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Are There any Association Between *Entamoeba gingivalis* and *Trichomonas tenax* in Dental and Periodontal Diseases? A Systematic Review and Meta-analysis

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Abstract

There are frequent epidemiological and experimental proofs to propose that *Entamoeba gingivalis* and *Trichomonas tenax* infections are ignored factors for the development of dental and periodontal diseases. For a more comprehensive review, the present study was designed and conducted, including a systematic search in PubMed, Scopus, Web of Science, and Google Scholar databases for retrieving related reports up to December 01, 2019. Finally, 48 studies (a total of 70 datasets), including 30 datasets with case-control designs (1711 patients and 614 controls) and 40 datasets with cross-sectional designs (a total of 7257 participants) met the eligibility criteria. Based on the random-effects model, the pooled prevalence of protozoan infections in dental and periodontal patients was estimated to be 25.2% (95% CI, 21.2-29.8%; 2210/8968). In case-control designed studies, the prevalence of protozoan infections in dental and periodontal patients (35.8%, 614/1711) was found to be significantly higher than in controls (14.4%, 198/1371; OR: 4.15, 95% CI: 2.92-5.92). In addition, *E. gingivalis* (OR: 3.81, 95% CI: 2.36-6.14) and *T. tenax* (OR: 5.08, 95% CI: 2.81-9.20) were significantly higher in dental and periodontal patients compared to the controls. Our results demonstrated an apparently significant association between these protozoan infections in dental and periodontal patients. In conclusion, more standardized experimental designs are proposed using the animal model, and longitudinal (cohort) studies support the association between these protozoan infections in dental and periodontal patients to understand whether *E. gingivalis* and *T. tenax* infections are a co-factor in the development of dental and periodontal patients.

Keywords: *Entamoeba gingivalis*, *Trichomonas tenax*, Dental diseases, Periodontal diseases

Background

Oral diseases are the most prevalent non-communicable diseases that affect people throughout their lifetime, causing pain, discomfort, deformity, and even death.[1,2] Dental caries and periodontal diseases (PD) have historically been considered the most important oral health complications worldwide.[3,4] As estimated by the Global Burden of Disease Study (2016), oral diseases affected nearly half of all people around the world (3.58 billion people),[5] and dental caries and PD are recognized as the first and second most common dental diseases (DD) worldwide, respectively.[6] As such, chronic periodontitis has affected about 750 million people throughout the world since 2010.[7]

Several risk factors are involved in DD and PD, including smoking, diabetes, HIV/AIDS, stress, poor diet, and family history of DD and the like.[8-10] Alongside these risk factors, oral microorganisms have a potentially vital role in dental plaque formation and are consequently recognized as a major cause of DD and PD.[11,12]

Periodontal tissues are a suitable site for the colonization and growth of different microorganisms such as bacteria, viruses, fungi, and protozoa.[13] There is evidence of tissue damage and induce inflammation by oral infectious agents, especially parasites.[14,15] Normally, two key parasitic microorganisms can colonize in the oral cavity, including the flagellate *Trichomonas tenax* and the amoeba *Entamoeba gingivalis*.[13,16] Although these anaerobic protozoans have been detected in teeth tartar and subgingival dental plaque, their role in the

pathophysiology of oral tissue damage is obscure, and little information is available about their pathogenic role in DD and PD.[16]

There are numerous reports of the prevalence of *E. gingivalis* and *T. tenax* infections among DD and PD patients throughout the world; nonetheless, up to now, there is no comprehensive study with statistical analysis in this regard. Henceforward, due to the important association of DD and PD with the presence of *T. tenax* and *E gingivalis* in the oral cavity, we conducted a systematic review and meta-analysis in order to shed light on whether *E. gingivalis* and *T. tenax* play a role in the occurrence of the DD and PD.

Methods

Search Strategy

Our comprehensive study was followed in agreement with the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" guidelines.[17] To assess the pooled prevalence and odds ratio (OR) of *E. gingivalis* and *T. tenax* infections in the DD and PD subjects, we systematically searched Google Scholar as a search motor engine and major databases (i.e., Scopus, PubMed, and Web of Science) for appropriate records until December 01, 2019. The searching process was performed using several medical subject heading (MeSH) terms; they included ("*Entamoeba gingivalis*" OR "*Entamoeba buccalis*" OR "*Entamoeba maxillaris*" OR "*Amoeba pyogenes*" OR "*Trichomonas tenax*" OR "*Trichomonas buccalis*") AND ("prevalence" OR "epidemiology") AND ("periodontal disease" OR "periodontitis" OR "dental disease" OR "dental caries" OR "dental decay"). Further, the references of the eligible records were manually searched to retrieve associated studies not recovered through the initial screening.

Eligibility Criteria

In the present research, four criteria were considered for including the studies. They were original research with case-control and cross-sectional designs and full texts about the prevalence of *T. tenax* and *E. gingivalis* infections in DD and PD patients and short reports and/or abstracts having relevant data published in the English language. The other inclusion criteria included reports with information on the total sample size and positive samples and available published online data up to December 01, 2019. On the other hand, articles without the above-mentioned criteria, including non-human or animal studies, studies that only included healthy people or the general population, case reports, all types of reviews, editorials, and/or letters were excluded from the study.

Study Selection and Data Extraction

The initial screening was conducted via the title (topics)/abstracts of the records, and the records were achieved accordingly; then, duplicated papers were removed by EndNote software. Two authors (A. F. and A. T.) screened and retrieved records through study criteria consideration. Next, two other researchers (Z. H. and S. B.) extracted the items of the studies, including the author's name, research publication year and execution time period, study design, geographical zone (continent/country), diagnostic method, precipitants age (mean or range), total sample size, positive case number, and the detected protozoans types in every study. All data were imported into the Microsoft Excel Worksheet, and two researchers (K. S. and H. R.) double-checked them. All controversial issues were resolved by the corresponding author's consensus (S. B).

Statistical Analysis and Data Synthesis

All statistical analyses were directed via comprehensive meta-analysis statistical software (Version 2.2, BioStat). The prevalence of *E. gingivalis* and *T. tenax* in DD and PD patients was estimated by using the random effects model. Moreover, for the possible association assessment, the OR with a 95% confidence interval (CI) was calculated for case-control studies. The I^2 method was applied to the heterogeneity assessment of the studies. Likewise, the small sample size effects and publication bias of the studies were determined by Egger's regression test; the probable publication bias was presented as the funnel plot. In all statistical analyses, $P < 0.05$ was considered statistically significant. The pooled prevalence of *E. gingivalis* and *T. tenax* in people with DD and PD was displayed as the forest plot, and the difference in the prevalence rate of *E. gingivalis* and *T. tenax* in DD and PD individuals and controls was presented by an OR and 95% CI. Finally, gender prevalence rates in DD and PD patients were compared using the OR.

Results

Study Characteristics

The selection steps of the present systematic review and meta-analysis studies are presented in the flow diagram (Figure 1). Briefly, 1242 papers were recovered in the initial search step; eventually, 48 records containing 70 datasets meeting the inclusion criteria remained for analysis after removing irrelevant and duplicated studies.[18-65] The included records were published between March 1970 and September 2019. The 48 studies were performed in 20 diverse geographical zones in four continents (12, 6, 6, 6, 5, 5, 4, 4, 4, 3, 3, 2, 2, 2, 1, 1, 1, 1, 1, and 1 datasets in Iran, Iraq, Turkey, Egypt, the USA, Poland, France, Nigeria, Venezuela, Mexico, China, Libya, Brazil, Pakistan, Romania, Sweden, Chile, Japan, Saudi Arabia, and India, respectively). Of the 70 datasets, 33, 36, and 1 datasets surveyed *E. gingivalis*, *T. tenax,* and mixed protozoans in DD and PD patients, respectively. As

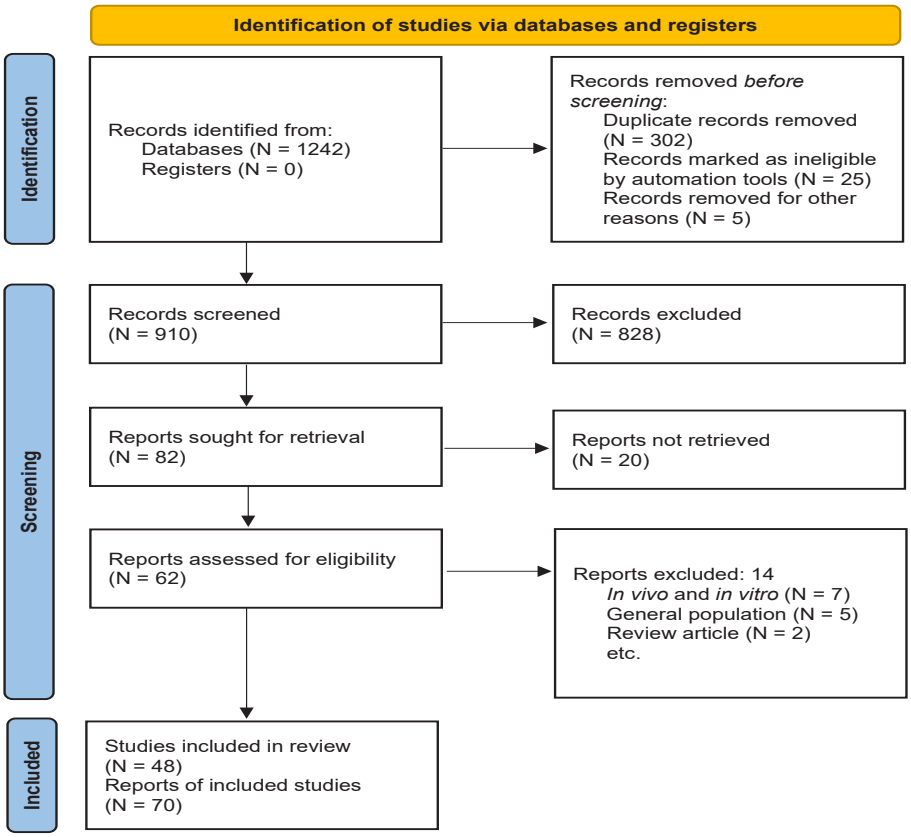

Figure 1. Flow Diagram of the Study Design Process

demonstrated in Tables 1 and 2, alongside the microscopic examination and culture, PCR had been used for the detection of *E. gingivalis* and *T. tenax* infections in 15.7% (11/70) of the studies. Regarding the type of study design, 30 and 40 studies were of case-control and cross-sectional type, respectively. The main characteristics of the case-control and cross-sectional studies are summarized in Tables 1 and 2, respectively.

The E. gingivalis and T. tenax Overall Prevalence in People With DD and PD

The pooled prevalence for both protozoan infections was 25.2% (95% CI: 21.2-29.8%) so that the prevalence of *E. gingivalis* and *T. tenax* among DD and PD patients was estimated at 36.1% (95% CI: 29.4-43.3%) and 17.4% (95% CI: 13.7-21.8%), respectively (Figures S1-S3, Supplementary file 1). In the subgroup analysis by continent, the highest pooled prevalence was observed in America at 30.3% (95% CI: 21.8-40.3%), followed by Europe at 29.1% (95% CI: 20.1-40.2%), Africa at 22.7% (95% CI: 15.1-32.6%), and Asia at 20.5% (95% CI: 14.5-28.1%), the details of which are provided in Table 3 and Figure S1.

The Pooled OR of Entamoeba gingivalis and Trichomonas tenax Based on Case-control Studies

The meta-analysis outcomes demonstrated a significant positive association between exposure to both protozoan infections (*E. gingivalis* and *T. tenax)* and DD and PD (OR: 4.15; 95% CI: 2.92-5.92%, Figure 2). Subgroup analysis considering the causative protozoan revealed a significantly higher prevalence of *E. gingivalis* (OR: 3.81; 95% CI: 2.36-6.14%) and *T. tenax* (OR: 5.08; 95% CI: 2.81-9.20%) in DD and PD patients compared to healthy controls (Figures S4 and S5).

In a subgroup analysis by gender in DD and PD patients, the prevalence in males was higher than in females (30.1% *vs.* 28.6%), but no significant association was found between males and females (OR: 1.15; 95% CI, 0.85-1.57%, Figure S6).

Publication Bias Results

According to Egger's regression test, a significant publication bias ($t=4.78$, $P=0.00$) was observed in the case-control studies, whereas publication bias was not significant in the cross-sectional studies ($t=1.73$, $P=0.09$). Figure 3 (funnel plot) shows the potential publication bias identification.

Discussion

Over the last century, it was postulated that only bacteria were microorganisms that contributed to formation of plaque and dental calculus. On the other hand, although

Table 1. Summary of Studies With Case-Control Design Investigating the Prevalence of *E. gingivalis* and *T. tenax* in DD and PD Patients and Control Subjects

First Author/Year	Country	Type of Parasite	Methods	Age Range or Mean Age* (y)	Type of Disease	Patients				Control Subjects			
						N	*T. tenax*	*E. gingivalis*	Mix	N	*T. tenax*	*E. gingivalis*	Mix
Dao et al (1983) [24]	USA	*E. gingivalis*	Microscopic; papanicolaou method	NR	Dental patients	113	_	67	_	96	_	31	_
El Azzouni and el Badry (1994) [60]	Egypt	*E. gingivalis*	Microscopic examination	25-40 years	Periodontitis	50		32		50		17	
Kikuta et al (1996) [47]	Japan	*T. tenax*	PCR	NR	Dental patients	9	5			10	0		
Lucht et al (1998) [19]	Sweden	*E. gingivalis*	Microscopic; trichrome stain	35- 38 years	Periodontal disease	13	_	10	_	2	_	0	_
Athari et al (2007) [33]	Iran	*T. tenax*	PCR (18S rRNA gene)	NR	Gingivitis/periodontitis	160	33	_	_	160	2	_	_
El-Sayed and Meabed (2008) [62]	Egypt	*T. tenax*	Microscopic Examination	20-50 years	Periodontitis	50	10			25	0		
El-Sayed and Meabed (2008) [62]	Egypt	*T. tenax*	PCR	20-50 years	Periodontitis	50	14			25	0		
El-Sayed and Meabed (2008) [62]	Egypt	*T. tenax*	Culture	20- 50 years	Periodontitis	50	13			25	0		
Acurero Osorio et al (2009) [38]	Venazuela	*E. gingivalis*	Microscopic examination; ferric hematoxylin stain	20-39 years	Dental/periodontal patients	25		5		25		0	
Acurero Osorio et al (2009) [38]	Venazuela	*T. tenax*	Microscopic examination; ferric hematoxylin stain	20-39 years	Dental/periodontal patients	25	1			25	0		
Ghabanchi et al (2010) [31]	Iran	*E. gingivalis*	Microscopic; Giemsa-stained	10-60 years	Periodontal disease	50	_	6	_	50		1	_
Ghabanchi et al (2010) [31]	Iran	*T. tenax*	Microscopic; Giemsa-stained	10-60 years	Periodontal disease	50	3	_	_	50	0	_	_
Abualqomsaan et al (2010) [32]	Turkey	Mix infection	Microscopic; Giemsa and trichrome-stain	NR	Periodontal disease	33	_	_	7	13	_	_	1
Onyido et al (2011) [63]	Nigeria	*E. gingivalis*	Microscopic examination; Eosin staining	NR	Dental patients	60		19		60		2	
Onyido et al (2011) [63]	Nigeria	*T. tenax*	Microscopic examination; Eosin staining	NR	Dental patients	60	21			60	0		
Norberg et al (2014) [39]	Brazil	*T. tenax*	Microscopic examination	NR	Periodontal disease	50	28			50	23		
Kashefi Mehr et al (2015) [25]	Iran	*T. tenax*	PCR (18S rRNA gene)	5-12 years	Periodontal disease	52	14	_	_	52	5	_	_
Luszczak et al (2016) [27]	Poland	*E. gingivalis*	Microscopic; safranin stained	18-81 years	Periodontal disease	102	_	83	_	32	_	20	_
Zawadzki et al (2016) [20]	Poland	*E. gingivalis*	Microscopic; Giemsa and trichrome-stain	28-40 and 41-53	Masticatory system disorders	48	_	10	_	48	_	4	_
Zawadzki et al (2016) [20]	Poland	*T. tenax*	Microscopic; Giemsa and trichrome-stain	28-40 and 41-53	Masticatory system disorders	48	22	_	_	48	7	_	_
El-Dardiry and Shabaan (2016) [64]	Egypt	*E. gingivalis*	Microscopic Examination; Iron & H stain	35-55	Gingivitis	80		23		80		15	
Garcia et al (2018)[40]	Mexico	*E. gingivalis*	PCR	16-80 (M=49.8)	Periodontal disease	102		40		105		32	
Ramamurthy et al (2018) [58]	India	*E. gingivalis*	Wet-smear	NR	Gingivitis/periodontitis	75		41		25		1	
Younis and Elamami (2019) [65]	Libya	*E. gingivalis*	Wet-smear	18-60 years	Periodontal disease	70		13		30		4	
Younis and Elamami (2019) [65]	Libya	*E. gingivalis*	Wet-smear	18-60 years	Periodontal disease	70	6			30	1		
Rahdar et al (2019) [59]	Iran	*E. gingivalis*	Microscopic examination	49.16	Periodontitis	25		3		25		0	
Rahdar et al (2019) [59]	Iran	*E. gingivalis*	PCR	49.16	Periodontitis	25		4		25		2	
Benabdelkader et al (2019) [28]	France	*T. tenax*	Real-time PCR	Adults	Periodontitis	106	45	_	_	85	24	_	_
Dubar et al (2019) [34]	France	*T. tenax*	PCR	38-65 (M=51)	Periodontitis	30	10	_	_	30	2	_	_
Dubar et al (2019) [34]	France	*E. gingivalis*	PCR	38-65 (M=51)	Periodontitis	30	_	26	_	30	_	4	_

Note. *E. gingivalis*: *Entamoeba gingivalis*; *T. tenax: Trichomonas tenax*; DD: Dental disease; PD: Periodontal diseases; PCR: Polymerase chain reaction.

Table 2. Summary of Studies With Cross-sectional Design Investigating the Prevalence of *E. gingivalis* and *T. tenax* in DD and PD Patients

Author, Year	Country	Type of Parasite	Methods	Age Range or Mean Age* (y)	Type of Disease	Total Patients	*T. tenax*	*E. gingivalis*
Wantland and Lauer (1970) [30]	USA	*E. gingivalis*	Wet-smear counts and culture	2-80 years	Dental patients	1036	_	302
Wantland and Lauer (1970) [30]	USA	*T. tenax*	Wet-smear counts and culture	2- 80 years	Dental patients	1036	199	_
Gottlieb and Miller (1971) [18]	USA	*E. gingivalis*	Microscopic; trichrome stain	NR	Periodontal disease	30	_	16
Linke et al (1989) [26]	USA	*E. gingivalis*	Wet-smear	20-68 years	Periodontal disease	10	_	10
Sefer et al (1989) [35]	Romania	*E. gingivalis*	Microscopic; Giemsa-stained smears	NR	Stomatological affections	78	_	18
Yali et al (1990) [46]	China	*E. gingivalis*	Microscopic examination	NR	Dental patients	100		76
Yali et al (1990) [46]	China	*T. tenax*	Microscopic examination	NR	Dental patients	100	6	
Favoreto and Machado (1995) [36]	Brazil	*E. gingivalis*	Microscopic examination; modified Papanicolaou staining	NR	Dental patients	100		62
Çeliksöz et al (2001) [42]	Turkey	*T. tenax*	Microscopic examination	NR	Dental patients	41	1	
Kurnatowska et al (2004) [43]	Poland	*T. tenax*	Microscopic examination	23-79 years	Periodontitis	91	34	
Ozumba et al (2004) [61]	Nigeria	*E. gingivalis*	Microscopic examination	NR	Dental patients	203		23
Ozumba et al (2004) [61]	Nigeria	*T. tenax*	Microscopic examination	NR	Dental patients	203	10	
Abdullatif (2005) [48]	Iraq	*E. gingivalis*	Microscopic; Heidenhair hematoxylin modified stain	38-60 years	Periodontitis	48		16
Gharavi et al (2006) [49]	Iran	*E. gingivalis*	Microscopic; Giemsa-stained smears	NR	Dental patients	120		50
Gharavi et al (2006) [49]	Iran	*T. tenax*	Microscopic; Giemsa-stained smears	NR	Dental patients	120	11	
Ullah et al (2007) [50]	Pakistan	*E. gingivalis*	Microscopic examination	NR	Dental patients	150		85
Ullah et al (2007) [50]	Pakistan	*T. tenax*	Microscopic examination	NR	Dental patients	150	5	
Dudko and Kurnatowska (2007) [44]	Poland	*T. tenax*	Microscopic examination	23-80 years	Periodontal disease	189	58	
Cuevas et al (2008) [37]	Mexico	*E. gingivalis*	Microscopic; trichrome stain	3-14 years	Periodontal disease	105		9
Cuevas et al (2008) [37]	Mexico	*T. tenax*	Microscopic; trichrome stain	3-14 years	Periodontal disease	105	13	
Yang et al (2009) [53]	China	*T. tenax*	Microscopic examination	NR	Dental patients	492	46	
Özçelik et al (2010) [29]	Turkey	*E. gingivalis*	Tech Lab Entamoeba Kit and Robinson Medium	12-75 years	Periodontal disease	220	_	56
Özçelik et al (2010) [29]	Turkey	*T. tenax*	Tech Lab Entamoeba Kit and Robinson Medium	12-75 years	Periodontal disease	220	10	_
Al-hamiary et al (2011) [51]	Iraq	*E. gingivalis*	Microscopic; Giemsa-stained smears	18-60 years	gingivitis/periodontitis	310		133
Al-hamiary et al (2011) [51]	Iraq	*T. tenax*	Microscopic; Giemsa-stained smears	18-60 years	gingivitis/periodontitis	310	64	
El Sibaei et al (2012) [21]	Egypt	*T. tenax*	Microscopic; Eosin, iodine and methylene blue stains	11-57 years	gingivitis/periodontitis	70	20	_
Maraghi et al (2013) [52]	Iran	*E. gingivalis*	Microscopic; Giemsa-stained smears	20-60 years	gingivitis/periodontitis	200		1
Maraghi et al (2013) [52]	Iran	*E. gingivalis*	Microscopic; Giemsa-stained smears	20-60 years	gingivitis/periodontitis	200	0	
Jabuk et al (2015) [54]	Iraq	*E. gingivalis*	Microscopic examination and culture	NR	Periodontal disease	100		53
Jabuk et al (2015) [54]	Iraq	*T. tenax*	Microscopic examination and culture	NR	Periodontal disease	100	27	
Yazar et al (2016) [45]	Turkey	*E. gingivalis*	Microscopic examination and culture	19-69 years	gingivitis/periodontitis	175		60
Yazar et al (2016) [45]	Turkey	*T. tenax*	Microscopic examination and culture	19-69 years	gingivitis/periodontitis	175	50	
Ismail et al (2017) [55]	Saudi Arabia	*T. tenax*	Microscopic examination	NR	gingivitis/periodontitis	58	7	
Derikvand et al (2018) [56]	Iran	*E. gingivalis*	Microscopic; Giemsa-stained smears	13-65 (M=39.2)	Periodontitis	76		13
Derikvand et al (2018) [56]	Iran	*T. tenax*	Microscopic; Giemsa-stained smears	13-65 (M=39.2)	Periodontitis	76	11	
Abdulhaleem et al (2018) [57]	Iraq	*T. tenax*	PCR	15-62 years	Dental patients	160	40	
Bisson et al (2018) [23]	France	*T. tenax*	Phase contrast microscopy	12.4-51.2 years	gingivitis/periodontitis	50	10	_
Traviezo Valles et al (2019) [41]	Venezuela	*E. gingivalis*	Microscopic examination	18-68 years	Dental patients	100		20
Traviezo Valles et al (2019) [41]	Venezuela	*E. gingivalis*	Microscopic examination	18-68 years	Dental patients	100	4	
Bracamonte-Wolf et al (2019) [22]	Chile	*T. tenax*	PCR amplification of the beta-tubulin gene	20-80 years	gingivitis/periodontitis	50	28	_

Note. E. gingivalis: *Entamoeba gingivalis; T. tenax: Trichomonas tenax;* DD: Dental disease; PD: Periodontal diseases; PCR: Polymerase chain reaction.

the main structure of these plaques is composed of a wide range of bacteria, some fungal, *Mycoplasma*, and protozoan species also have a neglected role in plaque formation and dental calculus. Recently, several epidemiological investigations have been performed on the prevalence of *E. gingivalis* and *T. tenax* infections in people with DD and PD in some countries; their presence in dental disorders led to the theory that these protozoans have a possible role in causing DD and PD. To fill this gap, the current systematic review and meta-analysis focused on the observational studies that evaluated the prevalence and association between *E. gingivalis* and *T. tenax* infections in subjects suffering from DD and PD. In this meta-analysis, it was found that the prevalence of *E. gingivalis* and *T. tenax* infections in DD and PD patients was significantly increased compared to the control group.

To date, the exact mechanism of the pathogenesis of these two protozoans in DD and PD has not been fully understood and is still under debate. Some virulence factors related to *T. tenax* have been partially discovered in recent papers. As it is evident, this flagellate has been documented that has the ability to secrete some proteolytic enzymes (e.g., metalloproteinase and cysteine proteinase), thereby leading to degrading collagen.[21] Moreover, no study has so far described the potential ability of this

Table 3. Sub-group Analysis of the Prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* Based on Continents

Continent	Number of Studies	Sample Size	Infected	Pooled Prevalence (95% CI)
Asia	26	3316	753	20.5% (14.5-28.1%)
Europe	17	1649	449	29.1% (20.1-40.2%)
America	15	2987	804	30.3% (21.8-40.3%)
Africa	12	1016	204	22.7% (15.1-32.6%)
Overall	70	8968	2210	25.2% (21.2-29.8%)

Note. CI: Confidence interval.

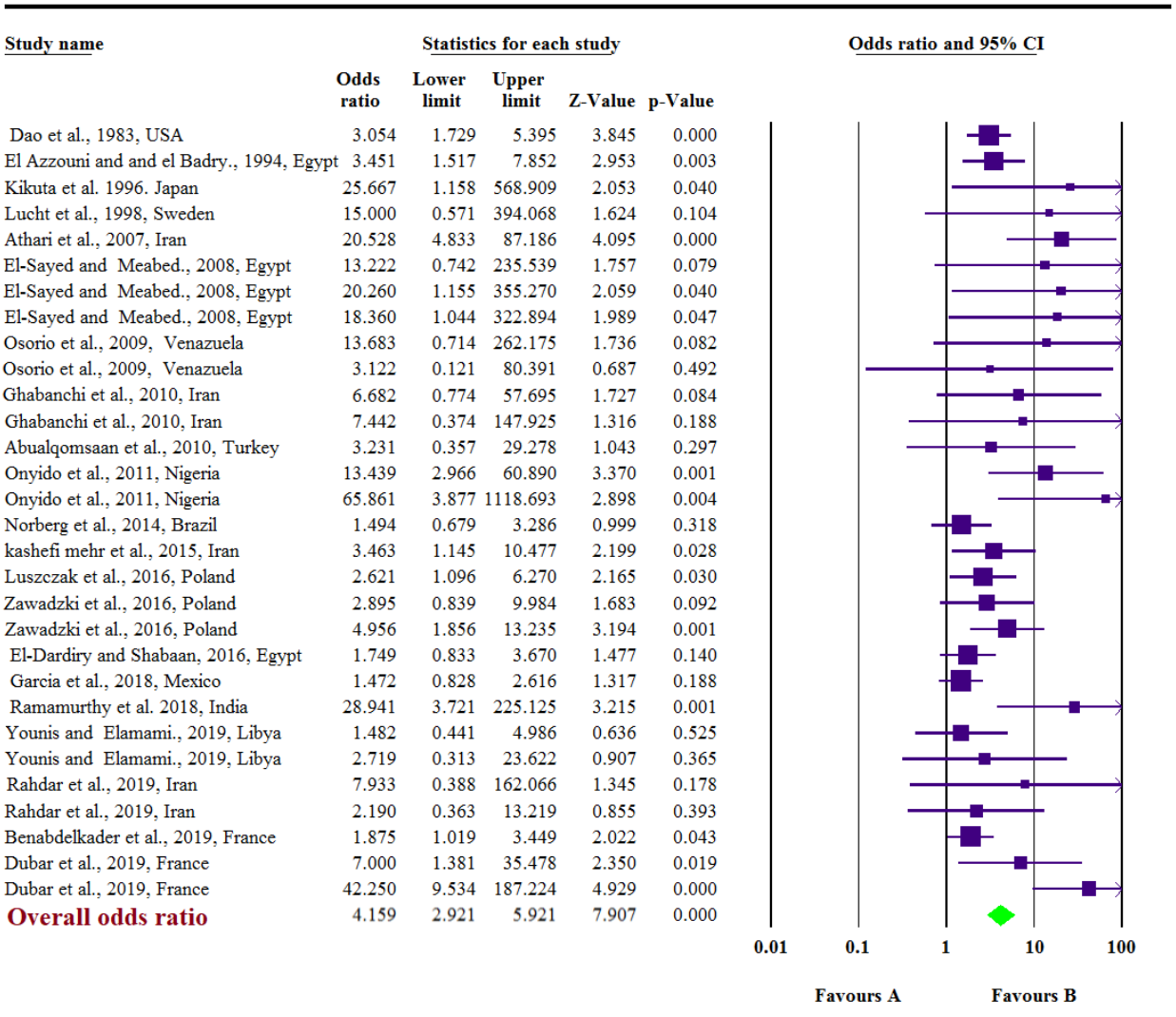

Figure 2. Forest Plot of Odds Ratios in terms of Prevalence of Protozoan (*E. gingivalis* and *T. tenax*) Infections in the DD and PD Patients in Case-control Studies. *Note. E. gingivalis*: *Entamoeba gingivalis*; *T. tenax: Trichomonas tenax*; DD: Dental disease; PD: Periodontal diseases

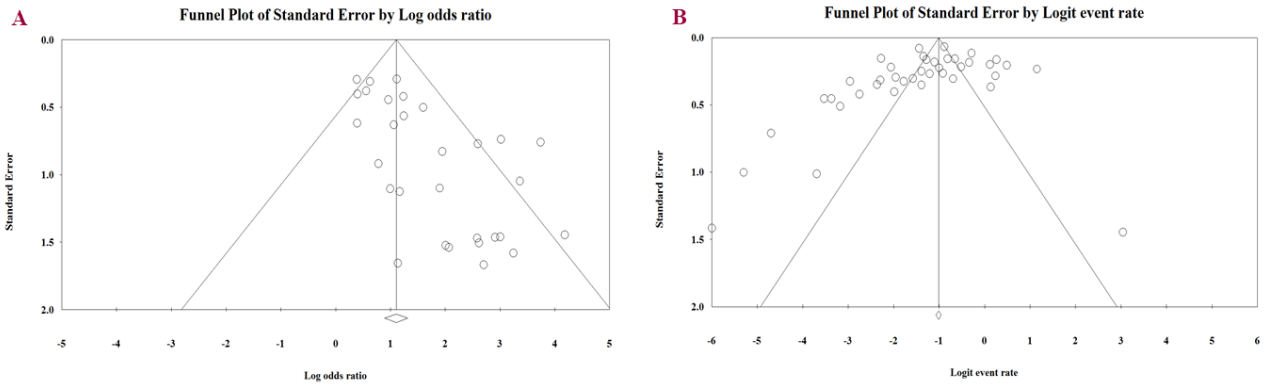

Figure 3. Publication Bias Using Funnel Plots: (A) Publication Bias in Studies With Case-control Design and (B) Publication Bias in Studies With a Cross-sectional Design

parasite in adhering to different eukaryotic cells through a wide range of iron-dependent surface adhesions, resulting in the cytotoxic effect by producing apoptotic bodies through the release of cysteine proteases.[66] These virulence factors can be indirectly involved in DD and PD so that conditions for the growth of periodontopathogenic such as anaerobic bacteria *Tannerella forsythia, Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis,* and *Fusobacterium nucleatum* have been prepared by inducing damage of the tooth-supporting tissue.[15,67] All the above-mentioned factors may support the possible impress of the mentioned protozoan in the initiation and progression of DD and PD.

The information on the pathogenicity of *E. gingivalis* is vaguer than *T. tenax*. In this regard, the genetic variation in *E. gingivalis* species appears to be important, and the genetic distance between the ST1 and ST2-kamaktli variants may indicate many differences in their biology and the possible association of these subtypes with various pathologies.[40,68] Thus, studies have demonstrated that the ST2 "kamaktli" variant is more genetically diverse than *E. gingivalis* ST1, which may indicate the hypothesis that strains cannot have the same pathogenicity and tropism.[68,69] Given that most epidemiological studies on this subject were performed by microscopy and culture methods, there is little information on the molecular epidemiology of this protozoan, thus it is recommended that molecular studies be conducted to gain a deeper understanding of this issue.

In this comprehensive study, we faced limitations, including differences in the sensitivity and specificity of techniques used in analyzed studies for parasite identification that the estimated prevalence can be affected by these differences, as well as limited reports from some geographical regions of the world about the prevalence of *E. gingivalis* and *T. tenax* among DD and PD patients. The other limitation were small sample sizes in some included studies and no further information about precipitants' complications condition, as well as differences in the number and type of variables and the quality of the analyzed studies.

In conclusion, the present study presented a relatively more comprehensive picture of the prevalence of *E. gingivalis* and *T. tenax* in subjects with DD and PD. The findings of this meta-analysis indicated that patients suffering from PD and DD were significantly more likely to be positive for *E. gingivalis* and *T. tenax* than healthy controls, suggesting that there is a significant association between these protozoans in the oral cavity and the occurrence of DD and PD.

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Supplementary Files
Supplementary file 1 contains Figures S1-S6.

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