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Review

Prevalence of *Trichomonas tenax* in the Population Affected by Periodontal Disease—A Review

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Abstract: Background and Objectives: *Trichomonas tenax* is a protozoan which participates in the human oral microflora. It is considered as a potential paradontopathogen. This microorganism is also reported in the respiratory tract. We aimed to analyze the available literature about the prevalence of *Trichomonas tenax* in the population affected by periodontal disease. Materials and Methods: Searching the Scopus, PubMed, and ScienceDirect databases with the keywords: "*Trichomonas tenax*" and "periodontal diseases" was able to identify several systematic reviews and original articles up until July 2023. All studies with patients suffering from periodontal disease, which mentioned the year of publication, the country, specified the detection methods, and included the total number of tested samples as well as the percentage of those infected with *Trichomonas tenax* were included. Irrelevant articles were excluded. Results: We found 137 studies, but only 64 studies about the distribution of *Trichomonas tenax* in patients with gum disease underwent qualitative analysis. The highest number of studies have been conducted in Iran, Poland and Iraq. Different methods have been used to detect the unicellular organism, each with a different specificity and sensitivity. Conclusions: Interest in *Trichomonas tenax* has grown considerably since 2000. Because of its association with periodontal disease, *Trichomonas tenax's* role in the inflammatory process should not be overlooked.

Keywords: oral protozoa; oral microflora; periodontal disease; *Trichomonas tenax*; respiratory diseases

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1. Introduction

It has been established that the oral cavity has a large number and a rich species composition of microorganisms forming its microflora [1]. Microorganisms adhere to various surfaces (the hard and soft tissues of the teeth, and the oropharyngeal mucosa) and form biofilms [2].

Trichomonas tenax is a flagellate, anaerobic unicellular microorganism that belongs to the genus Trichomonas, of the family Trichomonadidae. It is part of the oral microflora. Its name is derived from the Greek words "trichos" meaning "hair", "monas" meaning "simple organism" and the Latin word "tenere" meaning "hold fast" [3]. *Trichomonas tenax* was identified by Müller in the second half of the 18th century in solutions of tartar [4]. It was initially named *Cercaria tenax* and underwent several modifications until the final name, *Trichomonas tenax*, was accepted by K. Dobel in 1939 [5,6]. Additionally, the human organism is inhabited by two more representatives of the Trichomonadidae family—*Trichomonas vaginalis* and *Trichomonas hominis*. Their characteristics are presented in Table 1. It can be seen that *Trichomonas tenax* looks more like *Trichomonas vaginalis* than *Trichomonas hominis* [7]. *Trichomonas tenax* inhabits the oral cavity of humans, with a higher incidence in individuals with poor oral hygiene, the presence of tartar and periodontal disease, which can lead to tooth loss in adults. It is typically found between teeth, in saliva, in periodontal pockets and less commonly in tonsillar crypts [6]. There is some evidence for its presence in

the duct of the submandibular salivary gland [8]. This oral protozoan is transmitted via saliva, sneezing and coughing drops, kissing or using contaminated subjects and water [9]. Cases of non-oral localizations of *Trichomonas tenax* have been reported, including in sputum samples, bronchoalveolar lavage, pleural punctures in patients with diseases of the lower respiratory tract, the lungs and pleura during bronchiectasis and in lung abscesses, lung cancer and pyothorax. Pulmonary trichomoniasis is believed to develop after aspiration of the microorganism from the oropharynx into the airways [10,11]. The first case of *Trichomonas tenax* detection in the cerebrospinal fluid of patients with polymicrobial meningitis was reported in 1976 [12]. In 1987, its presence alongside a mixed oral bacterial microflora in pus from a subhepatic abscess following perforation of a gastric ulcer was reported [13]. In 1988, the protozoan was found in the mucus of the dilated ducts of patients with fibrocystic mastopathy [14]. *Trichomonas tenax* has been observed in an excised lymph node alongside the causative agent of tuberculosis, *Mycobacterium tuberculosis* [15]. Similar to *Trichomonas vaginalis*, *Trichomonas tenax* may also cause urogenital invasions [16]. Apart from humans, it has also been found in the oral cavities of domestic animals such as cats, dogs, horses, in the cloaca of birds and in the vaginas of monkeys [17,18].

Table 1. Comparative table showing the characteristics of Trichomonas species occurring in humans.

Characteristics \ Species	*Trichomonas tenax*	*Trichomonas vaginalis*	*Trichomonas hominis*
Host	human, dogs, cats, etc.	human	man, cattle
Localization in the body	oral cavity	vagina, urethra	intestinal tract
Size	5–16 × 2–15 µm	7–32 × 5–12 µm	8–20 × 3–14 µm
Number of blepharoplasts	1	1	2
Undulating membrane	does not cover the entire length of the cell (the membrane of *Trichomonas tenax* is longer than the membrane of *Trichomonas vaginalis)*		cover the entire length of the cell
Nucleus	rounded	extended	rounded
Chromatin	aggregated into uniformly distributed granules (in *Trichomonas tenax* they are larger and fewer in number)		rarely aggregated into granules, forms a layer around the nuclear membrane

Trichomonas tenax has been considered as a harmless commensal for a long time. Because of its association with periodontal disease, researchers have shifted their focus to its pathogenicity factors [19]. The secretory activity of *Trichomonas tenax* has been studied. Ribaux et al. reported the presence of fibronectin-like molecules on the surface of this flagellated microorganism in 1983. They examined two strains of *Trichomonas tenax*—one isolated from patients with ulcerative gingivitis and another maintained in culture for two years. Both strains exhibited positive fluorescence with anti-fibronectin antibodies. These fibronectin-like molecules are believed to facilitate adhesion to gingival cells and connective tissue and promote phagocytosis of the bacteria [20]. Bózner and Demeš continued the studies on proteolytic activity in cell extracts and culture filtrates of *Trichomonas tenax* using SDS-polyacrylamide gels, identifying seven different proteolytic bands. Among them, three are SH-dependent, with molecular weights in the range of 35–36 kDa, exhibiting inhibitory sensitivity characteristic of cysteine proteinases. The remaining four bands are SH-independent, with higher molecular weights (76–270 kDa), and their inhibition sensitivity suggests that they are metalloproteinases. Bózner and Demeš then investigated the degradation of collagens I, III, IV and V by secreted extracellular proteinases from *Trichomonas tenax*, concluding that degradation was temperature-dependent and that type IV collagen was the most efficiently degraded. These proteinases may play a role in peri-

odontal degradation [21]. *Trichomonas tenax* can produce hemolysins that destroy human, equine, and ovine erythrocytes. Two different types of hemolysins were identified. The first is protein-like, thermolabile and may be inhibited by cysteine proteinase inhibitors. The other is lipid-like, thermostable, not inhibited by proteinase inhibitors and resistant to the action of organic solvents. These hemolysins may serve as potential virulence factors [22]. Ribeiro et al. performed a study that revealed that *Trichomonas tenax* damages mammalian cells in vitro and behaves similarly to *Trichomonas vaginalis.* It phagocytoses portions of the mammalian cells, and also induces apoptosis in HeLa cells, suggesting that it behaves as a parasite [23]. In 2018, it was discovered that *Trichomonas tenax* lysates induce IL-8 synthesis by macrophages [19]. According to a study conducted in 2023, the protozoan exhibits toxicity to gingival cells, disrupts cell contacts, and leads to the synthesis of another inflammatory mediator (IL-6) by gingival and alveolar cells [24].

The objective of this article is to analyze the available studies on the prevalence of *Trichomonas tenax* in the population affected by periodontal disease.

2. Materials and Methods

2.1. Search Strategy

Several reviews and original articles in the Scopus, PubMed and ScienceDirect databases were examined. The articles had a publication period up to July 2023. We used the "Advanced search" extension and the following keywords: "*Trichomonas tenax*" and "periodontal diseases". Only full texts were included.

2.2. Inclusion and Exclusion Criteria

All studies involving individuals diagnosed with periodontal disease, with or without concomitant systemic disorders, were included. There were no geographical restrictions on selection. Other inclusion criteria included considering articles that contained information about the year of publication, country, reported detection methods, total number of samples tested, and the percentage of patients infected with the flagellate microorganism.

Duplicate articles from all three databases were excluded. In addition, articles were excluded if they were not relevant to the aims of the study, such as those involving animal studies or analyzing *Trichomonas tenax* in a site other than the oral cavity.

2.3. Statistical Analyses

For the statistical analyses and visual presentation of resumed studies, MS Excel 2016 software was used.

3. Results

3.1. Study Selection

We found 137 records from the Scopus, PubMed, and ScienceDirect databases that were potentially relevant. The study selection process followed the PRISMA flow diagram (Figure 1). Initially, duplicates were removed, leaving 102 articles for screening. Seventeen were excluded due to general irrelevancy based on either title or abstract, and nine were excluded due to the lack of a full text. Of the remaining seventy-six articles, twelve were further removed for various reasons, including the detection of *Trichomonas tenax* in animals, extraoral detection of *Trichomonas tenax*, and in vitro studies. Ultimately, sixty-four research papers underwent qualitative analysis.

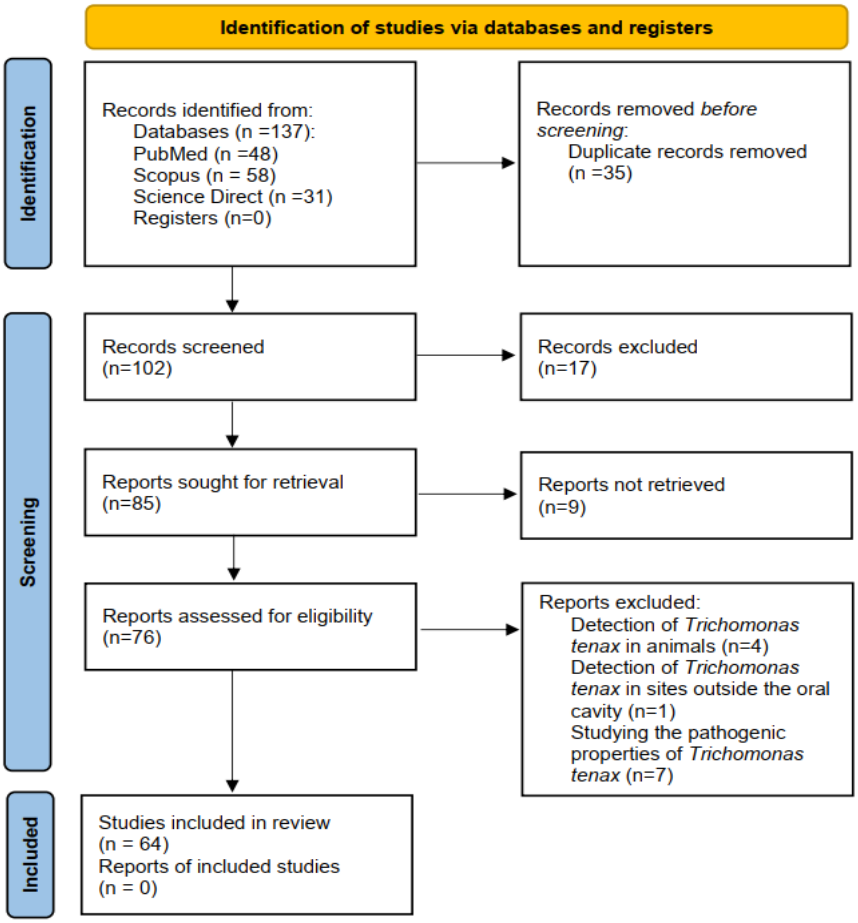

Figure 1. PRISMA flow diagram of the study selection process.

3.2. Demonstration of Our Results

For a better demonstration of the results, tables and figures were used. We divided the *Trichomonas tenax* prevalence studies in patients with gum disease by continent and country. Figure 2 illustrates the distribution by continents. We were able to find the following numbers of studies:

- Twenty-three studies carried out in Europe.
- Twenty-three studies carried out in Asia.
- Six studies carried out in Africa.
- Eight studies carried out in South America.
- Four studies carried out in North America.

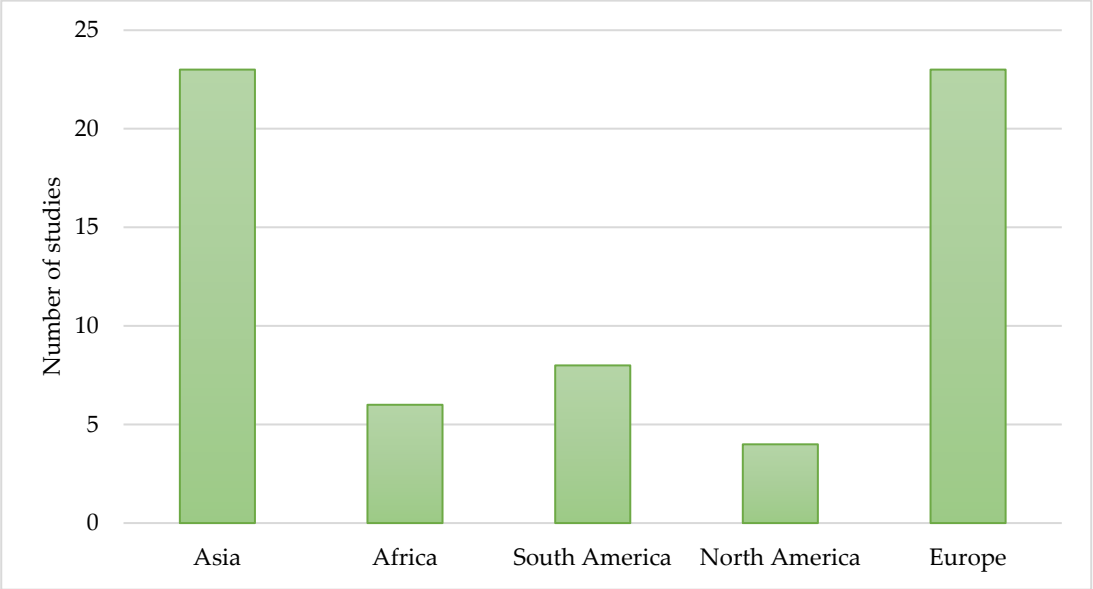

Figure 2. Number of studies available about the prevalence of *Trichomonas tenax*—distribution by continents. We were able to identify the fewest studies of *Trichomonas tenax* prevalence in North America, and the most in Asia and Europe.

Figure 3 shows the distribution by countries. Table 2 presents the total number of samples taken, whose number was 12,269, of which 2215 were infected with *Trichomonas tenax*. Tables 3–7 provide a detailed description of the studies, taking into account author, year of publication, country, number of samples tested, number of positive samples and detection method used. The detection methods used in the studies are those that were used to evaluate the presence of *Trichomonas tenax* in patients with gum disease. Their advantages and disadvantages, as well as the factors that may influence the spread of this microorganism, are mentioned in the Discussion section.

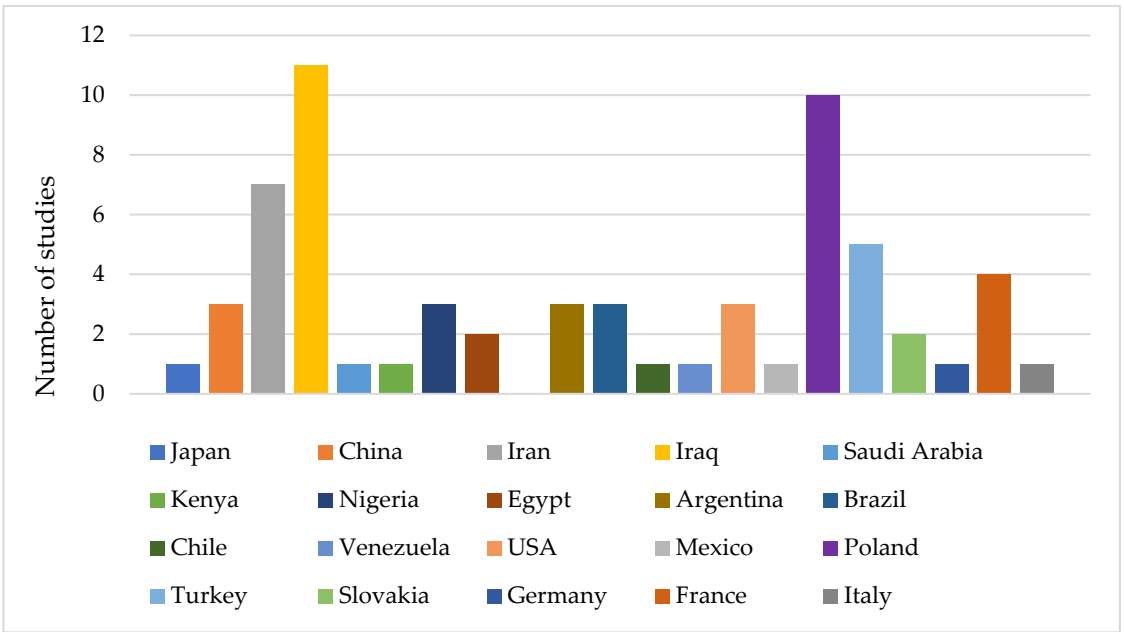

Figure 3. Number of studies available about the prevalence of *Trichomonas tenax*—distribution by countries. We found the fewest studies of *Trichomonas tenax* prevalence in Japan, Saudi Arabia, Kenya, Chile, Venezuela, Mexico, Italy, Germany and the most in Iraq, Poland and Iran.

Table 2. Percentage of samples infected with *Trichomonas tenax*.

Continent	Total Number of Collected Samples	Total Number of Samples Infected with *Trichomonas tenax*	% of Samples Infected with *Trichomonas tenax*
Europe	4531	790	17%
Asia	4931	713	14%
South America	345	114	33%
North America	1722	503	28%
Africa	740	95	12%

Table 3. *Trichomonas tenax* prevalence studies carried out in Europe.

Author(s)	Year	Country	Number of Tested Samples	Number of Samples Positive for *Trichomonas tenax*	Used Method for Detection
Feki et al. [25]	1981	France	300	84	Cultivation
Ferrara et al. [26]	1986	Italy	367	159	Light microscopy
Kurnatowska [27]	1990	Poland	452	69	Light microscopy
Kurnatowska et al. [28]	1990	Poland	1018	148	Light microscopy, biochemical methods
Vráblic et al. [29]	1991	Slovakia	176	7	Cultivation
Vráblic et al. [30]	1992	Slovakia	231	9	Cultivation
Kurnatowska et al. [31]	1998	Poland	936	90	Light microscopy
Celiksoz [27]	2001	Turkey	41	1	Light microscopy
Pardi et al. [32]	2002	Germany	30	9	Cultivation
Piekarczyk et al. [33]	2003	Poland	50	3	Light microscopy
Kurnatowska et al. [34]	2004	Poland	91	34	Light microscopy
Turkowicz et al. [35]	2004	Poland	54	3	PCR
Kurnatowska [27]	2004	Poland	22	16	PCR, light microscopy
Dudko [27]	2007	Poland	189	58	Light microscopy
Abualqomsaan et al. [36]	2010	Turkey	46	1	Light microscopy
Gedik et al. [37]	2010	Turkey	220	10	Tech Lab Entamoeba Kit and Robinson Medium
Yazar et al. [38]	2016	Turkey	175	50	Light microscopy, cultivation
Zawadzki et al. [39]	2016	Poland	48	22	Light microscopy
Zawadzki et al. [40]	2017	Poland	85	17	Light microscopy
Bisson et al. [19]	2018	France	50	10	Phase-contrast microscopy
Dubar et al. [41]	2019	France	30	10	PCR
Benabdelkader et al. [42]	2019	France	106	45	PCR
Arpağ and Kaya [43]	2020	Turkey	101	34	Light microscopy

Table 4. *Trichomonas tenax* prevalence studies carried out in Asia.

Author(s)	Year	Country	Number of Tested Samples	Number of Samples Positive for *Trichomonas tenax*	Used Method for Detection
Sato et al. [44]	1985	Japan	307	96	Cultivation
Li, 1988 [45]	1988	China	572	79	Light microscopy
Mahdi et al. [46]	1993	Iraq	143	12	Light microscopy
Xiufeng et al. [47]	2003	China	427	13	Unknown
Athari et al. [48]	2007	Iran	160	33	PCR
Kadir et al. [49]	2007	Iraq	156	18	Light microscopy
Marty et al. [50]	2009	China	492	46	Light microscopy
Ghabanchi et al. [51]	2010	Iran	50	3	Light microscopy
Ibrahim and Abbas [52]	2012	Iraq	60	28	Light microscopy
Hamad et al. [53]	2012	Iraq	500	56	Light microscopy
Mehr [54]	2015	Iran	52	14	PCR
Jabuk et al. [55]	2015	Iraq	100	27	Light microscopy, cultivation
Al-Khayat [56]	2016	Iraq	58	33	PCR
Khafari Ghosheh et al. [57]	2017	Iran	270	1	Light microscopy
Khadiga Ahmed Ismail and Mawaddah Ahmed Jastaniyyah [58]	2017	Saudi Arabia	56	7	Light microscopy
Derikvand et al. [59]	2018	Iran	76	11	Light microscopy, PCR
Hossein Mahmoudvand et al. [60].	2018	Iran	140	17	Light microscopy
Abdulhaleem et al. [61]	2018	Iraq	160	40	PCR
Jaffer et al. [62]	2019	Iraq	184	8	PCR
Hassan et al. [63]	2020	Iraq	310	64	PCR
Yaseen et al. [64]	2021	Iran	143	82	PCR
Sharifi et al. [65]	2020	Iraq	315	7	PCR
Hala Nadhim and Nadham Kadham [66]	2023	Iraq	200	18	Light microscopy

Table 5. *Trichomonas tenax* prevalence studies carried out in South America.

Author(s)	Year	Country	Number of Tested Samples	Number of Samples Positive for *Trichomonas tenax*	Used Method for Detection
Zdero et al. [67]	1999	Argentina	25	10	Light microscopy
Ponce De León et al. [68]	2001	Argentina	50	10	Light microscopy, cultivation
Nocito Mendoza et al. [69]	2003	Argentina	50	16	Light microscopy
Mabel et al. [70]	2009	Venezuela	25	1	Light microscopy
Albuquerque Júnior et al. [71]	2011	Brazil	42	12	Light microscopy
Bernaola-Paredes et al. [72]	2012	Brazil	53	9	Cultivation
Norberg [73]	2014	Brazil	50	28	Cultivation
Bracamonte-Wolf et al. [74]	2019	Chile	50	28	PCR

Table 6. *Trichomonas tenax* prevalence studies carried out in North America.

Author(s)	Year	Country	Number of Tested Samples	Number of Samples Positive for *Trichomonas tenax*	Used Method for Detection
Hinshaw [75]	1926	USA	186	49	Cultivation
Beatman [76]	1933	USA	350	132	Unknown
Wantland and Lauer [77]	1970	USA	1036	301	Light microscopy, cultivation
Cuevas et al. [78]	2008	Mexico	150	21	Light microscopy

Table 7. *Trichomonas tenax* prevalence studies carried out in Africa.

Author(s)	Year	Country	Number of Tested Samples	Number of Samples Positive for *Trichomonas tenax*	Used Method for Detection
Chunge et al. [79]	1988	Kenya	177	5	Light microscopy
Ozumba et al. [80]	2004	Nigeria	203	10	Light microscopy
Nagwa M. El-Sayed and Eman M. H. Meabed [81]	2008	Egypt	50	15	Light microscopy, cultivation, PCR
Onyido et al. [82]	2011	Nigeria	60	21	Light microscopy
El Sibaei et al. [83]	2012	Egypt	70	20	Light microscopy, cultivation
Ani et al. [84]	2020	Nigeria	180	24	Light microscopy

A relatively large number of studies have been conducted in Europe, with the majority carried out in Poland. In nine of these cases, light microscopy was used as a confirmatory method. Biochemical methods were also present in one of them, and PCR was present in two of them. Five studies were carried out in the European part of Turkey, all using light microscopy and one using cultivation. In France, interest in the protozoan began in 1979, leading to four studies, two of which used PCR as a confirmatory method. *Trichomonas tenax* was identified by cultivation in Germany and Slovakia, and by light microscopy in Italy. We mentioned the advantages and disadvantages of each detection method in the Discussion section.

In Asia, the majority of studies were found in Iraq. In five of them, *Trichomonas tenax* was identified by light microscopy. One of the studies used cultivation. Notably, half of the studies used molecular techniques for detection. In Iran, seven studies were found, half of which also employed molecular techniques. In China, two studies used light microscopy and one utilized an unknown method. In Japan and Saudi Arabia, microscopy and cultivation were used. In North America, South America and Africa the number of studies was lower compared to Asia and Europe. Light microscopy and cultivation were the main diagnostic methods used in the studies conducted in the USA and Mexico. In South America, we were able to find eight studies, with PCR being used in only one study. Most studies in Africa detected the unicellular microorganism by light microscopy or cultivation, with PCR being used in only one of them. In Australia, there was only one study that detected protozoan, but in canine samples.

4. Discussion

The relevance of the topic is supported by the fact that periodontal disease is a global societal problem, prevalent mainly in developed and developing countries, affecting both children and adults [85]. The literature emphasizes the importance of its etiology. Our

review aims to enrich the knowledge that exists about the etiology of periodontal disease, since, in addition to bacteria, protozoa such as *Trichomonas tenax* can also be involved in the inflammatory process. After analyzing the results, we have drawn some important conclusions about the prevalence of *Trichomonas tenax* and identified the factors associated with its distribution, which will be the focus of the discussion.

Regarding the prevalence of *Trichomonas tenax* in the oral cavity, our analysis revealed that it has been detected in samples of various biological material: most commonly in dental calculus or subgingival dental plaque, and less commonly in saliva, mouthwash or gingival crevicular fluid [71]. In one study, it was found in material from decayed dental cavities, suggesting that it may be associated with caries development [60].

Most articles do not provide information about the distribution of *Trichomonas tenax* by sex. However, some authors reported a higher incidence of this protist in men than women [27,29,77]. In addition, it has been found more in adults. Based on the systematic review of Eslahi et al., individuals aged 46–55 years showed the highest colonization by oral trichomonads [27]. Several articles highlight the presence of the single-celled microorganism in the pediatric population [29,30,78]. According to Vráblic et al. oral protozoa occur in older children. *Trichomonas tenax* invasion was found to be higher in children with Down syndrome and periodontitis than in healthy children in a study by Mehr et al. [54].

Oral trichomoniasis is more commonly associated with gingivitis and periodontitis. *Trichomonas tenax* was found more in patients with periodontitis than with plaque-induced gingivitis [74]. It is important to emphasize that most scientists have examined *Trichomonas tenax* alongside *Entamoeba gingivalis*, another protozoan that is part of the oral microflora. *Entamoeba gingivalis* is typically found in the early stages of periodontitis and *Trichomonas tenax* is associated with the progression of periodontitis [42,64,71,75,76]. The presence of *Trichomonas tenax* is usually less common than that of *Entamoeba gingivalis* in patients with gingivitis [27]. Associations of the flagellated protozoan with respiratory diseases have also been reported [10,11].

The prevalence of periodontal disease depends on various factors including the level of socioeconomic status, sanitary conditions (areas with bad hygiene may have higher risks of contamination and an increased spread of microorganisms), certain lifestyle factors (such as smoking, diet rich in sugary or acidic foods), health education, access to dental care, the population's immunological status and the presence of metabolic diseases such as diabetes [86]. A proportional relationship between the frequency of occurrence of *Trichomonas tenax* and poor oral hygiene, alcohol consumption and tobacco use has been reported by some authors [34,37,53]. Changes in oral ecology due to diabetes or reduced body resistance (HIV infection, treatment with immunosuppressants after organ transplantation) facilitate the reproduction of flagellate protozoa and the colonization of dental tissues [33,69]. Patients with masticatory system disorders, particularly those with congenital diseases, may also favor the presence of oral dysbiosis [39]. A study by Ponce de Leon et al. showed that the incidence of buccal parasites in patients with dental prostheses was greater [68]. *Trichomonas tenax* has also been found in peri-implantitis lesions [43].

From our analysis, it became evident that in some countries there is a notably high number of studies on the epidemiology of *Trichomonas tenax*. This is probably due to greater research interest in countries or regions where oral health problems, infectious and parasitic diseases are more prevalent. Previous research contributes to our understanding of why *Trichomonas tenax* is tested for more in Poland, Iran and Iraq. According to the study conducted by Muhammad Nazir et al., more than half of the adult populations in Poland and Iran suffer from periodontal disease. Additionally, Iran also has a high proportion of adolescents affected by it [87]. Factors such as low education, use of tobacco products and metabolic disorders can be considered as risk in Iran that lead to periodontitis and tooth loss in Iran. [88] Derikvand et al. found a significant association between parasite invasion and compromised oral hygiene in Iran [59]. An online-based survey in Iraq revealed low levels of awareness about oral health and periodontitis [89]. A similar

study conducted in Poland showed an insufficient knowledge about risk factors as well as the prophylaxis of periodontal disease [90]. Unlike Iran and Iraq, Poland has another demographic characteristic: a high proportion of the population is ageing, which plays a significant role in the increased incidence of dental diseases. Declining immunity in older individuals predisposes them to the development of oral and systemic pathology [70].

Additionally, we will discuss an issue that is critical to the positivity of the samples, namely the methods of detection. It is important to note that not all methods exhibit the same sensitivity and specificity. The limitations of the methods must be considered together with other factors, such as a limited number of samples, a small number of participants, and a lack of standardized protocols, which may account for the differences in the obtained results. Different methodologies have been used over the years to find *Trichomonas tenax*. The detection methods were unspecified in two studies and biochemical methods were used in one. We observed that many scientists have identified *Trichomonas tenax* through microscopic examination, with or without pre-staining, by visualizing pear-shaped or elliptical cells with several flagella [91]. Microscopic examination was the primary detection method in forty studies, with thirty-nine using light microscopy and one employing phase-contrast microscopy. The advantage of microscopy is the easy and quick visualization of trichomonads. Cultivation allows for the isolation and identification of *Trichomonas tenax*. We were able to find fourteen reports that detect the unicellular organism through cultivation in an axenic culture medium known as Diamond's medium, which was established in the second half of the 20th century [92]. However, we acknowledge the limitations of both of these methods, namely limited specificity and low sensitivity. Cultivation is also time-consuming. There is a high probability that the results may not be completely accurate in studies that have used these two methods because of their shortcomings. Furthermore, it is possible that the samples may have been contaminated with *Trichomonas hominis* or *Trichomonas vaginalis*, whose morphologies are particularly similar, as discussed in Table 1, causing false-positive results. As discussed earlier, most studies have examined *Trichomonas tenax* and *Entamoeba gingivalis* together. Diagnosis may be complicated by the tendency of *Trichomonas tenax* to form amoeba-like forms that may be overlooked by light microscopy [81]. These forms are visually similar to those of *Entamoeba gingivalis* and may lead to the misinterpretation of results. It should be noted that in some studies light microscopy and cultivation are complemented by Polymerase chain reaction (PCR), which increases diagnostic accuracy and reliability. PCR can detect the DNA of microorganisms even at very low concentrations, which is difficult or impossible with light microscopy. Molecular techniques are the only method used in some studies. These techniques have a high sensitivity and specificity and can be used for strain discrimination. The high sensitivity of PCR is highlighted in some studies, where it was able to detect the nucleic acid of the protozoan in samples that had previously been found to be negative by light microscopy [48,81]. The shortcomings of molecular techniques include the need for advanced laboratory skills and specialized equipment, as well as the possibility of contamination and false-positive results. We established that PCR was used in 15 studies. Some of the *Trichomonas tenax* genes, such as the beta-tubulin gene, 18S rRNA gene, rpb1 gene, have been analyzed by PCR [42,74,93]. Loop-mediated isothermal amplification (LAMP) is a relatively new method that requires a shorter run time and is more sensitive than PCR [94,95]. There was only one study in which LAMP was employed to detect *Trichomonas tenax*, but this used canine oral samples and the specific detection of ITS (internal transcribed spacers) and the 5.8S rRNA gene [96]. We observed that gaps currently exist in immunological diagnosis. Advances in diagnostic techniques and tools may contribute to increasing the number of epidemiological studies on *Trichomonas tenax*.

Despite these findings, there is still insufficient information regarding the distribution of *Trichomonas tenax* and there is a need for future research on its prevalence. Conducting more epidemiological studies could prove to be useful in understanding its prevalence as well as the social disparities among affected populations. Furthermore, future research may focus on the pathogenesis and impact of this microorganism on oral health, its genetics and

biology and its relationship with other microorganisms in the oral cavity. The development of more sensitive and specific methods for the diagnosis of *Trichomonas tenax* may facilitate a more accurate determination of its presence in the human mouth. Improved methods could help not only in the diagnosis of existing disease, but also in the screening of people with dental calculus. Because of its advantages, we may recommend the LAMP method as an efficient screening method in the future. Another critical area for potential future research is exploring the influence of the immune system on *Trichomonas tenax* and the potential development of immunological diagnostic methods. We may propose *Trichomonas tenax* to be a candidate for inclusion in the mandatory diagnostic panel for periodontitis. Its elimination will improve the prognosis of gum disease in these patients. In this context, studies on drug resistance and the impact of different toothpastes may be important. The involvement of *Trichomonas tenax* in the etiopathogenesis of dental caries requires further research.

5. Conclusions

Interest in *Trichomonas tenax* has significantly increased since 2000. Numerous studies have identified it in oral specimens from patients with periodontal disease, most commonly tartar or dental plaque, using a variety of methods. Studies have shown that there is a proportional relationship between its prevalence and poor personal hygiene and some behavioral factors. It occurs in older children and more frequently in adults with periodontitis than gingivitis. It may be a marker associated with the severity of periodontal disease. We found the largest number of studies detecting *Trichomonas tenax* in patients with gum disease in Iran, Iraq and Poland, where oral health problems are common. Consequently, *Trichomonas tenax's* role in the inflammatory process should not be overlooked, and therefore, this single-celled microorganism should be discussed in the diagnosis and treatment of patients with periodontal disease. Its eradication from the oral cavity can mitigate the risk of aspiration into the lungs and subsequent complications. For future research, it would be beneficial for researchers to focus on the epidemiology, the mechanisms of dental tissue damage and the immunological diagnosis, treatment and prevention of *Trichomonas tenax*.

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