

REVIEW

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A systematic review and meta-analysis on the global prevalence of microsporidia infection among dogs: a zoonotic concern

Ali Taghipour^{*†}, Saeed Bahadory[†] and Sasan Khazaei[†]

Abstract

Background: Microsporidiosis is an emerging zoonotic disease that is considered a global public health concern. Dogs are suggested as one of potential reservoirs for transmitting the microsporidia infection to humans. However, there is little data on distribution of microsporidia in dogs. The current study aimed to evaluate the global prevalence and genetic diversity of microsporidia infection among the dog population.

Methods: We searched four major databases for studies reporting the prevalence of microsporidia in dogs until 30 May 2020. A random-effects model was used to estimate the overall and the subgroup-pooled prevalence of microsporidia across studies.

Result: Finally, a total of 32 studies (including 37 datasets) from 17 countries were included in this meta-analysis. The overall prevalence (95% CI) of microsporidia infection was estimated at 23.1% (13.5–36.8%) using microscopic methods, 20.9% (14.6–29%) using serological methods, and 8.4% (6.1–11.5%) using molecular methods. Molecular methods showed that the highest number of reports was related to *Enterocytozoon bienersi* with a pooled prevalence of 6.5% (4.9–8.7%). Considering *E. bienersi* genotypes, most studies reported the PtEb IX (10 studies) and the D (eight studies) genotypes.

Conclusion: These results emphasize the role of a dog as a reservoir host for human-infecting microsporidia. In addition, monitoring programs for human-infecting microsporidia in animals with close contact to humans should be considered.

Keywords: Microsporidia infection, Dogs, Prevalence, Meta-analysis

Background

Microsporidiosis is an emerging disease caused by opportunistic unicellular organisms called microsporidia comprising more than 200 genera with approximately 1500 species. Microsporidia can infect a broad range of vertebrate and invertebrate hosts [1–3]. Epidemiological studies over the world showed that *Enterocytozoon bienersi* (*E. bienersi*) and *Encephalitozoon* species (*Enc. cuniculi*, *Enc. intestinalis*, and *Enc. hellem*) are the most

common species of microsporidia reported from humans and a wide range of animals [4, 5]. Microsporidia infection in immunocompetent individuals is mostly limited to mild or self-limiting diarrhea. This infection maybe reported from intestinal, pulmonary, ocular, muscular, and renal diseases in immunocompromised patients [6–9]. The main transmission routes of microsporidia seem to be fecal-oral transmission of spores from infected humans and animals through contaminated food and water together with inhalation of spores [2, 10, 11]. Human-infecting microsporidia have been frequently reported from domesticated animals, wild and laboratory animals, and birds highlighting the high probability of zoonotic transmission [12, 13].

* Correspondence: alitaghpor71@yahoo.com

[†]Ali Taghipour, Saeed Bahadory and Sasan Khazaei contributed equally to this work.

Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, P.O. Box 14115-111, Tehran, Iran



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From past to present, dogs play an important role in the psychological and physiological well-being of humans, especially children and elderly individuals [14, 15]. Nevertheless, dogs pose a potential health risk to human society, so that they can transmit more than 100 infectious zoonotic diseases to humans [16–19]. In relation to the One-Health approach, the health of a community is connected to the health of animals and the environment [20, 21]. Therefore, dogs play a major role in the transmission dynamics of microsporidia spores due to their wide niche and close contact to humans [12, 22, 23].

In recent years, a growing number of studies have been conducted on the prevalence of microsporidia infection among dogs, although there is a big gap in our knowledge in many countries. In this review, we described the global prevalence and genetic diversity of microsporidia infection in dogs.

Materials and methods

Systematic search strategy

In this review, the protocols recommended by the “preferred reporting items for systematic reviews and meta-analyses statement (PRISMA)” were followed for all eligible studies [24]. To assess the prevalence of microsporidia infection in dogs from a global scale, we performed a comprehensive systematic review and meta-analysis of literature (full text or abstracts) published online. Four major databases (PubMed, Web of Science, Scopus, and Google Scholar) were searched by two expert reviewers (S.B. and S.K.) for relevant studies published until 30 May 2020. Searching process was accomplished using MeSH terms alone or in combination: (“Microsporidiosis” OR “Microsporidia” OR “Microsporidium” OR “Microspora” OR “Nosema” OR “*Enterocytozoon bienersi*” OR “*Encephalitozoon spp.*”) AND (“Prevalence” OR “Epidemiology”) AND (“Dog” OR “Canine”). Additionally, the references of all selected articles were hand-searched to finding other relevant articles or their citations by searching in Google Scholar.

Eligibility criteria, study selection, and data extraction

Eligible articles were screened initially by title and abstract. All selected articles were imported into the End-Note X8 software (Thomson Reuters, New York, USA), and duplicated articles were checked and then removed. After removing duplicates, the full text of qualified records was retrieved and two expert investigators (S.B. and S.K.) evaluated the eligibility of the articles. Afterward, an investigator (A.T.) extracted the required information, and two others (S.B. and S.K.) rechecked them; any discrepancies of opinion or disagreement were resolved by consensus and discussion with the lead investigator (A.T.). In this systematic review, a strict protocol for the inclusion of papers was defined by our research

team. So, we included articles that they had all of the following inclusion criteria: (1) peer-reviewed original research articles or short reports or letters to the editors that studied the prevalence of microsporidia in dogs; (2) the papers with full-text or abstract in English without geographical restriction; (3) papers published online from the inception up to 30 May 2020 with a digital object identifier (DOI); (4) the investigations which examined microsporidia infection via at least one of the following methods, including molecular, serology, microscopy, or coproantigen, to detect the microorganism in feces and/or serum; and (5) those papers that provided the exact total sample size and positive samples. The papers were excluded if they did not meet the abovementioned criteria. Besides, we excluded articles from further considerations with the following characteristics: (1) the articles that reported microsporidia infection from humans and animals (except dogs); (2) specimens collected from the urine and tissues of dogs; (3) investigations of dogs which had been experimentally infected for other purposes, but not prevalent; (4) immunocompromised animals; and (5) all types of review papers, case reports, and case series.

The following variables were extracted for each study: first author’s last name, year of publication, country, types of samples tested (fecal and/or serum), diagnostic method, type of animals (pet or stray), the number of studied animals, and the number of animals with a positive test result. In addition, if possible, the prevalence was stratified by age (≤ 12 vs > 12 months of age) and gender of animals. Concerning molecular methods, the identified species/genotypes were also extracted. Since there were several types of dogs in the studies included, we divided dogs into (1) pet (domesticated) animals: “pet, household, sheltered or domesticated”; (2) stray animals: “stray, free-roaming or feral”; (3) unknown animals: “It is not clear whether the dogs are domesticated or stray.”

Study quality assessment

The Joanna Briggs Institute (JBI) checklist was used for quality assessment of the included articles [25]. This checklist contains ten questions with four options including, yes, no, unclear, and not applicable. Briefly, a study can be awarded a maximum of one star for each numbered item. The papers with a total score of 4–6 and 7–10 points were specified as the moderate and high quality, respectively. Based on the obtained score, the authors have decided to include (4–10 points) and exclude (≤ 3 points) the papers.

Data synthesis and statistical analysis

Comprehensive Meta-Analysis (V2.2, Bio stat) software was used to perform analysis. To minimize the biases,

we calculated the prevalence of microsporidia infection (the pooled prevalence and 95% confidence interval (CI)) for individual diagnostic methods (molecular, serology, microscopy methods) using the random-effects model (REM). The REM allows for a distribution of true effect sizes between published studies. Moreover, using subgroup analyses, the pooled prevalence of microsporidia infection was estimated according to the continents, countries, and type of animals. An odds ratio (OR) (and the corresponding 95% CI) was calculated using REM for each study to assess the association between microsporidia prevalence and risk factors such as age (animals aged 12 months or less and those more than 12 months) and gender (males and females). To calculate the heterogeneity between studies, t^2 statistic and the Q statistic were used. Publication bias was not estimated, because it is considered irrelevant for prevalence studies [26]. Moreover, due to different sensitivities and specificities of diagnostic methods, we assumed that our results would be “apparent” prevalence rates and did not represent true prevalence rates. Results are shown as forest plots of the pooled prevalence (with 95% CI) of microsporidia infection in dogs.

were found following the initial search of databases; after removing duplicates and/or non-eligible papers, 32 papers had eligibility to be included in this systematic review and meta-analysis [13, 23, 27–56]. Among these 32 studies, five studies had two datasets. In this regard, 32 studies containing 37 datasets were reviewed. The results of quality assessment according to JBI for eligible studies are mentioned in Tables 1, 3, and 4. The included articles in the present meta-analysis showed an acceptable quality. Out of the retrieved articles, 23, nine, and five datasets used molecular, serological, and microscopical methods, respectively. It should be noted that two studies used microscopic and coproantigen test for detection of microsporidia at the same time (the main characteristics of each study together with the types of implemented method are listed in Tables 1, 3 and 4, respectively). The results of the literature review and meta-analysis was separately considered for each of the three detection methods. Due to the low number of included studies in serological and microscopic methods, the subgroup analysis of continents and countries was performed only for molecular methods.

Results

Study characteristics

A flow diagram summarizing the study selection process is presented in Fig. 1. In brief, a total of 1431 records

The results based on molecular methods

As shown in Table 1, a total of 23 datasets from 12 countries have examined the prevalence of microsporidia species in dogs using molecular methods including 11

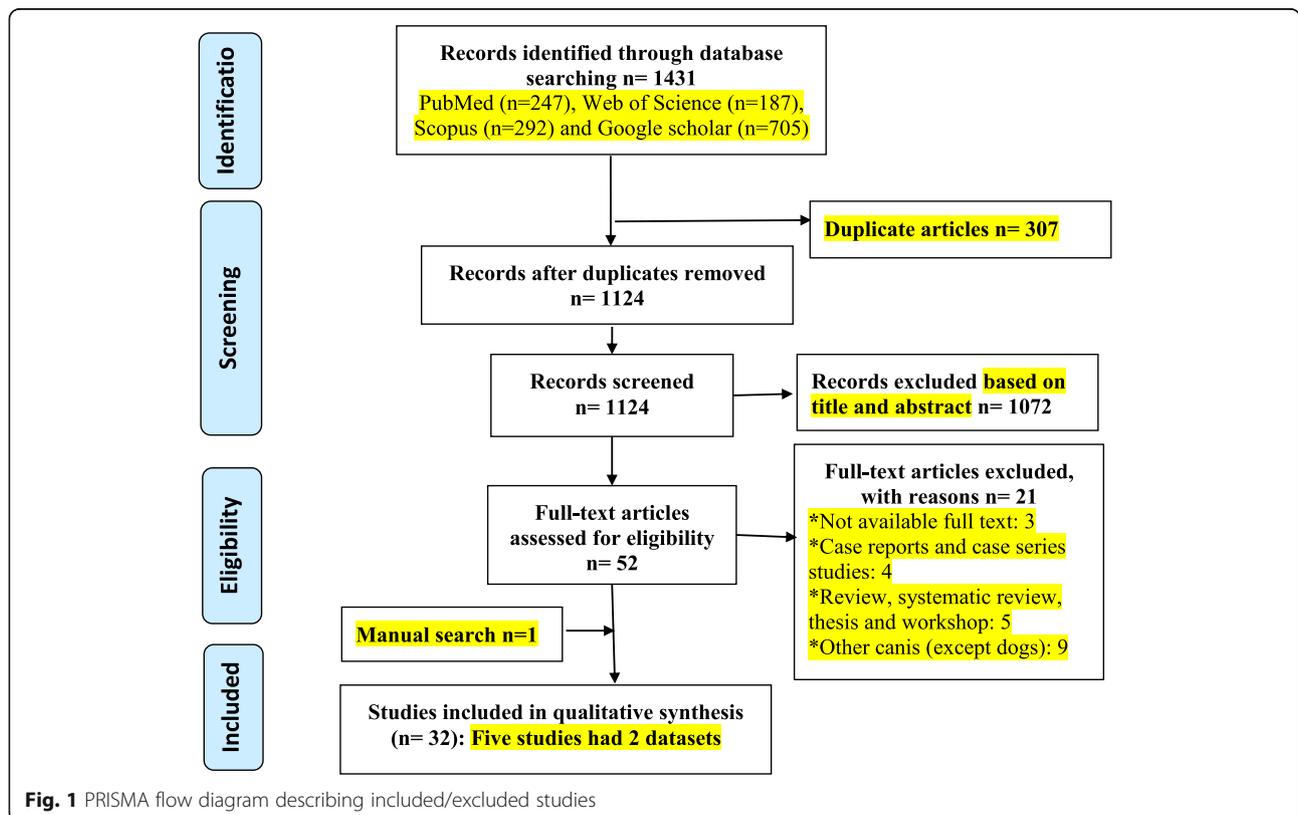


Table 1 All the studies investigating the global prevalence and genetic diversity of microsporidia infection in dogs according to molecular methods

Continent, country	First author	Publication Year	Type of sample	Diagnostic methods	Genes	Sample size	Total infected	<i>E. bienersi</i> (Genotypes)	<i>Enc. cucullii</i>	<i>Enc. intestinalis</i>	Quality assessment
Asia											
China	Zhang et al.	2011	Fecal	PCR	ITS	26	2	2 (CHN5(1)-CHN6(1))	-	-	8
	Karim et al.	2014	Fecal	Nested-PCR	ITS	348	54	54 (PtEb IX(26)-O(4)-EbpA(2)-pigEBITS5(1)-D(3)-CD1(1)-CD2(1)-CD3(1)CD4(1)-CD5(1)-CD6(1)-CD7(2)-CD8(4)-CD9(1)-CM1(2)-Peru8(1)-EbpC(1)-type IX(1))	-	-	10
Iran	Li et al.	2015	Fecal	Nested-PCR	SSU-rRNA and ITS	267	18	18 (EbpC/NED1(1)-EbpC(1)-NED2(1)-PtEb IX(14)-D(1))	-	-	10
	Xu et al.	2016	Fecal	Nested-PCR	ITS	485	29	29 (PtEb IX(28)-D(1))	-	-	10
	Wen-Chao et al.	2018	Fecal	Nested-PCR	ITS	315	27	27 (PtEb IX(16)-CHD1(2)-EbpC(4)-CHD2(2)-CHD3(3))	-	-	10
	Jamshidi et al.	2012	Fecal	PCR	SSU-rRNA	100	31	8 (unknown)	18	5	10
Japan	Askari et al.	2015	Fecal	Nested-PCR	SSU-rRNA	17	2	2 (unknown)	-	-	7
	Delrobaei et al.	2019	Fecal	Nested-PCR	SSU-rRNA and ITS	75	4	4 (D genotype)	-	-	9
	Abe et al.	2009	Fecal	Nested-PCR	ITS	79	2	2 (dog-specific genotype)	-	-	7
	Phrompraphai et al.	2019	Fecal	Nested-PCR	ITS	597	26	26 (PtEb IX)	-	-	10
Thailand	Mori et al.	2013	Fecal	Nested-PCR	ITS	206	0	0	-	-	8
Europe											
Spain	Lores et al.	2002	Fecal	PCR	SSU-rRNA	17	2	2 (unknown)	-	-	7
	^a Galvan-Diaz et al.	2014	Fecal	PCR	SSU-rRNA and ITS	73	14	7 (A genotype)	-	-	8
Portugal	Dashti et al.	2019	Fecal	Nested-PCR	ITS	237	2	2 (BEB6 and PtEb IX genotypes)	-	-	10
	^a Lobo et al.	2003	Fecal	PCR	SSU-rRNA	36	3	3 (Unknown)	-	-	7
	^a Matos et al.	2004	Fecal	PCR	SSU-rRNA	58	3	3 (Unknown)	-	-	7
Poland	Piekarska et al.	2016	Fecal	Nested-PCR	ITS	65	4	2 (PtEb IX(1)-D(1))	2	-	7
	Piekarska et al.	2017	Fecal	Nested-PCR	ITS	82	6	4 (PtEb IX(2)-D(2))	2	-	9
Switzerland	Mathis et al.	1999	Fecal	PCR	ITS and MSP	36	3	3 (D genotype)	-	-	7
Turkey	Duzlu et al.	2019	Fecal	Nested-PCR	SSU-rRNA, ITS and MSP	282	41	0	6	35	10
America											
Colombia	Santin et al.	2008	Fecal	PCR	ITS	120	18	18 (PtEb IX(16)-K(1)-Peru5(1))	-	-	9
Africa											
Egypt	Al-Herrawy et al. ^a	2016	Fecal	PCR	SIN1F/SIN1R and SSU-rRNA	108	23	14 (unknown)	-	19	9
Oceania											
Australia	Zhang et al.	2019	Fecal	Nested-PCR	ITS	342	15	15 (PtEb IX(9)-D(5)-VIC_dog1(1))	-	-	10

^aA study including two datasets, based on the diagnostic methods (molecular and microscopical)

datasets (2515 animals) in Asia, nine datasets (886 animals) in Europe, one dataset (108 animals) in Africa, one dataset (120 animals) in America, and one dataset (342 animals) in Oceania. The countries with the highest number of published reports were China (five studies), Iran (three studies), and Spain (three studies). PCR-based methods were used in all molecular studies. Of the 23 included datasets, *E. bienersi* (21/23; 91.30% studies), *Enc. cuniculi* (4/23; 17.39% studies), and *Enc. intestinalis* (3/23; 13.04% studies) had the highest number of reports (Table 1). As shown in the Table 1, the internal transcribed spacer (ITS) gene was used to determine the genotypes of *E. bienersi* (17/23 datasets; 73.91%). In this regard, the PtEb IX (10 studies) and the D (eight studies) genotypes of *E. bienersi* were the frequently reported genotypes (Table 1). The other reported *E. bienersi* genotypes are shown in Table 1. Nevertheless, genotyping was not performed in six studies, because they used only the small subunit ribosomal RNA (SSU-rRNA) gene (Table 1). The pooled molecular prevalence of microsporidia infection in dogs thought to be 8.4% (95% CI, 6.1–11.5%; 329/3971) (Supplementary Figure 1). According to the identified species, the prevalences were 11.6% (95% CI: 6.7–19.2%) for *Enc. intestinalis* (Supplementary Figure 2), 6.5% (95% CI: 4.9–8.7%) for *E. bienersi* (Fig. 2), and 4.5% (95% CI: 1.1–16.5%) for *Enc. cuniculi* (Supplementary Figure 3). In the subgroup analysis regarding continents, the highest pooled prevalence was observed in Africa 21.3%

(95% CI, 14.6–30%) followed by Europe 8.2% (95% CI, 5–13.2%), Asia 7.7% (95% CI, 4.6–12.5%), America 15% (95% CI, 9.7–22.6%), and Oceania 4.4% (95% CI, 2.7–7.1%) (Table 2). It should be noted that only one study has been conducted in America, Africa, and Oceania continents. In addition, the prevalence of microsporidia based on countries is categorized in Table 2.

The results based on serological detection techniques

A total of 1703 dogs from 9 datasets were evaluated for microsporidia sero-prevalence in dogs (Table 3). In this review, the ELISA (enzyme-linked immunosorbent assay) method was used in most of the datasets ($n = 4$), followed by IFA (immunofluorescence assay) method ($n = 3$), and DAT (direct agglutination test) method ($n = 2$). The included datasets represented seven different countries (i.e., three datasets from Slovakia, one dataset from Norway, one dataset from Colombia, one dataset from Brazil, one dataset from the USA, one dataset from Japan, and one dataset from Egypt). The pooled sero-prevalence of microsporidia in dogs was thought to be 20.9% (95% CI, 14.6–29%) (Supplementary Figure 4).

The results based on microscopic methods

A total of five datasets from four different countries that used microscopic methods were included in the systematic review and meta-analysis; although in two studies,

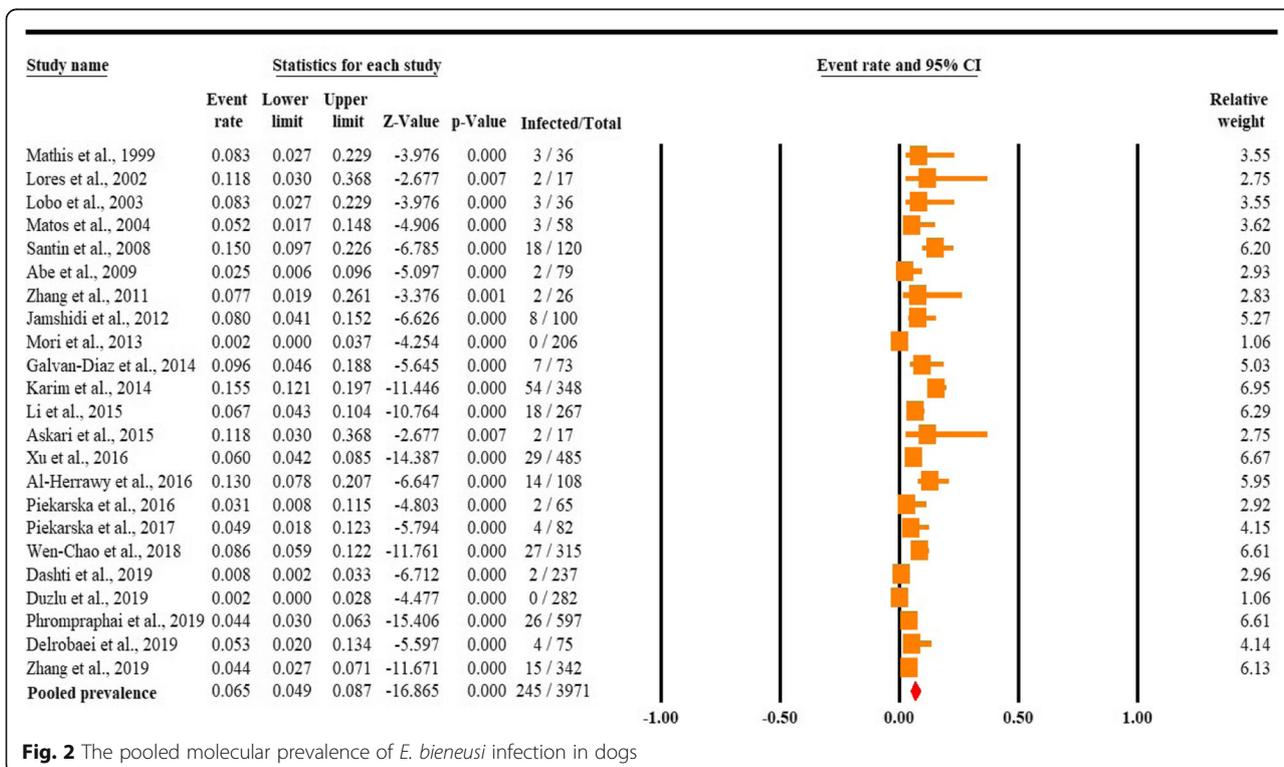


Table 2 Subgroup analysis of continents and countries based on molecular methods in dogs

Continents/countries	Datasets (n)	Sample size (n)	Infected (n)	Pooled prevalence % (95% CI)
Global	23	3971	329	8.4 (6.1–11.5)
Asia	11	2515	195	7.7 (4.6–12.5)
China	5	1441	130	8.6 (5.5–13.4)
Iran	3	192	37	13.7 (3.5–40.9)
Japan	2	676	28	4.2 (2.9–6)
Thailand	1	206	0	0
Europe	9	886	78	8.2 (5–13.2)
Spain	3	327	18	6.4 (0.9–33.5)
Portugal	2	94	6	6.6 (3–13.8)
Poland	2	147	10	6.8 (3.7–12.2)
Switzerland	1	36	3	8.3 (2.7–22.9)
Turkey	1	282	41	14.5 (10.9–19.2)
America	1	120	18	15 (9.7–22.6)
Colombia	1	120	18	15 (9.7–22.6)
Africa	1	108	23	21.3 (14.6–30)
Egypt	1	108	23	21.3 (14.6–30)
Oceania	1	342	15	4.4 (2.7–7.1)
Australia	1	342	15	4.4 (2.7–7.1)

microscopy and coproantigen methods were used, the reported prevalence rates were same (Table 4). As shown in the Table 4, no countries were available for America and Oceania continents. The techniques used for this diagnostic method are listed in Table 4. The pooled prevalence of microsporidia in dogs (from five datasets including 355 dogs) was 23.1% (95% CI, 13.5–36.8%) (Supplementary Figure 5).

Confounding factors

With regard to the type of dogs, infection rates were estimated to be 16% (95% CI, 10.4–23.8%) in unknown dogs, 12.8% (95% CI, 8.2–19.4%) in stray dogs, and 11.2% (95% CI, 7.9–15.8%) in pet (domesticated) dogs (Table 5 and Supplementary Table 1). Male dogs and

age > 12 months had a higher infection rate; however, these were not statistically significant (Table 5 and Supplementary Table 2).

Discussion

Over the years, zoonotic transmission of various species of microsporidia and the role of animals as reservoir for human infection are important issues in medical and veterinary practices [57, 58]. Dogs are considered one of the potential reservoirs for human-infecting microsporidia. According to our results, the prevalence of infection in the microscopic, serological, and molecular methods was estimated at 23.1%, 20.9%, and 8.4%, respectively. One of the most important reasons for the difference in prevalence is that each of the studied methods has its

Table 3 All the studies investigating the global prevalence of microsporidia in dogs according to serological methods

First author	Publication year	Country	Type of sample	Diagnostic methods	Sample size	Infected	Quality assessment
Stefkovic	2001	Slovakia	Serum	IFA	178	53	7
Akerstedt	2003	Norway	Serum	IFA	237	0	10
Halanova et al.	2003	Slovakia	Serum	IFA	193	73	10
Lindsay et al. ^a	2009	Brazil	Serum	DAT	113	27	10
Lindsay et al. ^a	2009	Colombia	Serum	DAT	254	19	10
Malcekova et al.	2010	Slovakia	Serum	ELISA	111	17	10
Sasaki et al.	2011	Japan	Serum	ELISA and WB	472	103	10
Cray and Rivas	2013	USA	Serum	ELISA	125	27	9
Abu-Akkada et al.	2015	Egypt	Serum	ELISA	20	8	8

IFA immunofluorescence assay, DAT direct agglutination test, ELISA Enzyme-linked immunosorbent assay, WB western blot

^aA study including two datasets, based on the countries

Table 4 All the studies investigating the global prevalence of microsporidia in dogs according to microscopy or coproantigens detection methods

First author	Publication year	Country	Type of sample	Diagnostic methods	Sample size	Infected	Quality assessment
Lobo et al. ^a	2003	Portugal	Fecal	MTS and IFA ^b	36	5	7
Matos et al. ^a	2004	Portugal	Fecal	MTS and IFA ^b	58	9	7
Al-Sadi et al.	2013	Iraq	Fecal	Multiple staining ^c	80	11	8
Galvan-Diaz et al. ^a	2014	Spain	Fecal	WS	73	32	8
Al-Herrawy et al. ^a	2016	Egypt	Fecal	MTS	108	36	9

MTS modified trichrome staining, IFA immunofluorescence assay for coproantigens survey, WS Weber’s staining

^aA study including two datasets, based on the diagnostic methods (molecular and microscopical)

^bMTS and IFA: both methods reported the same prevalence

^cMultiple staining: Giemsa, quick-hot Gram-chromotrope, Weber-green modified trichrome, Ryan-blue modified trichrome, and Calcofluor white stains

own sensitivity and specificity [59, 60]. Traditionally, light microscopy using specific staining techniques is one of the ways to diagnose microsporidia infection [61]. However, this method is difficult and needs a well-experienced person due to its similarity to bacteria or yeasts in stool samples [31, 62]. In the current study, the results of the microscopic prevalence are higher than the other two methods, which may be due to misdiagnosis with other elements and microorganisms. Nevertheless, nowadays molecular techniques are considered the gold standard for detection and differentiation of microsporidia at the species/genotype levels [63, 64]. The main advantage of molecular methods is higher sensitivity and specificity, along with easier interpretation [65, 66]. In this regard, the pooled prevalence obtained by molecular methods (8.4%) can be considered as “true” prevalence. On the other hand, although serological techniques (i.e., DAT, IFA, and ELISA) are more sensitive than the microscopic methods, high costs and false-positive reactions are considered disadvantages. In addition, there is no reliable serological method for detection of *E. bienewisi* [36, 67].

According to the molecular analysis, *E. bienewisi* is a complex species with numerous genotypes, diverse host spectrum and pathogenicity, and wide distribution [68–70]. The genetic data on *E. bienewisi* diversity rely almost exclusively on the analysis of the ITS region, which provides valuable information about the transmission and pathogenic potential of this parasite [70]. As the

molecular results of this review showed, most of the reports were related to *E. bienewisi* the genotypes PtEb IX and D. In this regard, the genotype PtEb IX, a dog-specific genotype, could have an exclusive dog-to-dog transmission that may cause some gastrointestinal disorders in dogs [12, 71]. As shown in Table 1, zoonotic transmission between dogs and humans is possible for the genotypes D, A, Peru 5, Peru 8, and type IV that most of these genotypes have been reported in immunocompromised individuals [5, 70, 72–75]. This finding proposed that dogs and humans can either infect each other or be infected from the same environmental sources (i.e., water sources and vegetables). However, more samples from these animals and humans should be genotyped to confirm the zoonotic transmission of these genotypes.

According to formal reports, the approximate population of pet and stray dogs has been estimated at 471 million and 200 million worldwide, respectively [76] (<https://www.statista.com/statistics/1044386/dog-and-cat-pet-population-worldwide/>) (<https://www.npr.org/2017/12/29/574598877/no-easy-answer-to-growing-number-of-stray-dogs-in-the-u-s-advocate-says>). In this regard, the top 10 countries with the largest population of dogs include the USA, Brazil, China, Russia, Japan, Philippines, India, Argentina, UK, and France (<https://www.mapsofworld.com/world-top-ten/countries-with-most-pet-dog-population.html>). In this review, most studies have been conducted in developed countries,

Table 5 Subgroup analysis of animal type, age and gender in dogs

Variables		Sample size (n)	Infected (n)	Pooled prevalence % (95% CI)	Odds ratio (OR) (95% CI)
Type of dogs	Pet (domestic) dogs	2847	287	11.2 (7.9–15.8)	-
	Stray dogs	917	126	12.8 (8.2–19.4)	
	Unknown	2083	336	16 (10.4–23.8)	
Age	≤ 12 months	339	22	8.3 (2.8–21.9)	0.76 (0.23–2.48)
	> 12 months	1177	98	10.5 (6.2–17)	
Gender	Male	783	104	13.8 (9.2–20.1)	1.08 (0.74–1.59)
	Female	569	62	11.9 (7.9–17.5)	

especially European countries, where laboratory tools are easily accessible compared to developing countries. Therefore, some key countries (countries with the highest number of dogs) have few or no studies, and the need for further studies and more attention to microsporidia infection in dogs in these countries is emphasized.

The results of this meta-analysis showed that the prevalence of microsporidia infection in stray dogs were higher than that in pet (domesticated) dogs. Since stray dogs are freely scattered in the environment and easily access various environmental sources, they can be considered an important reservoir of microsporidia. As a result, it is recommended that health officials and health policymakers provide health strategies to prevent stray dogs from entering urban and rural areas in order to control infections.

In the current meta-analyses, we observed a higher microsporidia prevalence in dogs aged > 12 months and male dogs, but these were not statistically significant. One of the reasons for the higher prevalence of the infection in older age can be related to the higher risk of exposure to microsporidia.

As in our previous meta-analysis studies [19, 77, 78], the strengths of this study include the rigorous methodology, the comprehensive literature search in four international databases, the large total sample size, and analysis of several subgroups. Moreover, this study has some limitations and the results presented here should be interpreted with regard to these limitations. Limitations compose inclusion of reports with a lack of information on age, gender, symptoms, and risk factors; failed online registration (PROSPERO) due to the data were already extracted, some reports with low sample size, variations in the sensitivity and specificity of diagnostic methods, and the possibility of missing some studies during search strategy. Because of these limitations, it should be noted that our results may not reflect the true prevalence, and the reported numbers are apparent prevalence. Nevertheless, we believe what we report here is very close to true microsporidia prevalence in dogs from a global perspective.

Conclusion

We have found that there is a high burden of microsporidia infection in dogs. Moreover, our results have shown that *E. bieneusi* the genotypes PtEb IX and D are the common species/genotypes in dogs. These data should be taken into consideration by the health authorities, physicians, veterinarians, and dog's owners. Furthermore, this study signifies the importance of periodical monitoring of dogs for microsporidia. We suggest additional investigations to further clarify the prevalence and genetic diversity of microsporidiosis in the world to guide the development of appropriate public health interventions.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s41182-020-00265-0>.

Additional file 1: Supplementary Figure 1. The pooled molecular prevalence of microsporidia infection in dogs. **Supplementary Figure 2.** The pooled molecular prevalence of *Enc. intestinalis* infection in dogs. **Supplementary Figure 3.** The pooled molecular prevalence of *Enc. cuniculi* infection in dogs. **Supplementary Figure 4.** The pooled seroprevalence of microsporidia infection in dogs. **Supplementary Figure 5.** The pooled prevalence of microsporidia infection in dogs.

Additional file 2: Supplementary Table 1. Excel spreadsheet of extracted data from included studies for type of dogs.

Additional file 3: Supplementary Table 2. Excel spreadsheet of extracted data from included studies for age and gender of dogs.

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Authors' contributions

All authors contributed to study design. AT contributed to all parts of the study. AT, SB, and SK contributed to study implementation. AT collaborated in the analysis and interpretation of data. AT collaborated in the manuscript writing and revision. All the authors commented on the drafts of the manuscript and approved the final version of the article.

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Availability of data and materials

All data generated or analyzed during study are included in this systematic review and meta-analysis

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

None declared.

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