rattus; <i>Rattus norvegicus</i>	6 (3/3); 6 (3/3)	Male	NR / NR	Ligature (5-0 Silk)	1st mandibular molar bilateral	1; 7
Tian et al. (2021)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5); 10 (5/5)	Male	18-20 / 6	Ligature (5-0 Silk); Ligature (5-0 Silk) & Bacteria inoculation	2nd maxillary molar (360°) bilateral	14; 28; 56

Hu et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4); 10 (5/5)	Male	NR / 11	Ligature (0.25mm diameter Wire)	1st maxillary molar	72
Ren et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	220-250 / 6	Ligature (0.2mm diameter Wire)	2nd maxillary molar (360°) bilateral	14
Gu et al. (2021)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-220 / 11-13	Ligature (3-0 Silk)	1st mandibular molar bilateral	7
Zeng et al. (2021)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	NR / 6-10	Ligature (5-0 Silk)	2nd maxillary molar (360°)	28
Fernandes et al. (2021)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	140-160 / 6	Ligature	1st mandibular molar bilateral	15
Yang et al. (2021)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 8	Bacteria inoculation	NA	56
Jiang et al. (2021)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (8/4)	NR	NR / NR	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	8
Camargo et al. (2021)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	200-250 / 12	Ligature (4-0 Silk)	1st mandibular molar bilateral	30
Akiyama et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Female	NR / 8	Ligature (5-0 Silk)	1st mandibular molar	10
Zhou et al. (2022A)	China	China; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	289 / 36	Bacteria inoculation	2nd maxillary molar (360°) bilateral	10
Yang et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	254 / 8	Ligature (5-0 Silk)	2nd maxillary molar (360°)	14
Sun et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	180-220 / 6	LPS	NA	21
Lin et al. (2022A)	Taiwan	Taiwan; Muridae; Rattus; <i>Rattus norvegicus</i>	15 (10/5); 15 (10/5)	NR	NR / 8	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	7; 14
Zhang et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 10	Ligature (3-0 Silk)	1st maxillary molar (360°) Right	14
Li et al. (2022B)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Female	200-250 / NR	Ligature (0.2mm diameter Wire)	2nd maxillary molar (360°) bilateral	60
Suh et al. (2022)	USA	California; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	19 / 8	Ligature (6-0 Silk)	2nd maxillary molar (360°) bilateral	98
Gong et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Both	NR / 10	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	14
Zhou et al. (2022B)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 10	Ligature & LPS (NR Steel wire and Silk)	Interproximal between 1st and 2nd maxillary molar & Interproximal between 2nd and 3rd maxillary molar	30
Wu et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-220 / 7-9	Ligature & LPS (NR Silk)	2nd maxillary molar (360°)	56

Spuldaro et al. (2022)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	350 / 10	Ligature (4-0 Silk)	2nd maxillary molar (360°)	154
Zhou et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	NR / 9	Ligature (NR Silk)	2nd maxillary molar (360°)	8
Lari et al. (2022)	USA	Los Angeles; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 7	LPS	NA	42
Yang et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	254 / 8	Ligature (5-0 Silk)	2nd maxillary molar (360°)	14
Alpan & Çalışır (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	350-400 / NR	Ligature (4-0 Silk)	1st mandibular molar left	10
Chen et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (9/3)	Male	20 / 6	Ligature (5-0 Silk)	2nd maxillary molar (360°) left	3
Jeon et al. (2022)	Korea	Korea; Muridae; Rattus; <i>Mus musculus</i>	15 (8/7)	Male	NR / 7-9	Bacteria inoculation	NA	21
Li et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	20-22 / 11-12	Ligature (NR Silk)	Interproximal between 1st and 2nd maxillary molar & Interproximal between 2nd and 3rd maxillary molar	10
Silveira et al. (2022)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	180 (150/30)	Male	200-250 / 8-12	Ligature (n.10 Cotton)	1st mandibular molar left	7
Hao et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	18 (9/9)	Both	NR / 7	Oral gavage	NA	56
Peng et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4)	Male	200–230 / NR	LPS	NA	2
Lektemur et al. (2022)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	NR	365–420 / 14	Ligature (4-0 Silk)	1st mandibular molar right	NR
França et al. (2022)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Female	180–240 / NR	Ligature (NR Nylon)	2nd maxillary molar (360°) left	11
Liu et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	60 (30/30)	Male	180–220 / 7	Ligature (3-0 Silk)	1st mandibular molars and bilateral maxillary 2nd molars	3
Doğan et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	246,56 / 11-13	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	14
Caetano et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Female	204 / 16	Ligature (3-0 Nylon)	1st mandibular molar bilateral	20
Bi et al. (2022)	China	Shanghai; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 7-8	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	20
Kang et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	20 / 6	Oral gavage	NA	154
Bertolini et al. (2022)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	17 / 10	Ligature (6-0 Nylon)	2nd maxillary molar (360°) bilateral	15

Silva et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	38 (19/19)	Male	180-220 / 10	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	14
Tang et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Female	NR / 8	Ligature	2nd maxillary molar (360°)	14
dos Santos et al. (2022)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	150-200 / 8	Ligature (NR Cotton)	1st mandibular molar	14
Li et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	20-22 / 11-12	Ligature (NR Silk)	Interproximal between 1st and 2nd maxillary molar & Interproximal between 2nd and 3rd maxillary molar	10
Miyashita et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6); 12 (6/6); 12 (6/6); 12 (6/6)	Male	NR / 11	Ligature (6-0 Silk)	2nd maxillary molar (360°) bilateral	1; 3; 5; 7
Yigit et al. (2022)	Turkey	Turkey; Muridae; Rattus; <i>Rattus norvegicus</i>	18 (10/8)	Male	200 / NR	Ligature (3-0 Silk)	2nd maxillary molar (360°)	14
Hao et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	18 (9/9)	Both	NR / 7	Oral gavage	NA	56
Hiura et al. (2022)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	19 (9/10)	Male	NR / 8	Ligature (6-0 Silk)	2nd maxillary molar (360°) left	7
Wu et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	200-220 / 7-9	Ligature & LPS (NR Silk)	2nd maxillary molar (360°)	56
Nagao et al. (2022)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 7-9	Bacteria inoculation	NA	28
Qin & Min (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	NR / 6-7	Bacteria inoculation	NA	56
Novaes et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8); 16 (8/8); 16 (8/8)	Male	250-300 / NR	Ligature (n.24 Cotton)	1st mandibular molar bilateral	7; 15; 30
Da Ponte Leguizamón et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8)	Male	25 / 8	Ligature (5-0 Silk)	1st maxillary molar bilateral	15
Zhang et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	10 (5/5)	Male	NR / 10	Ligature (3-0 Silk)	1st maxillary molar (360°) Right	14
Li et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	20-22 / 11-12	Ligature (NR Silk)	Interproximal between 1st and 2nd maxillary molar & Interproximal between 2nd and 3rd maxillary molar	10
Yuan et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	32 (16/16)	Male	180-210 / 6	Ligature (3-0 Silk)	1st mandibular molar bilateral	14
Abdalla et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 8	Ligature (6-0 Silk)	2nd maxillary molar (360°)	14
Kondo et al. (2022)	Japan	Japan; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 6	Ligature (3-0 Nylon)	2nd maxillary molar (360°)	14

de Mello-Neto et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	13 (7/6)	Male	386-485 / 14	Ligature (5-0 Silk)	1st mandibular molar right	15
Zhu et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10)	Male	NR / 8	Ligature (5-0 Silk)	1st mandibular molar left	14
Satomi et al. (2022)	Japan	Japan; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8); 8 (4/4)	Male	NR / 7-8	Ligature (5-0 Silk)	2nd maxillary molar (360°) right	14
Jiang et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4)	Male	250 / 7	Ligature & LPS (NR Wire)	1st maxillary molar (360°) Right	28
Li et al. (2022)	China	China; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Female	20-22 / 11-12	Ligature (NR Silk)	Interproximal between 1st and 2nd maxillary molar & Interproximal between 2nd and 3rd maxillary molar	10
Guan et al. (2022)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	180-220 / 6-8	Ligature (4-0 Nylon)	1st maxillary molar bilateral	112
Maia et al. (2022)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	170-250 / NR	Ligature (NR Cotton)	1st mandibular molar	44
Silva et al. (2022)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	38 (19/19)	Male	180-220 / 10	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	14
Ohsugi et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	9 (5/4)	Male	NR / 9	Ligature (6-0 Silk)	2nd maxillary molar (360°) right	8
Alccayhuaman et al. (2022)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	28 (16/12)	Both	NR / 10-12	Ligature (4-0 Silk)	Interproximal between 1st and 2nd maxillary molars bilateral	12
Lee et al. (2022)	Korea	South Korea; Muridae; Rattus; <i>Mus musculus</i>	20 (10/10); 20 (10/10)	Both	NR / 12	Ligature (6-0 Silk and 4-0 Silk)	1st mandibular molar bilateral	7; 28
Zhao et al. (2023)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	100 / 6	Ligature (3-0 Silk)	2nd maxillary molar (360°) bilateral	28
Kaminska et al. (2023)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	14 (7/7)	Female	NR / 12	Bacteria inoculation	NA	21
Shi et al. (2023)	China	China; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	Male	NR / 4-5	Ligature (5-0 Silk)	2nd maxillary molar (360°) bilateral	84
Wang et al. (2023)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	32 (16/16)	Female	NR / 24	Bacteria inoculation	NA	42
Gao et al. (2023)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	12 (6/6)	Male	25-30 / 8	Ligature & Bacteria inoculation (5-0 Silk)	2nd maxillary molar (360°) bilateral	14
Zhang et al. (2023)	China	China; Muridae; Rattus; <i>Rattus norvegicus</i>	8 (4/4); 8 (4/4)	Male	350-400 / 10-12	Ligature (3-0 Silk and 0.2mm diameter Wire)	between 1st and 2nd maxillary	21
Oliveira et al. (2023)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	150 / 14	Ligature (4-0 Silk)	1st mandibular molar right	39
Song et al. (2023)	China	China; Muridae; Rattus; <i>Mus musculus</i>	16 (10/6)	NR	NR / 8	LPS	NA	28

Franchin et al. (2023)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	8 (4/4); 8 (4/4); 8 (4/4)	Male	20-22 / NR	Ligature (n.4 Cotton)	1st mandibular molar left	3; 5; 9
de Oliveira et al. (2023)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	230-250 / 8	Ligature (NR Cotton)	1st mandibular molar bilateral	14
Oliveira et al. (2023)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	150 / 14	Ligature (4-0 Silk)	1st mandibular molar right	39
Lee et al. (2023)	Korea	South Korea; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	NR / 16	Ligature (NR Floss)	Interproximal between 1st and 2nd mandibular molars	28
Joseph et al. (2023)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	24 (12/12)	Both	NR / 9-13	Oral gavage	NA	56
Ma et al. (2023)	China	China; Muridae; Rattus; <i>Mus musculus</i>	16 (8/8); 16 (8/8)	Male	18-20 / NR	Bacteria inoculation; Ligature (6-0 Silk) & Bacteria inoculation	NA	14
Li et al. (2023)	China	China; Muridae; Rattus; <i>Mus musculus</i>	30 (15/15)	Male	NR / 8	Bacteria inoculation	NA	21
Melo et al. (2023)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	12 (6/6)	Male	180-220 / 6	Ligature (3-0 Nylon)	2nd maxillary molar (360°) left	11
Tamura et al. (2023)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5)	NR	NR / 10	Bacteria inoculation	NA	14
Pelegrin et al. (2023)	Brazil	Brazil; Muridae; Rattus; <i>Rattus norvegicus</i>	20 (10/10)	Male	NR / 14-18	Ligature (n.3 Cotton)	1st mandibular molar bilateral	15
Franchin et al. (2023)	Brazil	Brazil; Muridae; Rattus; <i>Mus musculus</i>	8 (4/4); 8 (4/4); 8 (4/4)	Male	20-22 / NR	Ligature (n.4 Cotton)	1st mandibular molar left	3; 5; 9
Liu et al. (2023)	NR	NR; Muridae; Rattus; <i>Rattus norvegicus</i>	16 (8/8)	Male	NR / 6	Ligature (NR Wire)	1st maxillary molar	NR
Santos et al. (2023)	NR	NR; Muridae; Rattus; <i>Mus musculus</i>	10 (5/5); 10 (5/5)	Male	NR / 6-8	Oral gavage	NA	30; 60

N – Number of Animals; NR – Not Reported; NA– Not Applicable.

3. QUALITY ASSESSMENT

Two hundred and fifty-four (56.2%; n= 253) were considered to have a moderate ROB, while one hundred and ninety-seven (43.7%; n=197) had a high ROB.

More specifically, more than 75% of the included articles showed a low ROB in four items: baseline characteristics (97.8%; n=438), random allocation (77.5%; n=349), incomplete outcome reporting (84.7%; n=379), and other bias (100%; n=450). On the other hand, more than 60% of the included articles showed a high ROB in two items: blind outcome assessors (62%; n=278) and selective outcome reporting (100%; n=450). In terms of allocation concealment, blinding and random outcome assessment, 100% of the articles are not clear.

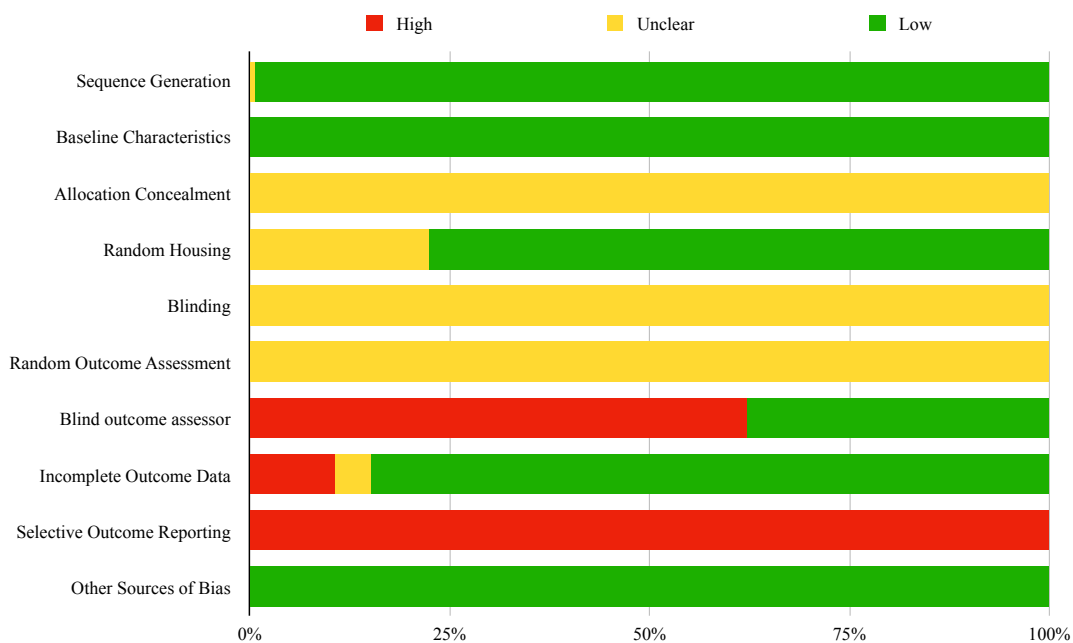


Figure 4 | *Summary Plot of the Methodological quality of included studies*

4. SYNTHESIS OF RESULTS

4.1. Local repercussions

We first analysed the different types of induced periodontitis models to determine where the greatest ABL was achieved (Table 2). Overall, the results confirmed a statistically significant difference (n=459; p<0.0001) with high heterogeneity ($I^2 = 99.5\%$). Overall, the model of periodontitis induced by a combination of ligature and periodontal inoculation with bacterial LPS was the method with the highest ABL (n=10; effect=2.46; 95% CI: 2.11-2.86; $I^2=99.5\%$), followed by induction by ligature (n=290; effect=2.39; 95% CI: 2.21-2.58; $I^2=99.4\%$) and induction by ligature with oral inoculation of bacteria associated with periodontitis (n=34; effect=2.11; 95% CI: 1.79-2.49; $I^2=99.5\%$). This was followed by LPS induction (n=38; effect=2.01; 95% CI: 1.67-2.42; $I^2=99.6\%$), bacterial inoculation (n=57; effect=1.60; 95% CI: 1.36-1.89; $I^2=99.0\%$) and finally oral gavage (n=30; effect=1.28; 95% CI: 1.20-1.37; $I^2=97.5\%$). The heterogeneity was considered substantial.

In addition, we examined the effect of animal species on ABL in each of the periodontitis induction methods (Table 2). We found that in the model of periodontitis induced by a combination of ligature and inoculation with LPS, *Mus musculus* had a higher ABL (n=3; effect=2.98; p<0.0001) compared to *Rattus norvegicus* (n=7; effect=2.24; p<0.0001), whereas in the induction method by ligature, *Rattus norvegicus* obtained a higher ABL (n=220; effect=2.54; p<0.0001) compared to *Mus musculus* (n=69; effect=1.95; p<0.0001).

Table 2 | *ABL according to animal species*

Induction method (circumferential ABL)	n	Effect	95% CI	p-value	I ²
Overall	459	2.14	2.02; 2.27	<0.0001	99.5
Bacteria inoculation	57	1.60	1.36; 1.89	<0.0001	99.0
<i>Mus musculus</i>	44	1.61	1.30; 1.99	<0.0001	99.2
<i>Rattus norvegicus</i>	13	1.62	1.29; 2.03	<0.0001	98.2
Ligature	290	2.39	2.21; 2.58	<0.0001	99.4
<i>Canis Lupus</i>	1	-	-	-	-
<i>Mus musculus</i>	69	1.95	1.80; 2.24	<0.0001	99.2
<i>Rattus norvegicus</i>	220	2.54	2.32; 2.78	<0.0001	99.4
Ligature + Bacteria inoculation	34	2.11	1.79; 2.49	<0.0001	99.5
<i>Mus musculus</i>	17	2.23	1.70; 2.91	<0.0001	99.6
<i>Oryctolagus cuniculus</i>	1	-	-	-	-
<i>Rattus norvegicus</i>	16	2.03	1.63; 2.54	<0.0001	99.5
Ligature + LPS	10	2.46	2.11; 2.86	<0.0001	99.5
<i>Mus musculus</i>	3	2.98	2.56; 3.46	<0.0001	96.0
<i>Rattus norvegicus</i>	7	2.24	1.87; 2.68	<0.0001	93.4

LPS	38	2.01	1.67; 2.42	<0.0001	99.6
<i>Mus musculus</i>	7	3.48	1.87; 6.48	<0.0001	99.8
<i>Oryctolagus cuniculus</i>	1	-	-	-	-
<i>Rattus norvegicus</i>	30	1.78	1.54; 2.06	<0.0001	98.6
Oral gavage	30	1.28	1.20; 1.37	<0.0001	97.5
<i>Mus musculus</i>	24	1.29	1.19; 1.40	<0.0001	97.9
<i>Rattus norvegicus</i>	6	1.26	1.12; 1.41	<0.0001	92.4

n – Number of studies; CI – Confidence Interval; I²– I squared.

We compared the method of induction of periodontitis based on the sex of the animal species (Table 3). There were statistically significant differences between the sexes for the bacterial inoculation method (p=0.0367), the combination of ligature with bacterial inoculation (p<0.0001) and oral gavage (p<0.0001). On the other hand, in the ligature method, the differences were not statistically significant (p=0.9658). Finally, we were unable to make comparisons for the methods combining ligature with periodontal inoculation with LPS and isolated LPS.

When testing for publication bias, funnel asymmetry did not impact the estimates made (Table 3).

Table 3 | *ABL according to the sex of the animal*

Induction method (circumferential ABL)	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Overall						
Bacteria inoculation						
Female and Male	1	-	-	-	-	-

Female	17	1.71	1.45; 2.01	0.0367	98.6	
Male	29	1.57	1.39; 1.78	0.0367	99.1	5.67; 3.02; 0.0719
NR	10	1.19	0.98; 1.44	0.0367	97.6	
Ligature						
Female and Male	3	2.07	1.07; 4.03	0.9658	97.9	-
Female	39	2.36	2.01; 2.76	0.9658	99.3	5.55; 3.09; 0.0803
Male	233	2.38	2.23; 2.55	0.9658	99.4	2.22; 1.49; 0.1408
NR	15	2.28	1.74; 2.97	0.9658	99.0	-
Ligature + Bacteria inoculation						
Female	3	1.71	1.21; 2.40	<0.0001	99.0	
Male	30	2.10	1.74; 2.52	<0.0001	99.5	
NR	1	-	-	<0.0001	-	-
Ligature + LPS						
Female	1	-	-	-	-	-
Male	9	2.66	2.57; 2.75	0.2852	99.6	
LPS						
Female	1	-	-	-	-	-
Male	36	1.99	1.55; 2.54	0.2544	99.6	
NR	1	-	-	-	-	-
Oral gavage						
Female and Male	2	-	-	-	-	-

Female	13	1.19	1.09; 1.29	<0.0001	97.6	
Male	14	1.37	1.26; 1.48	<0.0001	93.9	
NR	1	-	-	-	-	-

n – Number of studies; CI – Confidence Interval; I² – I squared; SE - Standard Error.

We analysed, using a sub-group meta-analysis, whether the type of ligature used influenced the ABL results (Table 4). Cotton n.3 ligature achieved the highest ABL (n=18; effect=3.36; 95% CI: 2.53-4.47; p<0.0001) with high heterogeneity (I²=99.4%), followed by nylon n.3 (n=26; effect=3.28; 95% CI: 2.67-4.03; p<0.0001) and silk 3-0 (n=44; effect=2.54; 95% CI: 2.11-3.05; p<0.0001).

When considering the type of ligature, there were significant differences between the different types reported (p<0.0001) (Table 4).

Although there is considerable heterogeneity, the estimates for cotton ligatures were relatively stable and unaffected by ligature thickness (n=65; estimate=-0.02; SE=0.02; p-value>0.05), induction time (n=65; estimate=0.01; SE=0.01; p-value>0.05) and number of teeth (n=65; estimate=0.08; SE=0.12; p-value>0.05), as confirmed by meta-regression (Table 3). Similarly, for nylon type ligatures, ligature thickness (n=41; estimate=-0.31; SE=0.06; p-value=0.001), induction time (n=41; estimate=-0.01; SE=0.00; p-value=0.001) and number of teeth (n=41; estimate=-0.66; SE=0.14; p-value=0.001) significantly influenced the results. For silk ligatures, ligature thickness (n=143; estimate=-0.11; SE=0.05; p-value=0.01) influenced the outcome, but the other variables were relatively stable. Finally, for wire ligatures, thickness influenced the outcome (n=16; estimate=12.84; SE=6.45; p-value=0.01), while the remaining variables were relatively stable.

When testing for publication bias, asymmetry was found in studies using the silk bandage induction method (n=143; bias=5.09; SE=1.82; p=0.0034) (Table 4). No publication bias was found for the remaining estimates.

Table 4 | *ABL according to the type of ligature*

Induction method (circumferential ABL)	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Ligature type						
Cotton	65	2.35	2.03; 2.73	<0.0001	99.2	3.94; 2.28; 0.0884
n.3	18	3.36	2.53; 4.47	<0.0001	99.4	1.10; 4.94; 0.8261
n.4	14	1.53	1.27; 1.86	<0.0001	96.9	-0.63; 3.19; 0.8459
n.5	2	-	-	<0.0001	-	-
n.10	1	-	-	-	-	-
n.24	3	1.64	1.42; 1.88	<0.0001	82.1	-
NR	27	2.35	1.86; 2.99	<0.0001	99.1	-
Meta-regression per ligature thickness: Estimate=-0.02; SE=0.02; p-value >0.05						
Meta-regression per time of periodontitis induction: Estimate=0.01; SE=0.01; p-value >0.05						
Meta-regression per number of teeth: Estimate=0.08; SE=0.12; p-value >0.05						
Elastic	3	1.82	1.50; 2.23	<0.0001	79.3	-
Floss	2	-	-	-	-	-
Nylon	41	3.25	2.49; 4.23	<0.0001	99.4	0.75; 3.05; 0.7676
2.0mm	2	-	-	-	-	-
3-0	26	3.28	2.67; 4.03	<0.0001	99.5	0.84; 4.96; 0.8669
4-0	1	-	-	-	-	-
5-0	3	1.89	1.48; 2.40	<0.0001	90.6	-
6-0	4	1.71	1.37; 2.14	<0.0001	97.6	-

NR	5	3.07	2.44; 3.86	<0.0001	92.0	-
Meta-regression per ligature thickness: Estimate=-0.31; SE=0.06; p-value= 0.001						
Meta-regression per time of periodontitis induction: Estimate=-0.01; SE=0.00; p-value= 0.001						
Meta-regression per number of teeth: Estimate=-0.66; SE=0.14; p-value= 0.001						
Silk	143	2.20	1.98; 2.45	<0.0001	99.4	5.09; 1.82; 0.0034
2-0	1	-	-	-	-	-
3-0	44	2.54	2.11; 3.05	<0.0001	99.2	10.16; 2.67; 0.0005
4-0	33	2.41	1.91; 3.03	<0.0001	99.7	6.25; 5.58; 0.2630
5-0	31	2.48	2.05; 3.01	<0.0001	98.8	0.33; 2.62; 0.9001
6-0	15	1.25	0.95; 1.64	<0.0001	98.0	1.76; 2.90; 0.5540
7-0	2	-	-	-	-	-
NR	17	1.72	1.25; 2.39	<0.0001	98.9	-
Meta-regression per ligature thickness: Estimate=-0.11; SE=0.05; p-value= 0.01						
Meta-regression per time of periodontitis induction: Estimate=-0.00; SE=0.00; p-value >0.05						
Meta-regression per number of teeth: Estimate=-0.14; SE=0.08; p-value >0.05						
Wire	16	2.14	1.61; 2.86	<0.0001	99.5	-2.06; 6.26; 0.7473
0.2	13	1.89	1.58; 2.27	<0.0001	96.3	-
0.25	1	-	-	-	-	-
NR	2	-	-	-	-	-
Meta-regression per ligature thickness: Estimate=12.84; SE=6.45; p-value= 0.01						
Meta-regression per time of periodontitis induction: Estimate=0.00; SE=0.01; p-value >0.05						

Meta-regression per number of teeth: Estimate=0.01; SE=0.24; p-value >0.05
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n – Number of studies; CI – Confidence Interval; I² – I squared; SE - Standard Error.

4.2. Systemic repercussions

4.2.1. Cytokines

We also examined the effect of periodontitis on systemic levels of interleukin-1 beta (IL-1 β) (Table 5). Sensitivity analyses showed that studies including four teeth had higher levels of IL-1 β (n=3; effect=6.03; 95% CI: 2.01-18.13; p=0.0066; I²=99.0%), whereas studies including only one tooth had lower levels (n=32; effect=3.73; 95% CI: 2.62-5.33; p=0.0066; I²=99.7%). When evaluating the meta-regressions, we can see that the number of teeth involved is influenced by the induction time (n=99; estimate=-0.01; SE=0.01; p-value < 0.0001).

We found that in the ligature-induced periodontitis model, *Rattus norvegicus* obtained higher levels of IL-1 β (n=62; effect=3.27; p=0.2443) than *Mus musculus* (n=8; effect=2.22; p=0.2443). Similarly, in the induction method combining ligature with bacterial inoculation, *Rattus norvegicus* obtained higher levels of IL-1 β (n=7; effect=4.45; p=0.5844) compared to *Mus musculus* (n=4; effect=3.59; p=0.5844).

If we compare the induction method by ligature with the combination of ligature and bacterial inoculation, we find that the same species has higher levels of IL-1 β , namely *Rattus norvegicus*.

Overall, we were able to confirm that the method that induces higher levels of IL-1 β is oral gavage (n=5; effect=5.27; 95% CI: 2.22-12.50; p<0.0001; I²=98.5%).

Table 5 | *IL-1 β according to animal species*

IL-1 β	n	Effect	95% CI	p-value	I ²
Overall	99	3.15	2.55; 3.89	<0.0001	99.8
Number of involved teeth					

1	32	3.73	2.62; 5.33	0.0066	99.7
2	46	2.12	2.12; 3.42	0.0066	99.8
4	3	6.03	2.01; 18.13	0.0066	99.0
6	1	-	-	-	-
NR	2	-	-	-	-
NA	15	3.02	2.05; 4.45	0.0066	99.2
Meta-regression per time of periodontitis induction: Estimate=-0.01; SE=0.01; p-value <0.0001					
Bacteria inoculation	4	1.73	1.10; 2.73	<0.0001	99.3
<i>Mus musculus</i>	2	-	-	-	-
<i>Rattus norvegicus</i>	2	-	-	-	-
Ligature	71	3.12	2.40; 4.07	<0.0001	99.9
<i>Minipig</i>	1	-	-	-	-
<i>Mus musculus</i>	8	2.22	1.47; 3.33	0.2443	99.3
<i>Rattus norvegicus</i>	62	3.27	2.43; 4.39	0.2443	99.9
Ligature + Bacteria inoculation	11	4.14	2.84; 6.03	<0.0001	99.1
<i>Mus musculus</i>	4	3.59	1.71; 7.55	0.5844	98.2
<i>Rattus norvegicus</i>	7	4.45	3.58; 5.56	0.5844	94.9
Ligature + LPS	1	-	-	-	-
LPS	7	2.26	1.62; 3.15	<0.0001	98.0
<i>Mus musculus</i>	1	-	-	-	-
<i>Rattus norvegicus</i>	6	1.61	1.36; 1.91		
Oral gavage	5	5.27	2.22; 12.50	<0.0001	98.5
<i>Mus musculus</i>	5	5.27	2.22; 12.50	<0.0001	98.5

n – Number of studies; CI – Confidence Interval; I² – I squared.

When considering the type of ligature, cotton ligature was the one that yielded the highest levels of IL-1 β (n=13; effect=6.64; 95% CI: 2.77-15.96, p=0.0197), with high heterogeneity ($I^2 = 99.6\%$), more than twice that of other ligature types (Table 6).

Despite the high heterogeneity, the estimates for cotton ligature were relatively stable and unaffected by induction time (n=13; estimate=0.00; SE=0.04; p-value>0.05) and tooth number (n=13; estimate=0.80; SE=0.44; p-value=0.1), which was confirmed by meta-regression. Similarly, for nylon ligatures, the number of teeth significantly influenced the final analyses (n=13; estimate=0.98; SE=0.14; p-value <0.0001), while the induction time was relatively stable. Finally, for silk ligatures, induction time (n=36; estimate=-0.02; SE=0.01; p-value>0.05) and number of teeth (n=36; estimate=-0.14; SE=0.13; p-value>0.05) did not significantly influence the results.

Table 6 | *IL-1 β according to the type of ligature*

IL-1 β	n	Effect	95% CI	p-value	I^2
Ligature type					
Cotton	13	6.64	2.77; 15.96	0.0197	99.6
n.3	5	7.36	1.68; 32.29	0.0002	99.8
n.4	1	-	-	-	-
NR	7	5.02	2.72; 9.26	0.0002	97.7
Meta-regression per time of periodontitis induction: Estimate=0.00; SE=0.04; p-value >0.05					
Meta-regression per number of teeth: Estimate=0.80; SE=0.44; p-value=0.1					
Nylon	13	2.66	2.03; 3.48	0.0197	96.5
2.0mm	2	-	-	-	-
3-0	26	3.28	2.67; 4.03	<0.0001	99.5
4-0	1	-	-	-	-
5-0	3	1.89	1.48; 2.40	<0.0001	90.6
6-0	4	1.71	1.37; 2.14	<0.0001	97.6
NR	5	3.07	2.44; 3.86	<0.0001	92.0
Meta-regression per time of periodontitis induction: Estimate=-0.13; SE=0.14; p-value >0.05					
Meta-regression per number of teeth: Estimate=0.98; SE=0.14; p-value <0.0001					

Silk	36	2.09	1.67; 2.62	0.0197	99.7
3-0	15	2.22	1.47; 3.36	<0.0001	99.7
4-0	9	2.12	1.37; 3.30	<0.0001	98.6
5-0	5	2.12	1.53; 2.95	<0.0001	95.0
6-0	1	-	-	-	-
7-0	1	-	-	-	-
NR	5	1.43	1.21; 1.70	<0.0001	98.1
Meta-regression per time of periodontitis induction: Estimate=-0.02; SE=0.01; p-value >0.05					
Meta-regression per number of teeth: Estimate=-0.14; SE=0.13; p-value >0.05					
Wire	2	-	-	-	-

n – Number of studies; CI – Confidence Interval; I² – I squared.

Looking at IL-6 levels, we found that the LPS induction model produced the highest systemic levels (n=5; effect=4.78; 95% CI: 1.71-12.22; I²= 98.7%), followed by bacterial inoculation (n=4; effect=4.59; 95% CI: 0.82-25.56; I²= 99.8%) and ligature induction (n=35; effect=3.60; 95% CI: 2.25-5.76; I²= 99.9%) (Table 7). We also have induction by ligature with oral inoculation of bacteria associated with periodontitis (n=5; effect=2.25; 95% CI: 1.86-2.72; I²=86.6%) and finally by oral gavage (n=4; effect=1.67; 95% CI: 1.07-2.62; I²=97.1%). Heterogeneity was considered to be substantial.

When evaluating the meta-regressions, we can see that the number of teeth involved is influenced by the induction time (n=55; estimate=-0.44; SE=0.27; p-value <0.0001) (Table 7).

In addition, we observed that in the ligature induction method, *Rattus norvegicus* obtained higher IL-6 levels (n=27; effect=4.33; p=0.0297), more than twice as much as *Mus musculus* (n=8; effect=1.92; p=0.0297), although the differences are statistically significant.

Table 7 | *IL-6 according to animal species*

IL-6	n	Effect	95% CI	p-value	I ²
Overall	55	3.27	2.32; 4.61	<0.0001	99.9
Meta-regression per time of periodontitis induction: Estimate=-0.44; SE=0.27; p-value <0.0001					
Bacteria inoculation	4	4.59	0.82; 25.56	<0.0001	99.8
<i>Mus musculus</i>	3	6.40	0.30; 135.20	0.3980	99.4
<i>Rattus norvegicus</i>	1	-	-	-	-
Ligature	35	3.60	2.25; 5.76	<0.0001	99.9
<i>Mus musculus</i>	8	1.92	1.21; 3.06	0.0297	99.2
<i>Rattus norvegicus</i>	27	4.33	2.46; 7.62	0.0297	99.9
Ligature + Bacteria inoculation	5	2.25	1.86; 2.72	<0.0001	86.6
<i>Mus musculus</i>	2	-	-	-	-
<i>Rattus norvegicus</i>	3	2.33	2.02; 2.70	0.7884	89.2
Ligature + LPS	2	-	-	-	-
LPS	5	4.78	1.71; 12.22	<0.0001	98.7
<i>Mus musculus</i>	1	-	-	-	-
<i>Rattus norvegicus</i>	4	3.33	1.15; 9.69	0.0049	98.7
Oral gavage	4	1.67	1.07; 2.62	<0.0001	97.1
<i>Mus musculus</i>	4	1.67	1.07; 2.62	-	97.1

n – Number of studies; CI – Confidence Interval; I²– I squared.

When considering the type of ligature, cotton ligature yielded the highest levels of IL-6 (n=11; effect=5.90; 95% CI: 2.40-14.51, p=0.0001) with high heterogeneity (I² = 99.9%), being reported more than twice as high as the other types (Table 8).

Despite the high heterogeneity, the estimates for cotton ligature were relatively stable and unaffected by induction time (n=11; estimate=0.00; SE=0.04; p-value>0.05) or number of teeth (n=11; estimate=-0.72; SE=0.52; p-value>0.05), which was confirmed by meta-regression. Similarly, the variables were stable in the silk type ligatures.

Table 8 | *IL-6 according to the type of ligature*

IL-6	n	Effect	95% CI	p-value	I ²
Ligature type					
Cotton	11	5.90	2.40; 14.51	0.0001	99.9
n.3	7	15.24	2.82; 82.23	0.0048	99.9
n.4	3	1.43	1.32; 1.54	0.0048	68.3
NR	1	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=0.00; SE=0.04; p-value >0.05					
Meta-regression per number of teeth: Estimate=-0.72; SE=0.52; p-value >0.05					
Nylon	1	-	-	-	-
Silk	14	1.72	1.35; 2.21	<0.0001	98.9
3-0	5	1.69	1.05; 2.73	<0.0001	99.3
5-0	4	2.87	1.05; 2.73	<0.0001	99.3
6-0	2	-	-	-	-
7-0	1	-	-	-	-
NR	2	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=-0.00; SE=0.01; p-value >0.05					
Meta-regression per number of teeth: Estimate=0.17; SE=0.30; p-value >0.05					
Wire	2	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared.

When investigating the effect of periodontitis on the systemic level of IL-10, we found that in the ligature-induced periodontitis model, *Mus musculus* obtained higher levels of IL-10 (n=4; effect=1.03; p=0.099) compared to *Rattus norvegicus* (n=23; effect=0.81; p=0.099) (Table 9).

When evaluating the meta-regressions, we can see that the number of teeth involved is influenced by the induction time (n=44; estimate=1.21; SE=0.18; p-value <0.0001).

Overall, we were able to confirm that the method that induces higher levels of IL-10 is induction by ligature with oral inoculation of bacteria associated with periodontitis (n=9; effect=2.19; 95% CI: 1.04-4.58; p=0.0013; I²= 98.4%).

Table 9 | *IL-10 according to animal species*

IL-10	n	Effect	95% CI	p-value	I ²
Overall	44	1.17	1.01; 1.36	0.0349	99.2
Meta-regression per time of periodontitis induction: Estimate=1.21; SE=0.18; p-value <0.0001					
Bacteria inoculation	2	-	-	-	-
Ligature	27	0.84	0.72; 0.97	0.0013	95.4
<i>Mus musculus</i>	4	1.03	0.82; 1.31	0.0990	98.4
<i>Rattus norvegicus</i>	23	0.81	0.67; 0.96	0.0990	98.8
Ligature + Bacteria inoculation	9	2.19	1.04; 4.58	0.0013	98.4
<i>Mus musculus</i>	2	-	-	-	-
<i>Rattus norvegicus</i>	7	1.77	0.74; 4.26	0.0344	98.7
Ligature + LPS	1	-	-	-	-
LPS	3	0.95	0.64; 1.42	0.0013	95.4
<i>Rattus norvegicus</i>	3	0.95	0.64; 1.42	0.0013	95.4
Oral gavage	2	-	-	-	-

n – Number of studies; CI – Confidence Interval; I² – I squared.

When considering the type of ligature, the 3-0 silk ligature yielded the highest levels of IL-10 (n=4; effect=2.27; 95% CI: 0.86-6.00; p<0.0001) with high heterogeneity (I²=99.5%), followed by cotton (n=6; effect=1.33; 95% CI: 0.94-1.90; p=0.1111) with high heterogeneity (I²=98.5%) (Table 10).

Although there is considerable heterogeneity, the estimates for cotton and silk ligatures were relatively stable and not influenced by induction time or number of teeth. On the contrary, for nylon ligature, induction time (n=6; estimate=-1.30; SE=0.19; p-value

<0.0001) and number of teeth (n=6; estimate=-1.30; SE=0.19; p-value<0.0001) influenced the results, which was confirmed by meta-regression.

Table 10 | *IL-10 according to the type of ligature*

IL-10	n	Effect	95% CI	p-value	I ²
Ligature type					
Cotton	6	1.33	0.94; 1.90	0.1111	98.5
n.3	1	-	-	-	-
NR	5	1.48	1.01; 2.17	0.0202	98.8
Meta-regression per time of periodontitis induction: Estimate=-0.01; SE=0.01 p-value >0.05					
Meta-regression per number of teeth: Estimate=0.29; SE=;0.18 p-value >0.05					
Nylon	6	0.27	0.19; 0.39	<0.0001	92.5
3-0	5	0.23	0.18; 0.31	<0.0001	75.0
NR	1	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=-1.30; SE=0.19; p-value <0.0001					
Meta-regression per number of teeth: Estimate=-1.30; SE=0.19; p-value <0.0001					
Silk	14	1.02	0.84; 1.24	0.8422	99.0
3-0	4	2.27	0.86; 6.00	<0.0001	99.5
4-0	1	-	-	-	-
5-0	4	1.11	0.82; 1.51	<0.0001	98.5
7-0	1	-	-	-	-
NR	4	0.66	0.50; 0.87	<0.0001	98.9
Meta-regression per time of periodontitis induction: Estimate=0.02; SE=0.02; p-value >0.05					
Meta-regression per number of teeth: Estimate=0.41; SE=0.24; p-value >0.05					
Wire	1	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared.

We verified that in the model of periodontitis induced by bacterial inoculation, *Mus musculus* (n=6; effect=1.75; p=0.1032) and *Rattus norvegicus* (n=3; effect=1.75; p=0.1032) obtained similar results in terms of tumor necrosis factor alpha (TNF- α) levels. On the other hand, in the ligature induction method, *Rattus norvegicus* obtained higher levels of TNF- α (n=56; effect=2.82; p<0.0001) compared to *Mus musculus* (n=5; effect=1.26; p<0.0001).

When evaluating the meta-regressions, we can verify that the induction time variable remains relatively stable and does not significantly influence the result (n=97; estimate=-0.21; SE=0.10; p-value=0.05) (Table 11).

Overall, we were able to verify that the method that induces higher levels of TNF- α is oral gavage (n=7; effect=3.11; 95% CI: 0.82-11.71; p=0.4468; I²= 99.9%), followed by induction by ligature with oral inoculation of bacteria associated with periodontitis (n=8; effect=2.76; 95% CI: 1.92-3.98; p=0.4468) and induction by ligature (n=62; effect=2.67; 95% CI: 2.07-3.43; p=0.4468) (Table 11). Then we have the induction by bacterial inoculation (n=9; effect=2.55; p=0.4468) and by LPS (n=8; effect=2.55; p=0.4468) with the same levels of TNF- α .

Table 11 | *TNF- α according to animal species*

TNF- α	n	Effect	95% CI	p-value	I ²
Overall	97	2.67	2.21; 3.20	<0.0001	99.9
Meta-regression per time of periodontitis induction: Estimate=-0.21; SE=0.10; p-value= 0.05					
Bacteria inoculation	9	2.55	1.73; 3.76	0.4468	97.8
<i>Mus musculus</i>	6	1.75	1.14; 2.68	0.1032	98.5
<i>Rattus norvegicus</i>	3	1.75	1.14; 2.68	0.1032	89.5
Ligature	62	2.67	2.07; 3.43	0.4468	99.9
<i>Canis Lupus</i>	1	-	-	-	-
<i>Mus musculus</i>	5	1.26	0.96; 1.65	<0.0001	95.0
<i>Rattus norvegicus</i>	56	2.82	2-12; 3.76	<0.0001	100

Ligature + Bacteria inoculation	8	2.76	1.92; 3.98	0.4468	99.6
<i>Mus musculus</i>	2	-	-	-	-
<i>Rattus norvegicus</i>	6	2.99	1.96; 4.57	0.4685	99.7
Ligature + LPS	3	1.87	1.42; 2.46	0.4468	80.2
LPS	8	2.55	1.46; 4.46	0.4468	98.9
<i>Rattus norvegicus</i>	8	2.55	1.46; 4.46	0.4468	98.9
Oral gavage	7	3.11	0.82; 11.71	0.4468	99.9
<i>Mus musculus</i>	7	3.11	0.82; 11.71	0.4468	99.9

n – Number of studies; CI – Confidence Interval; I² – I squared.

Considering the type of ligature, the 3-0 nylon ligature achieved the highest levels of TNF- α (n=11; effect=4.91; 95% CI: 3.18-7.67; p=0.1762; I²=98.3%), followed by cotton n.3 (n=8; effect=4.90; 95% CI: 2.00-11.99; p=0.0029; I²=99.9%) (Table 12). On the other hand, silk (n=25; effect=1.72; p<0.0001) and wire (n=2; effect=1.42; p=0.0745) ligatures had higher TNF- α levels (Table 12).

Although there is considerable heterogeneity, the estimates for nylon and silk ligatures were relatively stable and not influenced by induction time or number of teeth, which was confirmed by meta-regression. On the other hand, for cotton ligatures, the induction time significantly influenced the results (n=15; estimate=-0.05; SE=0.02; p-value=0.01), while the number of teeth was relatively stable.

Table 12 | *TNF- α according to the type of ligature*

TNF- α	n	Effect	95% CI	p-value	I ²
Ligature type					
Cotton	15	2.50	1.74; 3.59	<0.0001	99.8
n.3	8	4.90	2.00; 11.99	0.0029	99.9
n.4	2	-	-	0.0029	-
NR	5	1.17	1.04; 1.31	0.0029	84.1
Meta-regression per time of periodontitis induction: Estimate=-0.05; SE=0.02; p-value= 0.01					

Meta-regression per number of teeth: Estimate=-0.43; SE=0.32; p-value >0.05					
Nylon	13	4.37	3.23; 5.90	<0.0001	98.9
3-0	11	4.91	3.18; 7.67	0.1762	98.3
NR	2	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=-0.17; SE=0.16; p-value=0.1					
Meta-regression per number of teeth: Estimate=-0.12; SE=0.69; p-value >0.05					
Silk	25	1.72	1.46; 2.03	<0.0001	99.6
3-0	11	2.28	1.56; 3.32	<0.0001	99.7
4-0	5	1.12	0.76; 1.65	<0.0001	99.0
5-0	3	1.23	0.94; 1.61	<0.0001	96.1
6-0	1	-	-	-	-
7-0	1	-	-	-	-
NR	3	1.51	0.76; 3.00	<0.0001	98.7
Meta-regression per time of periodontitis induction: Estimate=0.01; SE=0.00; p-value= 0.05					
Meta-regression per number of teeth: Estimate=0.06; SE=0.06; p-value >0.05					
Wire	2	1.42	0.97; 2.10	0.0745	96.1
0.25	1	-	-	-	-
0.2	1	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared.

4.2.2. Cells

Looking at the levels of tartrate-resistant acid phosphatase positive cells (TRAP+), we found that the ligature induction model with oral inoculation of bacteria associated with periodontitis produced the highest systemic levels (n=4; effect=4.91; 95% CI: 2.92-8.27) with high heterogeneity (I²=95.2%), followed by ligature (n=22; effect=3.29; 95% CI: 2.66-4.07) with high heterogeneity (I²=94.9%) and LPS induction (n=3; effect=3.24; 95% CI: 2.81-3.72) with low heterogeneity (I²=14.2%) (Table 13). Induction by bacterial inoculation (n=5; effect=2.93; 95% CI: 2.14-4.02; I²=93.2%) was also observed.

When evaluating the meta-regressions, we can verify that the number of teeth involved is not influenced by the induction time (n=37; estimate=-0.13; SE=0.25; p-value>0.05).

Furthermore, we observed that in the ligature induction method, *Rattus norvegicus* obtained higher levels of TRAP+ (n=15; effect=3.73; p=0.1974) compared to *Mus musculus* (n=7; effect=2.75; p=0.1974). However, in the induction method combining ligature with bacterial inoculation, *Mus musculus* (n=3; effect=4.75; p=0.681) showed higher levels compared to the induction method by ligature.

Table 13 | TRAP+ according to animal species

TRAP +	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Overall	37	3.36	2.93; 3.86	<0.0001	93.8	2.44; 1.03; 0.0230
Meta-regression per time of periodontitis induction: Estimate=-0.13; SE=0.25; p-value >0.05						
Bacteria inoculation	5	2.93	2.14; 4.02	0.5487	93.2	-
<i>Mus musculus</i>	4	2.94	1.93; 4.48	0.8152	91.1	-
<i>Rattus norvegicus</i>	1	-	-	-	-	-
Ligature	22	3.29	2.66; 4.07	0.5487	94.9	1.83; 1.36; 01949
<i>Mus musculus</i>	7	2.75	2.08; 3.64	0.1974	96.7	-
<i>Rattus norvegicus</i>	15	3.73	2.58; 5.40	0.1974	92.2	
Ligature + Bacteria inoculation	4	4.91	2.92; 8.27	0.5487	95.2	-
<i>Mus musculus</i>	3	4.75	2.52; 8.96	0.6810	95.9	-

<i>Rattus norvegicus</i>	1	-	-	-	-	-
Ligature + LPS	2	-	-	-	-	-
LPS	3	3.24	2.81; 3.72	0.5487	14.2	-
<i>Mus musculus</i>	2	-	-	-	-	-
<i>Rattus norvegicus</i>	1	-	-	-	-	-
Oral gavage	1	-	-	-	-	-

n – Number of studies; CI – Confidence Interval; I² – I squared; SE – Standard Error.

Regarding the type of ligature, the nylon ligature showed the highest TRAP+ levels (n=3; effect=4.26; 95% CI: 1.33-13.65; p=0.0146; I²=62.6%), followed by the silk ligature (n=9; effect=3.49; 95% CI: 2.51-4.86; p<0.0001; I²=97.8%) and finally the cotton ligature (n=9; effect=2.86; 95% CI: 2.18-3.74; p<0.0001; I²=75.1%) (Table 14).

Meta-regression analyses confirmed that in nylon and silk ligatures, induction time and number of teeth involved are relatively stable variables that do not influence the results. On the other hand, in cotton ligatures, the number of teeth significantly influenced the results (n=9; estimate=1.04; SE=0.14; p-value<0.0001), while the induction time was relatively stable.

Table 14 | *TRAP+ according to the type of ligature*

TRAP +	n	Effect	95% CI	p-value	I ²
Ligature type					
Cotton	9	2.86	2.18; 3.74	<0.0001	75.1
n.3	4	2.82	1.82; 4.36	0.0547	69.8
n.24	3	4.07	2.99; 5.54	0.0547	0.0
NR	2	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=-0.02; SE=0.01; p-value >0.05					
Meta-regression per number of teeth: Estimate=1.04; SE=0.14; p-value <0.0001					
Nylon	3	4.26	1.33; 13.65	0.0146	62.6
3-0	1	-	-	-	-

5-0	1	-	-	-	-
NR	1	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=-0.32; SE=0.14; p-value= 0.05					
Meta-regression per number of teeth: Estimate=-1.51; SE=1.87; p-value >0.05					
Silk	9	3.49	2.51; 4.86	<0.0001	97.8
3-0	2	-	-	-	-
5-0	4	3.24	2.16; 4.87	<0.0001	89.7
6-0	1	-	-	-	-
7-0	2	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=-0.02; SE=0.02; p-value >0.05					
Meta-regression per number of teeth: Estimate=0.07; SE=0.44; p-value >0.05					
Wire	1	-	-	-	-

n – Number of studies; CI –Confidence Interval; I²– I squared.

Regarding osteoclast levels, we found that the ligature induction model with oral inoculation of bacteria associated with periodontitis produced the highest systemic levels (n=12; effect=9.76; 95% CI: 6.01-15.85; p<0.0001; I²=95.9%), followed by ligature (n=52; effect=5.48; 95% CI: 4.41-6.82; p<0.0001; I²=97.1%) and finally induction by bacterial inoculation (n=3; effect=4.42; 95% CI: 1.45-13.49; p<0.0001) with moderate heterogeneity (I²=51.3%) (Table 15).

We found that in the ligature-induced periodontitis model, *Mus musculus* obtained higher levels of osteoclasts (n=16; effect=5.65; p=0.9494) than *Rattus norvegicus* (n=36; effect=5.41; p=0.9494). Likewise, in the induction method combining ligature with bacterial inoculation, *Mus musculus* obtained higher levels of osteoclasts (n=8; effect=15.99; p=0.0002) compared to *Rattus norvegicus* (n=4; effect=3.88; p=0.0002). If we compare the induction method by ligature with the combination of ligature and bacterial inoculation, we find that the same species has higher osteoclast levels in *Mus musculus*.

When evaluating the meta-regressions, we can verify that the number of teeth involved is not influenced by the induction time (n=70; estimate=-0.35; SE=0.15; p-value =0.05).

When testing for publication bias, a funnel asymmetry was found both in studies using ligature induction (n=52; bias=4.77; SE=1.26; p=0.0004) and in studies using ligature induction combined with bacterial inoculation (n=12; bias=7.24; SE=2.67; p=0.0217) (Table 15).

Table 15 | *Osteoclast according to animal species*

Osteoclast	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Overall	70	5.85	4.88; 7.02	<0.0001	96.9	5.04; 1.03; <0.0001
Number of involved teeth						
Meta-regression per time of periodontitis induction: Estimate=-0.35; SE=0.15; p-value=0.05						
Bacteria inoculation	3	4.42	1.45; 13.49	<0.0001	51.3	-
<i>Mus musculus</i>	2	-	-	-	-	-
<i>Rattus norvegicus</i>	1	-	-	-	-	-
Ligature	52	5.48	4.41; 6.82	<0.0001	97.1	4.77; 1.26; 0.0004
<i>Mus musculus</i>	16	5.65	3.95; 8.09	0.9494	96.7	
<i>Rattus norvegicus</i>	36	5.41	4.11; 7.12	0.9494	97.2	
Ligature + Bacteria inoculation	12	9.76	6.01; 15.85	<0.0001	95.9	7.24; 2.67; 0.0217
<i>Mus musculus</i>	8	15.9 9	9.05; 28.27	0.0002	91.8	-
<i>Rattus norvegicus</i>	4	3.88	2.36; 6.38	0.0002	94.0	-
Ligature + LPS	1	-	-	-	-	-
LPS	2	-	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared; SE – Standard Error.

When considering the type of ligature, the 3-0 nylon ligature showed the highest level of osteoclasts (n=5; effect=8.54; 95% CI: 6.35-11.48; p<0.0001; I²=74.1%), followed by the 6-0 silk ligature (n=4; effect=5.79; 95% CI: 2.24-14.97; p=0.1549; I²=98.2%) (Table 16).

When examining for publication bias, a funnel asymmetry was found in studies using silk ligature induction (n=39; bias=4.87; SE=1.11; p<0.0001) (Table 16).

Table 16 | *Osteoclast according to the type of ligature*

Osteoclast	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Ligature type						
Cotton	1	-	-	-	-	-
Nylon	5	8.54	6.35; 11.48	<0.0001	74.1	-
3-0	5	8.54	6.35; 11.48	<0.0001	74.1	-
Silk	39	4.56	3.69; 5.63	<0.0001	94.5	4.87; 1.11; <0.0001
3-0	4	4.40	1.44; 13.43	0.1549	94.5	-
4-0	20	4.98	3.63; 6.84	0.1549	93.2	-
5-0	5	3.03	2.26; 4.07	0.1549	88.3	-
6-0	4	5.79	2.24; 14.97	0.1549	98.2	-
NR	6	4.77	3.32; 6.86	0.1549	89.6	-
Meta-regression per number of teeth: Estimate=-0.24; SE=0.13; p-value=0.1						
Wire	0	-	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared; SE – Standard Error.

Finally, we looked at the levels of inflammatory cells.

We verified that in the model of periodontitis induced by ligature with bacterial inoculation, *Mus musculus* (n=4; effect=6.02; p=0.0273) and *Rattus norvegicus* (n=7; effect=6.02; p=0.0273) obtained similar results in the levels of inflammatory cells. On the other hand, in the ligature induction method, *Rattus norvegicus* obtained higher levels of inflammatory cells (n=25; effect=14.54; p<0.0001) compared to *Mus musculus* (n=5; effect=1.86; p<0.0001). Overall, *Rattus norvegicus* has higher levels of inflammatory cells (Table 17).

When evaluating the meta-regressions, we can verify that the number of teeth involved is influenced by the induction time (n=46; estimate=-0.78; SE=0.24; p-value=0.01).

When testing for publication bias, a funnel asymmetry was found in studies using ligature induction (n=30; bias=12.25; SE=2.57; p<0.0001) (Table 17).

Table 17 | *Inflammatory cells according to animal species*

Inflammatory cells	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Overall	46	7.09	5.18; 9.68	<0.0001	99.3	8.73; 2.19; 0.0003
Number of involved teeth						
Meta-regression per time of periodontitis induction: Estimate=-0.78; SE=0.24; p-value= 0.01						
Bacteria inoculation	3	1.33	1.13; 1.55	<0.0001	0	-
<i>Rattus norvegicus</i>	3	1.33	1.13; 1.55	<0.0001	0	-
Ligature	30	10.26	6.91; 15.25	<0.0001	99.4	12.25; 2.57; <0.0001
<i>Mus musculus</i>	5	1.86	1.15; 3.03	<0.0001	94.6	-
<i>Rattus norvegicus</i>	25	14.54	9.28; 22.78	<0.0001	99.5	
Ligature + Bacteria inoculation	11	4.17	2.31; 7.56	<0.0001	98.3	-6.11; 4.83; 0.2382
<i>Mus musculus</i>	4	6.02	3.02; 11.96	0.0273	90.7	-
<i>Rattus norvegicus</i>	7	6.02	3.02; 11.96	0.0273	98.4	-
Ligature + LPS	0	-	-	-	-	-
LPS	2	-	-	-	-	-
Oral gavage	0	-	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared; SE – Standard Error.

When considering the type of ligature, the 3-0 nylon ligature showed the highest levels of inflammatory cells (n=3; effect=109.82; 95% CI: 90.86-132.73; p<0.0001) with low heterogeneity (I²=0%), followed by the 3-0 silk ligature (n=4; effect=14.61; 95% CI: 7.61-28.04; p<0.0001; I²=97.4%) (Table 18).

No publication bias was identified (Table 18).

Although there is substantial heterogeneity, the estimates for nylon ligature were influenced by both induction time and number of teeth. On the other hand, the estimates for silk ligatures were relatively stable in the variable number of teeth but influenced by induction time (n=20; estimate=-0.05; SE=0.02; p-value =0.01), which was confirmed by meta-regression (Table 18).

Table 18 | *Inflammatory cells according to the type of ligature*

Inflammatory cells	n	Effect	95% CI	p-value	I ²	Egger test (Bias; SE; p-value)
Ligature type						
Cotton	1	-	-	-	-	-
Nylon	4	50.90	18.21;142.27	<0.0001	96.6	-
3-0	3	109.82	90.86; 132.73	<0.0001	0	-
5-0	1	-	-	-	-	-
Meta-regression per time of periodontitis induction: Estimate=0.59; SE=0.09; p-value <0.0001						
Meta-regression per number of teeth: Estimate=3.93; SE=0.52; p-value <0.0001						
Silk	20	8.33	5.02; 13.81	<0.0001	98.8	5.44; 4.59; 0.2520
3-0	4	14.61	7.61; 28.04	<0.0001	97.4	-
4-0	11	13.56	6.85; 26.80	<0.0001	98.3	-
5-0	5	1.86	1.15; 3.03	<0.0001	94.6	-
Meta-regression per time of periodontitis induction: Estimate = -0.05; SE=0.02; p-value =0.01						
Meta-regression per number of teeth: Estimate = -0.48; SE=0.33; p-value >0.05						
Wire	0	-	-	-	-	-

n – Number of studies; CI – Confidence Interval; I²– I squared; SE – Standard Error.

IV. DISCUSSION

1. SUMMARY OF THE MAIN RESULTS

The main aim of this systematic review was to summarise the protocols used to induce periodontitis in animal models and their respective local and systemic repercussions. This systematic review concluded that the models of ligature-induced periodontitis caused 139% ABL compared to the control group and the combination of ligature with LPS caused 146% ABL, which were the models that showed the highest ABL and best mimicked periodontitis.

Furthermore, there were no statistically significant differences between males and females in the ligature-induction periodontitis method. On the other hand, there was a higher ABL in males with the oral gavage induction method and with the combination of ligature and bacterial inoculation, with statistically significant differences between the genders. Overall, males had a higher ABL.

Regarding the type of ligature, the greater the ligature thickness, the higher the ABL, namely in cotton n.3 (236%), nylon 3-0 (228%) and silk 3-0 (154%) ligatures. Similarly, the larger the ligature thickness, the higher the levels of systemic inflammatory markers, namely we have more IL-1 β in cotton n.3 ligatures (636%), more IL-6 in cotton n.3 (1424%), more IL-10 in silk 3-0 (127%), more TNF- α in nylon 3-0 (391%), more osteoclast in nylon 3-0 (754%) and finally more inflammatory cells in nylon 3-0 (10882%).

Overall, regarding inflammatory cytokines, IL-1 β is higher when induced by oral gavage (427%), IL-6 when induced by LPS (378%), IL-10 when induced by ligature combined with bacterial inoculation (119%) and finally TNF- α when induced by oral gavage (211%). On the other hand, the combination of ligature with bacterial inoculation is the method that achieves the highest systemic levels of TRAP+ (391%) and osteoclasts (876%). Finally, inflammatory cells reach higher systemic levels when periodontitis is induced by ligature (926%).

2. QUALITY OF THE EVIDENCE AND POTENTIAL BIASES IN THE REVIEW PROCESS

The strengths and limitations of this systematic review are mainly related to the methodological quality of the included studies. This review was conducted according to evidence-based guidelines and included an extensive literature search, a comprehensive analysis, and a rigorous methodology with a reproducible search strategy. However, the included studies were not so linear.

Almost all the articles included in this systematic review are of moderate quality, the rest are of low quality, and none are of high quality. None of the studies met the requirements for "allocation concealment", "blinding" and "random outcome assessment". In addition, none met the requirement for "selective outcome reporting", meaning this bias should be considered in future research. All the above are essential to minimise ROB.

The wide variety of methods used to induce periodontitis in animal models was demonstrated by the high heterogeneity between studies and should be considered as it may lead to high levels of heterogeneity and make it difficult to draw definitive conclusions.

It was not possible to make comparisons in the anaesthetic parameters, since there is a large variability between the types of anaesthesia and most articles lacked information on the final amount of anaesthetic given to each animal. The variability of LPS conditioned their analysis, and these were all grouped together in the same subgroup, making it impossible to carry out specific meta-analyses. For the same reason, it was not possible to perform subgroup analyses of the induction sequence because of its variability. Furthermore, the analyses relating to the type of tooth involved could not be compared either, seeing only three studies were carried out on incisors. In addition, few studies present systemic data, and it is difficult to compare different induction methods. Finally, most studies have been conducted in males, which suggests that more research is needed in females to assess their behaviour. However, there appears to be no differences between females and males in most of the studies, showing that the use of females to induce periodontitis does not affect the results. These are some of the variables that may influence the results. Therefore, our analysis and interpretation of the results was limited

and could be extended in the future provided that the studies gather the information detailed above.

3. AGREEMENTS AND DISAGREEMENTS WITH OTHER REVIEWS OR STUDIES AND CLINICAL RELEVANCE

Most studies report on the reliability of induction methods, but none have investigated which induction method is best for higher ABL and its associated systemic effects.

In the results of Molon et al. (2012), induction by ligature combined with *P. gingivalis* was significantly higher than induction by ligature alone, suggesting that bone loss in the latter group was mainly induced by the trauma of ligature placement (54). However, in our study, ligature showed 139% ABL compared to the control group, while its combination with periodontopathogenic bacteria showed 111% ABL, i.e., they showed statistically significant differences.

According to Molon et al. (2016), induction using ligature is an effective approach for ABL, whereas oral gavage is not (68). These findings are consistent with our results, considering that the meta-analysis showed that induction by ligature has more than 100% ABL compared to induction by oral gavage.

Duan et al. (2015) study concluded that female animals were more susceptible to ABL in the ligature and oral gavage induction models (80). In contrast, our analysis found that only bacterial inoculation induction confirmed this with statistically significant differences between the sexes.

Regarding the type of ligature, our research showed that cotton n.3 caused greater ABL, namely 236% compared to the control group. Similarly, Abe et al (2013) reported that ligature placement in mice caused ABL through the accumulation of endogenous bacteria, therefore, the greater the width of the ligature, the greater the accumulation of bacteria and, consequently, the greater the ABL and systemic impact (66).

Human periodontitis can be classified as localised or generalised depending on its extent and distribution (<30% or >30% of teeth involved) (13). Therefore, one would expect that

the greater the number of teeth involved in the induction of periodontitis, the greater the resulting systemic impact. Be that as it may, our results have not shown this to be so linear.

In Saadi-Thiers et al. (2013), the authors indicated that the highest levels of pro-inflammatory cytokines IL-6 and IL-1 β were observed in the combination model of ligature with bacterial inoculation compared to the other methods (81). However, our results report that the cytokine IL-1 β shows higher levels when induced by oral gavage (427%) with a difference of more than 100% compared to the combination of ligature with bacterial inoculation. Similarly, IL-6 shows higher levels when induced by LPS (378%) with a difference of more than 250% compared to the combination of ligature with bacterial inoculation. In the same study, data shows that osteoclast levels are significantly higher with induction by the combination of ligature with bacterial inoculation compared to induction by ligature or oral gavage. Furthermore, our meta-analysis of the induction subgroups showed that osteoclasts are present in a higher percentage (876%) with the combination of ligature and oral inoculation, given that induction by ligature had 448% osteoclasts. However, we did not obtain data on osteoclasts in induction by oral gavage and it is not possible to confirm or refute Saadi-Thiers et al. (2013) on this parameter (81).

With regard to inflammatory cells, our results reported high levels in the induction by ligature (926%), as shown by Molon et al. (2016) (68). In the study by Molon et al. (2016), an increased inflammatory infiltrate was observed as a result of the local effects of ligature, whereas any significant inflammation after induction of periodontitis by both bacterial inoculation and oral gavage were reported (68). However, our meta-analysis was unable to obtain data on this inflammatory parameter following induction by oral gavage and therefore could not fully confirm this statement.

4. FUTURE PERSPECTIVES

The animal model of periodontitis induction and their systemic effects are fundamental to the scientific community to better understand the systemic impact of periodontitis in humans. In the future, more studies are essential to further investigate systemic effects of periodontitis, considering the limited information available on the systemic effects after

periodontitis induction in animal model. Also, it is of utmost importance to study the long-term effects and disease progression in these animal models. These investigations will allow for a better identification of the biological mechanism at the intersection of periodontitis and systemic diseases, which may lead to new therapeutic targets.

The translation of results from animal models to human studies is essential and of great clinical relevance. Therefore, the development of clinical trials and observational studies in humans is necessary to validate theories developed in preclinical research. These suggestions can contribute to the scientific progress and improvement of therapeutic strategies in the field of periodontology.

V. CONCLUSION

According to this systematic review, although most of the periodontitis induction models are effective in inducing ABL, the ligature-induced periodontitis method and the combination of ligature with LPS were the models that best mimicked periodontitis and had the highest ABL.

In terms of systemic impact, the ligature-induced periodontitis method and the combination of ligature with oral inoculation were the models that showed the greatest ability to modulate the host response through systemic inflammatory changes.

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APPENDICES

Supplementary information 1 – PRISMA Checklist (71)

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	13-26
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	26
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	27
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	27-28
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	27-28
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	29
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	29
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	

Section and Topic	Item #	Checklist item	Location where item is reported
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	29-30
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	30
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	30
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	30
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	30
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	30
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	30
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	30
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	30
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	31
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	32-56

Section and Topic	Item #	Checklist item	Location where item is reported
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	57
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	58-82
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	58-82
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	58-82
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	58-82
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	83
	23b	Discuss any limitations of the evidence included in the review.	84-87
	23c	Discuss any limitations of the review processes used.	84-86
	23d	Discuss implications of the results for practice, policy, and future research.	86-87
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	27
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	

Section and Topic	Item #	Checklist item	Location where item is reported
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Supplementary information 2 – PRISMA Abstract Checklist (71)

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	No
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	No
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	No
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	No
Registration	12	Provide the register name and registration number.	No