Review

Prevalence of Trichomonas tenax in the Population Affected by Periodontal Disease – a Review

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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, participating 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*: The searching with keywords: ,,*Trichomonas tenax"* and "periodontal diseases'' in Scopus, PubMed and ScienceDirect databases was able to identify several systematic reviews and original articles until July 2023. All studies with suffering from periodontal disease people, mentioned year of publication, country, specified detection methods, 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 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 different specificity and sensitivity. *Conclusions*: Interest in *Trichomonas tenax* has grown considerably since 2000. Because of its association with periodontal disease, *Trichomonas tenax* role in the inflammatory process shouldn't be overlooked.

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

1. Introduction

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

Trichomonas tenax is a flagellate, anaerobic unicellular microorganism that belongs to the genus Trichomonas, family Trichomonadidae. It enters into the composition 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]. It was first identified by Müller in the second half of the 18th century in solutions of tartar [4]. The name *Cercaria tenax* was given during this time – it underwent several modifications until acceptance of the final name *Trichomonas tenax* by K. Dobel in 1939 [5,6]. In addition, 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 shows that *Trichomonas tenax* looks more like *Trichomonas vaginalis* than *Trichomonas hominis* [7]. *Trichomonas tenax* inhabits the oral cavity of humans, and its incidence is higher in persons with poor oral hygiene, the presence of tartar and periodontal disease, which is a cause of tooth loss in adults. It is usually found between teeth, in saliva, in periodontal pockets, carious cavities and less commonly in tonsillar crypts [6]. There is an evidence of its presence in the duct of the submandibular salivary gland [8]. It is transmitted via saliva, sneezing and coughing drops, kissing or using contaminated subjects and water [9]. Cases of non-oral localizations of

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Trichomonas tenax have been reported. It has been detected in sputum samples, bronchoalveolar lavage, pleural punctures in patients with diseases of the lower respiratory tract, lung and pleura – bronchiectasis, lung abscess, lung cancer, pyothorax. Pulmonary trichomoniasis is thought to develop after aspiration of the microorganism into the airways from the oropharynx [10,11]. In 1976, the first case of trichomonas detected in the cerebrospinal fluid of patients with polymicrobial meningitis is reported [12]. In 1987, the presence of *Trichomonas tenax* and an accompanying mixed oral bacterial microflora in pus from a subhepatic abscess following perforation of a gastric ulcer was reported [13]. In 1988, the protozoan was detected in mucus of dilated ducts of patients with fibrocystic mastopathy [14]. *Trichomonas tenax* is found in an excised lymph node together with the causative agent of tuberculosis, *Mycobacterium tuberculosis* [15]. Similar to *Trichomonas vaginalis*, *Trichomonas tenax* may also cause urogenital invasions [16]. Besides humans, it has also been found in the oral cavity of domestic animals – cats, dogs, horses, in the cloaca of birds and in the vagina of monkeys [17,18].

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

Species / **Characteristics**	*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	doesn't 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

For a long time *Trichomonas tenax* has been considered as a harmless commensal. Because of its association with periodontal diseases, researchers focused on its pathogenicity factors [19]. The secretory activity of *Trichomonas tenax* has been investigated. In 1983, fibronectin-like molecules on the surface of the flagellated microorganism are reported. These molecules probably promote adhesion to gingival cells and phagocytosis of the bacteria [20]. *Trichomonas tenax* secretes cysteine proteinases and metalloproteinases [21]. There is an evidence that it can produce hemolysins that degrade erythrocytes of humans, horses, and sheep [22]. *Trichomonas tenax* damages mammalian cells in vitro and behaves similarly to *Trichomonas vaginalis* [23]. In 2018 it was found that its lysates induce IL-8 synthesis by macrophages [19]. According to a study performed in 2023, *Trichomonas tenax* 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 recent article is to analyze the available studies about the prevalence of *Trichomonas tenax* in the population affected by periodontal disease.

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2. Materials and Methods

2.1. Search strategy

Several systematic reviews and original articles in Scopus, PubMed and ScienceDirect databases were reviewed. The articles had a publication period up to July 2023. They were found using the following keywords: ,,*Trichomonas tenax"* and "periodontal diseases''.

2.2. Inclusion and Exclusion criteria

The review included all studies in which the people had periodontal disease. The studies with mentioned year of publication, country, specified detection methods, total number of tested samples as well as the percentage of those infected with *Trichomonas tenax* also were considered. Irrelevant articles, like those involving animal studies or analyzing *Trichomonas tenax* in different than oral cavity location were excluded.

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 Scopus, PubMed, ScienceDirect databases that were potentially relevant. Study selection process was described using PRISMA flow diagram (Figure 1). Duplicate results were removed – so only 102 articles were screened. The sample was further reduced to 85 after removing the papers with inappropriate title and abstract. Other 9 ones were rejected due to lack of full text. Of the remaining 76 articles, 12 were removed due to various reasons: detection of *Trichomonas tenax* in animals with periodontal disease, extraoral detection of *Trichomonas tenax*, studies that emphasized possible pathogenic mechanisms of the unicellular organism. There were 64 papers that underwent qualitative analysis.

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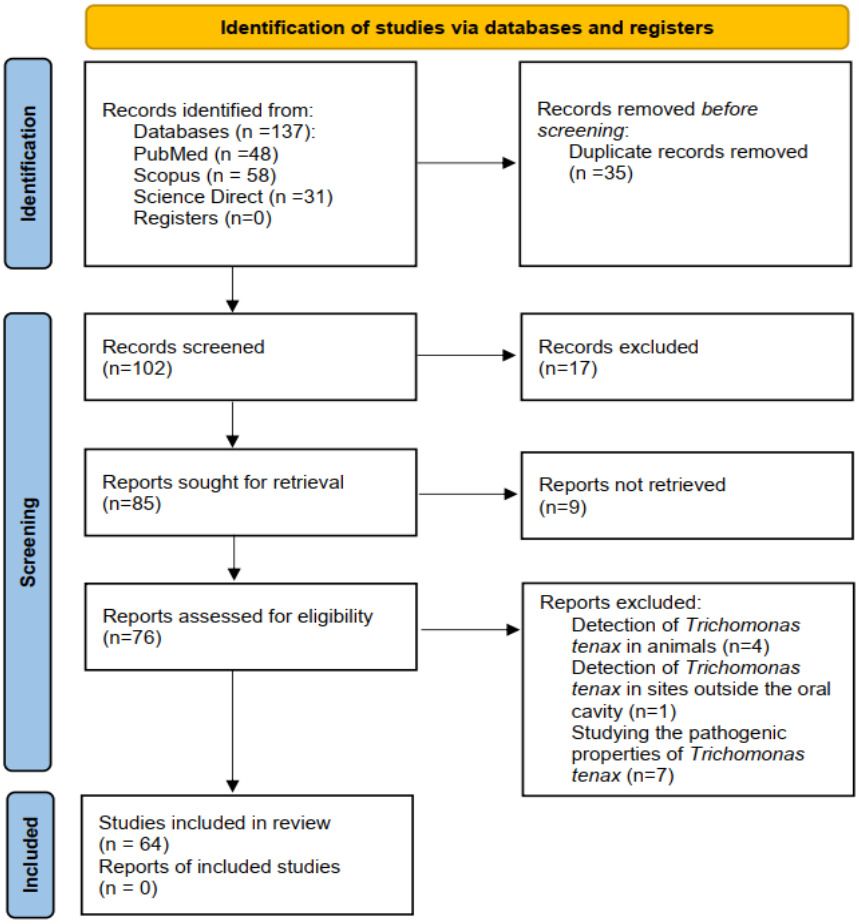

Figure 1. PRISMA flow diagram of the study selection process.

3.2. Demonstration of our results

We divided Trichomonas tenax prevalence studies in patients with gum disease by continent and country to analyze the epidemiological distribution. Figure 2 shows the distribution by continents. We were able to find:

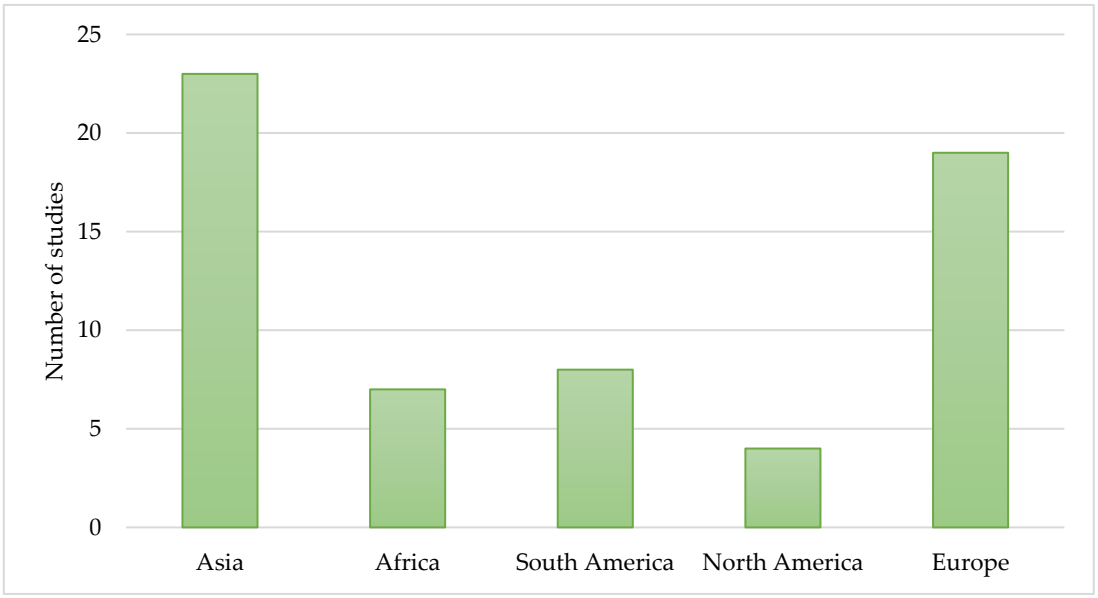

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Figure 2. Number of available studies about the prevalence of *Trichomonas tenax* – distribution by continents.

- 23 studies carried out in Europe
- 23 studies carried out in Asia
- 6 studies carried out in Africa
- 8 studies carried out in South America
- 4 studies carried out in North America

Figure 3 shows the distribution by countries. In presenting the results, we would like to draw attention to the methods of detection. We mentioned the advantages and disadvantages of each one in the „Discussion" section. A relatively large number of studies have been conducted in Europe. The majority of these were carried out in Poland. The similarities in nine of these cases were such that 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 started in 1979 and since then there have been 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.

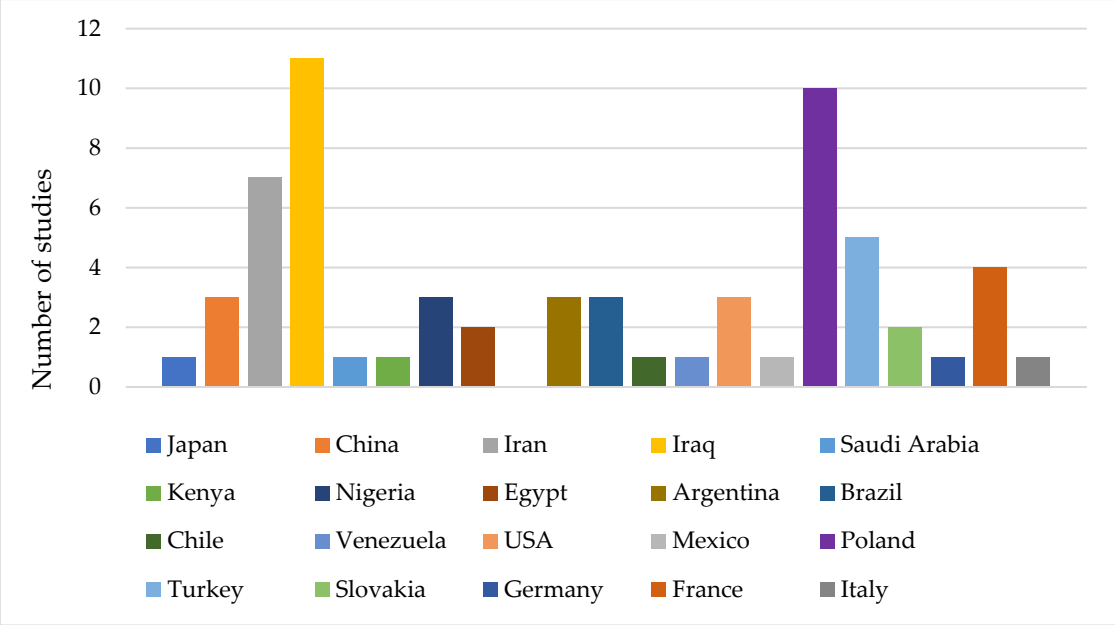

Figure 3. Number of available studies about the prevalence of *Trichomonas tenax* – distribution by countries.

In Asia, most studies were found in Iraq. In five of them, *Trichomonas tenax* was identified by light microscopy. One of the studies used cultivation. It is noteworthy that half of the studies used molecular techniques for detection. In Iran, we found seven studies, half of which also used molecular techniques. In China, two of the studies used light microscopy and one with unknown method. In Japan and Saudi Arabia microscopy and cultivation were used. In North America, South America and Africa the number of studies is lower than 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. PCR was used only in one research. The most studies in Africa detected the unicellular microorganism by light microscopy or cultivation. PCR was used again in only one of them. In Australia, there was only one study that detected protozoan, but in canine samples.

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For a better presentation of the results, tables were used. Table 2 shows the total number of taken samples, whose number was 12269, of which 2215 were infected with *Trichomonas tenax*. Tables 3–7 illustrate a detailed description of the studies, taking into account author, year of publication, country, number of samples tested, number of positive samples and used detection method.

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

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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	2017	Saudi Arabia	56	7	Light microscopy

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[58]					
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

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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

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. The discussion is focused on the role of *Trichomonas tenax* as an object of interest in some countries and on the advantages and disadvantages in the methodology used to detect this microorganism.

As a result of our analysis, it became clear that in some countries the number of studies about epidemiology of *Trichomonas tenax* are notably high. This is probably due to several factors: firstly, knowing the etiology of periodontal disease and secondly, the high prevalence of periodontal disease in some countries. The prevalence of periodontal disease depends on the level of socioeconomic status, sanitary conditions (in areas with bad hygiene the risks of contamination and spread of the microorganism may be greater), certain lifestyle factors (smoking, diet rich in sugary or acidic foods), health education, access to dental care, the population's immunological status, presence of metabolic diseases such as diabetes, genetic disorders (Down syndrome), presence of dental implants. [43,54,86]. These factors interact and may explain why *Trichomonas tenax* may be more prevalent in some countries compared to others. Previous research contributes to our understanding why *Trichomonas tenax* is tested more in Poland, Iran and Iraq. According to the study conducted by Muhammad Nazir et al. more than half of adult population in Poland and Iran have a periodontal disease. Additionally, Iran also has a high proportion of adolescents with it. [87] Low education, use of tobacco products, metabolic disorders can be considered as risk factors in Iran that lead to periodontitis and tooth loss. [88] An online-based survey in Iraq showed low levels of awareness about oral health and periodontitis. [89] A similar study was conducted in Poland, which revealed an insufficient knowledge about risk factors as well as prophylaxis of periodontal disease. [90]

Additionally, used methods for detection, are also important for the positivity of the samples. It is important to note that not all methods have the same sensitivity and specificity. The shortcomings of the methods must be taken into account with some other factors such as limited number of samples, small number of participants, lack of standardized protocols, which may explain the differences in the obtained results. Different methodologies have been used over the years to find

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Trichomonas tenax. Detection methods are unknown in two of the studies and biochemical methods are used in one of them. We found that many scientists have detected *Trichomonas tenax* by microscopic examination, with or without pre-staining, by visualizing pear-shaped or elliptical cells with several flagella [91]. The number of studies in which we detected microscopic examination as the main detection method was forty, of which thirty nine with light microscopy and one with phase-contrast microscopy. The advantage of microscopy is the easy and quick visualization of trichomonads. Cultivation allows isolation and identification of *Trichomonas tenax*. We were able to find fourteen reports that identify the unicellular organism by cultivation in an axenic culture medium known as Diamond's medium, that is established in the second half of the 20th century [92]. We acknowledge the limitations of these both methods – limited specificity and low sensitivity. Cultivation also is time-consuming. There is a high probability that the results are not completely accurate in studies that have used these two methods because of their shortcomings. It is also possible that the samples may have been contaminated with *Trichomonas hominis* or *Trichomonas vaginalis*, whose morphology is too similar and discussed in Table 1, causing false-positive results. It is noteworthy that in some studies light microscopy and cultivation are complemented by Polymerase chain reaction (PCR), which increases diagnostic accuracy and reliability. PCR can detect 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 high sensitivity and specificity and can be used for strain discrimination. Limitations of molecular techniques are: requiring advanced laboratory skills and specialized equipment, possibility of contamination and false positive results. We established that PCR is used in 15 studies. Some of the *Trichomonas tenax* genes such as the beta-tubulin gene, 18S rRNA gene, rpb1 gene have been analysed by PCR. [42,74,93]. Loop-mediated isothermal amplification (LAMP) is relatively new method, requires less run time and is more sensitive than PCR [94,95]. There is only one study in which LAMP was used to detect *Trichomonas tenax*, but from oral canine samples by specific detection of the ITS (internal transcribed spacers) and 5.8S rRNA gene [96]. We noticed that current gaps in immunological diagnosis exist.

Despite these results, there is an insufficient information on *Trichomonas tenax* distribution and need for future research about the prevalence of it. More epidemiological studies may be useful to understand prevalence and social differences in related populations. Future research may further 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. The methodology will help both in the diagnosis of already occurring disease and in screening individuals with dental calculus Because of its advantages, we may suggest the LAMP method as an efficient screening method in the future. Another key area for potential future research is the influence of the immune system on it and the potential development of immunological diagnostic methods. We may propose *Trichomonas tenax* to be included in the mandatory diagnostic panels for periodontitis. Its elimination will improve the prognosis of the gum disease in these patients. In this context, drug resistance studies may be important, as well as the influence of different toothpastes.

5. Conclusions

Interest in *Trichomonas tenax* has grown considerably since 2000. There are a number of studies that have identified it in oral specimens of patients with periodontal disease using various methods. Its role in the inflammatory process should not be overlooked, hence *Trichomonas tenax* should be discussed in the diagnosis and treatment of the patients with this disease. Its eradication in the oral cavity will prevent the risk of aspiration into the lungs and subsequent complications.

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