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REVIEW

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Global molecular epidemiology of microsporidia in pigs and wild boars with emphasis on *Enterocytozoon bieneusi*: A systematic review and meta-analysis

Ali Taghipour[1] | **Saeed Bahadory**[2] | **Sasan Khazaei**[2] | **Leila Zaki**[2] | **Sheida Ghaderinezhad**[2] | **Jila Sherafati**[2] | **Amir Abdoli**[1,3]

[1] Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran

[2] Department of Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[3] Department of Parasitology and Mycology, Jahrom University of Medical Sciences, Jahrom, Iran

Correspondence
Ali Taghipour, Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran.
Email: a.taghipour@jums.ac.ir, alitaghipor71@yahoo.com
Amir Abdoli, Department of Parasitology and Mycology, Jahrom University of Medical Sciences, PO Box 74148-46199, Ostad Motahari Avenue, Jahrom, Iran.
Email: a.abdoli25@gmail.com

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Abstract

Background: Microsporidia are spore-forming intracellular pathogens with worldwide prevalence, causing emerging infections in humans and animals. *Enterocytozoon bieneusi* is a zoonotic species of microsporidia and is responsible for more than 90% of cases of microsporidiosis in humans and animals. Pigs and wild boars are important animal reservoirs of microsporidia. Hence, we aimed to estimate the global prevalence of microsporidia and genetic diversity of *E. bieneusi* in pigs and wild boars through a set of systematic review and meta-analysis (PRISMA) guidelines.

Methods: Four databases (Web of Science, PubMed, Scopus and Google Scholar) were searched between January 1, 2000 and April 30, 2021. Regarding meta-analysis, the random-effect model was employed by forest plot with 95% confidence interval (CI).

Results: After exclusion of irrelevant articles and duplication removal, 33 papers, including 34 datasets (30 datasets for domestic pigs and 4 for wild boars) finally meet the inclusion criteria to undergo meta-analysis. The pooled prevalence rates of microsporidia infection in domestic pigs and wild boars were 37.6% (95% CI: 30.8–44.9%) and 8.1% (95% CI: 2.1–26.8%), respectively. While, the pooled prevalence rates of *E. bieneusi* were 35% (95% CI: 28.4–42.2%) in domestic pigs and 10.1% (95% CI: 1.7–42.4%) in wild boars. The genotypes EbpA was the most reported genotype in domestic pigs and wild boars. Male animals had higher prevalence rates of microsporidia infection than females (27 vs. 17.4%, OR = 1.91; 95% CI, 0.77–4.71%).

Conclusion: This study indicates the important role of domestic pigs and wild boars as animal reservoir hosts of microsporidia. Thereby, strategies for control and prevention of these zoonotic pathogens should be designed in pigs and wild boars.

KEYWORDS
domestic pig, *Enterocytozoon bieneusi*, microsporidia, systematic review, wild boar

1 | INTRODUCTION

Microsporidia are a group of ubiquitous and obligate intracellular pathogens, cause an emerging infection in humans and animals worldwide (Santín & Fayer, 2011, Qiu et al., 2019). To date, 220 genera and 1700 species of microsporidia have been recognized (Han et al., 2021), which *Enterocytozoon bieneusi* and *Encephalitozoon* species (*Enc. hellem, Enc. intestinalis* and *Enc. cuniculi*) are the most important species causing infection in humans and animals worldwide (Henriques-Gil et al., 2010, Karimi et al., 2020). However, *E. bieneusi* is responsible for more than 90% of the human and animal cases of microsporidiosis (Wang et al., 2018c). Microsporidia are spore-forming pathogens and their spores shed through faces and urine of infected hosts, while contamination of the environment, soil and water are the main sources of infection (Ryan et al., 1993). Accordingly, most of the infections are predominantly transmitted via consumption of contaminated food or water containing microsporidian spores in human and animals (Stentiford et al., 2016, Henriques-Gil et al., 2010). Although numerous studies have reported vertical transmission of microsporidia from mother to offspring in several animal species, this transmission route has not yet been observed in human hosts (Becnel & Andreadis, 1999, Goertz et al., 2007). The outcomes of microsporidiosis may differ in humans and animals according to the species of microsporidia and host immune status (Karimi et al., 2020). In immunocompetent individuals, microsporidiosis is oftentimes asymptomatic, although it can lead to mild or self-limiting infections (Sak et al., 2011). In those with a weakened immune system (organ transplant recipients, chemotherapy patients and those with HIV/AIDS), microsporidiosis can cause widespread signs and severity such as renal diseases, sinusitis, ocular infection, persistent diarrhoea, encephalitis, poor coordination or even may result in death if not treated (Weber et al., 1993, Chupp et al., 1993, Nagpal et al., 2013). Although not all microsporidian genera and species are zoonotic, only 17 microsporidian species have been reported in humans (Han et al., 2021). According to the published literature, microsporidia infections have increased significantly in multiple host species such as rodents, birds, fish, insects, pets, wild and livestock animals, suggesting a possible zoonotic transmission (Taghipour et al., 2021b, 2020b, 2021a). Accordingly, some microsporidia are considered as zoonotic pathogens in the emerging infectious diseases or pathogens category and have worldwide clinical importance (Santín & Fayer, 2011). The One Health approach acknowledges that animal and environmental health are inextricably linked to human health and constitute a community biomass so that their respective well-beings are interdependent (Mazet et al., 2009). It has been observed that pigs can be infected with microsporidia spores at an early age, consequently the animals excrete spores throughout their lives which can contaminate the environment (Sak et al., 2008). Therefore, using untreated pig manure as fertilizer that may be a potential source of infection in humans and animal population, which in turn can be a risk to public health (Zhao et al., 2014, Luo et al., 2019). In many countries of the world, pigs are the major sources of meat production (Dione et al., 2017, Tisdell, 2013). However, pigs and their meat may act as reservoirs for zoonotic transmission of microsporidia to humans (Dashti et al., 2020, Němejc et al., 2014). While both pigs and wild boars could be a source of human microsporidiosis, the epidemiology of microsporidia in these animals has been poorly investigated. To this end, the aim of this study is to provide a global estimation of molecular prevalence and genetic diversity of microsporidia infection in pigs and wild boars through a systematic review and meta-analysis protocol.

2 | METHODS

2.1 | Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol was conducted in line with the checklist (Moher et al., 2015). In summary, a systematic search for epidemiological studies was undertaken in four international databases (Scopus, PubMed, Web of Science databases and Google Scholar) between January 1, 2000 and April 30, 2021. Applied medical subject headings terms were (“Microsporidia” OR “Microsporidiasis” OR “Microspora” OR “*Enterocytozoon bieneusi*”) AND (“Epidemiology” OR “Prevalence”) AND (“Pig” OR “Swine” OR “Boar” OR “*Sus scrofa*”) alone and/or in appropriate combinations. It should be noted that the scientific name of the wild boar is *Sus scrofa*. In addition to searching electronic databases, the bibliographic list of initially found articles were manually searched to find other relevant citations.

2.2 | Eligibility criteria and study selection

The following conditions were considered for inclusion of the studies: (1) published studies up to April 30, 2021 that contain information such as the total sample size and the infection rate. (2) Original studies and brief reports with case-control or cross-sectional designs. (3) English full text or abstract that had no geographical limitations. (4) Studies that used molecular detection techniques. (5) The studied populations included pigs and boars. Studies that did not meet any of these conditions were excluded. Review articles, case reports, letter to the editor, drug trials, as well as reports with unclear and/or confusing data were excluded.

2.3 | Data collection

All included studies were screened and their details were inserted into the tables including the author’s name, year of publication, continent, countries, type of animals (pig or boar), gender, types of molecular method, total sample sizes and number of positive samples. Moreover, the type of genes that were used for molecular methods along with the identified *E. bieneusi* genotypes was extracted for each study.

2.4 | Quality assessment

In this review, the Joanna Briggs Institute (JBI) checklist was used for quality assessment of included studies (Institute, 2014). Accordingly, four questions with yes, no, unclear and not applicable were considered. Depending on the comprehensive information and the quality of the studies, we gave a score between 1 and 10. Studies with a final score of 4–10 were included, among them, 4–7 and 7–10 scores papers were considered moderate and high quality, respectively; studies with lower scores than mentioned values were excluded (≤3 points).

2.5 | Data synthesis and statistical analysis

The BioStat software version 2.2 was used for statistical analysis (Taghipour et al., 2021c, 2020a). For estimation of microsporidia infection among pigs and boars, we used the random-effects model (REM) with 95% confidence intervals (CIs). The inter-study distribution was facilitated by the REM-based procedure to calculate the true effect sizes. For sub-group analysis, the pooled prevalence was established based on the type of animals (pig or boar) as well as continent and country. The REM-based odd ratio (OR) calculation was performed to estimate the association between prevalence of microsporidia and the animal gender. The I^2 index was applied to calculate heterogeneity between studies (Khademvatan et al., 2019, Taghipour et al., 2020c). Publication bias was not estimated because this study was from gathered data of the prevalence studies (Hunter et al., 2014). For representing the pooled prevalence of microsporidiosis (with 95% CI) in pigs and boars, we used the forest plot diagram.

3 | RESULTS

In the systematic search (Figure 1), 1708 unique publications were initially retrieved. Among them, many duplicates or non-eligible articles were removed and only 33 papers were finally eligible to undergo meta-analysis. Of note, one out of 33 studies possessed more than one dataset (Table 1), so that 34 datasets (30 datasets for domestic pigs and 4 for wild boars) were reviewed and required data were extracted. In Table 1, the main characteristics of each study with quality assessment according to JBI are classified. All of the included articles had suitable quality. Polymerase chain reaction (PCR) was applied for microsporidia detection and genotyping in all of the included studies. The included studies were from 12 countries in four continents, including Asia (24 datasets, 8766 animals), Europe (seven datasets, 775 animals), America (two datasets, 293 animals), and Africa (one dataset, 96 animals) (Table 1). China possessed the most published literature with 16 studies (17 datasets). Most studies focused on *E. bieneusi* and only three studies reported *Enc. cuniculi* (2) *Enc. intestinalis* (1) among domestic pigs and wild boars (Al-Herrawy, 2016; Němejc et al., 2014; Reetz et al.,

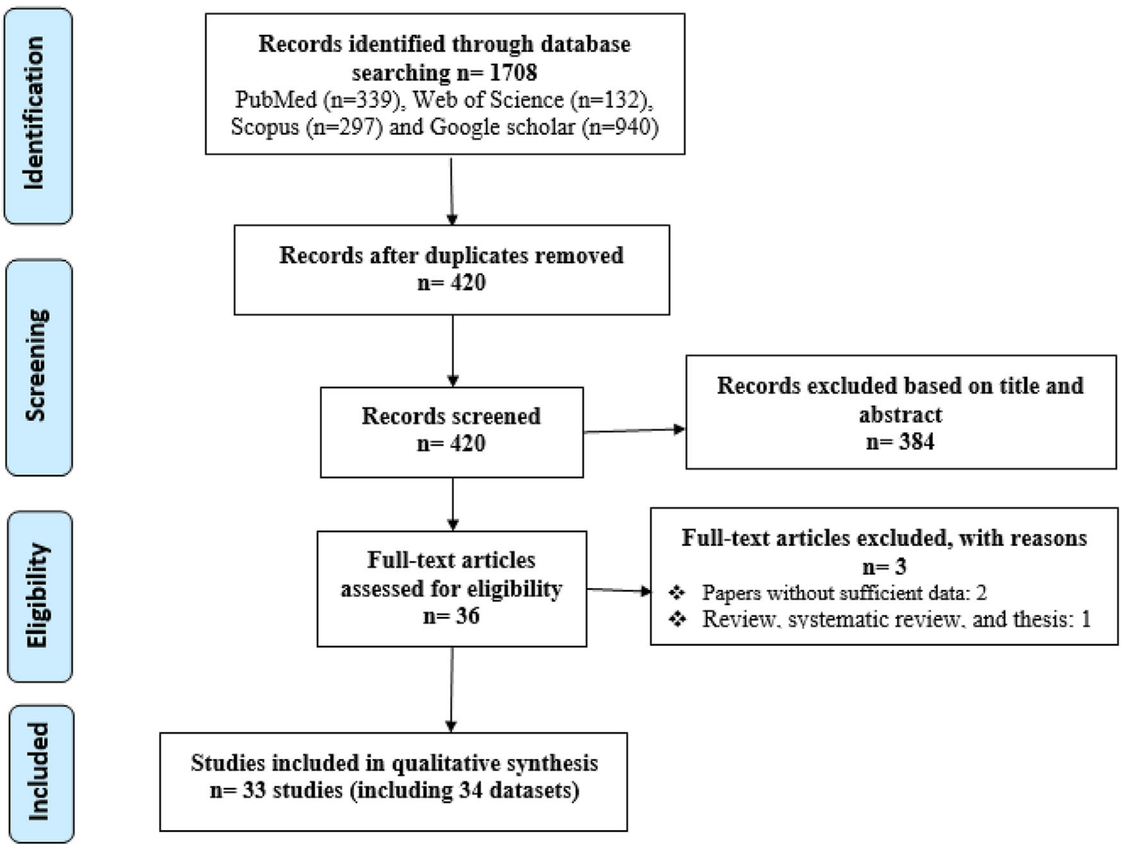

FIGURE 1 PRISMA flow diagram describing included or excluded studies

TABLE 1 All the studies investigating the global prevalence of microsporidia species in domestic pigs and wild boars according to molecular methods

Continent/ country/ reference	Diagnostic method	Gene	Animal	Sample size	Infected by microsporidia	*E. bieneusi*	*Enterocytozoon bieneusi* (genotypes [n; number])	QA
America								
Brazil								
Fiuza et al. (2015)	PCR	ITS	Domestic pigs	91	54	54	CS-1 (7), EbpA (7), H (1), O (3), PigEb1 (5), PigEb2 (16), PigEb3 (2), PigEb4 (16), PigEb5 (1), PigEb6 (2), PigEb7 (1), PigEb8 (1), PigEb9 (1), PigEb10 (1), PigEb11 (1), PigEb12 (1), PigEb13 (1), PigEb14 (1), PigEb15 (1), PigEb16 (1) and PigEb17 (1)	8
USA								
Buckholt et al. (2002)	Nested-PCR	ITS	Domestic pigs	202	36	36	Unknown	7
Asia								
China								
Zhao et al. (2014)	Nested-PCR	ITS	Domestic pigs	95	85	85	EbpA (30), D (19),H (18),O (11),CS-1 (1),LW1 (1), HLJ-I to HLJ-IV (4) and unknown (1)	8
Li et al. (2014)	PCR	ITS	Domestic pigs	113	51	51	EbpC (12), CS-1 (7), O (6), EbpA (5), CS-8 (5), Henan-IV (4), CS-4 (2), and unknown (10)	8
Wan et al. (2016)	Nested-PCR	ITS	Domestic pigs	563	267	267	CS-4 (32), EbpC (48), CC-1 (2), EbpB (28), EbpB/EbpC (7), CS-1 (1), CS-10 (1), EbpA (14), Henan-IV (2), O (4), CS-1/EbpC (1), CS-3/EbpA (2), CHN7/O (1), PigEBITS5/Henan-IV (1), EbpA/EbpC (11), EbpA/Henan-IV (1), EbpC/Henan-IV (1), EbpC/O (30) and unknown (80))	9
Li et al. (2017)	Nested-PCR	ITS	Wild boars (*Sus scrofa*)	357	147	147	EbpC (85), F (22), CHG19 (11), CHC5 (10), WildBoar 10 (6), WildBoar 8 (5), WildBoar 9 (2), WildBoar 7 (1), PigEBITS5 (1), D (1), WildBoar 11 (1), RWSH4 (1) and SC02 (1)	9
Wang et al. (2018a)	Nested-PCR	ITS	Domestic pigs	897	408	408	EbpC(60), EbpA (55), pigEbITS5 (17), LW1 (12), H (12), CM8 (11), G (10), CHG19 (7), CHS5(6), HN-1 (6), HN-2 (2), HN-3 (1), HN-4 (1) and unknown (208)	9
Shi et al. (2018)	Nested-PCR	ITS	Domestic pigs	129	30	30	CHC5(3), CHG19(7), EbpD(9), EbpA(2), EbpC(4) and novel genotype YNZ1(5)	8
Wang et al. (2018b)	Nested-PCR	ITS	Domestic pigs	560	442	442	SYLA5 (56), CHG19 (32), SLTC3 (15), CHC5 (31), SZZA2 (6), EbpA (19), SLTC2 (56), SZZD1 (81), PigEBITS5 (12), SHZA1 (2), SZZC1 (3), H (4), PigEB4 (3), SYLC1 (1), Henan-IV (3), SLTC1 (2), SYLA1 (2), SYLA2 (1), CHS5 (1), D (1), SMXB1 (1), SMXC1 (1), SZZB1 (1), SZZA1 (1), SYLA3 (1), SMXD1 (1), SYLA4 (1), SYLD1 (1), CHG3(1), SZZD2 (1), SHZC1 (1), SMXD2 (1) and unknown (99)	9

(Continues)

TABLE 1 (Continued)

Continent/ country/ reference	Diagnostic method	Gene	Animal	Sample size	Infected by microspordia	*E. bieneusi*	*Enterocytozoon bieneusi* (genotypes [n; number])	QA
Zou et al. (2018)	Nested-PCR	ITS	Domestic pigs	396	125	125	EbpC (87), EbpA (17), KIN-1 (2), PigEBITS5 (3), CHS5 (1), Henan-IV (6), G (1), H (1), D (1), ZJ1 (1), ZJ2 (1), GD1 (1), YN1 (1), YN2 (1) and YN3 (1)	9
Li et al. (2019a)	Nested-PCR	ITS	Domestic pigs	801	389	389	CHC5 (2), CS-1 (5), CS-4 (20), CS-7 (3), CS-9 (1), D (17), EbpA (129), EbpC (168), EbpD (5), H (2), PigEb4 (12), PigEBITS5 (19), WildBoar8 (3), XJP-II (2) and XJP-III (1)	9
de Silva et al. (2019)	PCR	ITS	Domestic pigs	1190	645	645	EbpC (520), EbpA (93), CHG19 (17), CHC5 (4), XZP-II (4), H (2), I (2), D (1), Henan-III(1), XZP-I (1) and XZP-II (4)	9
Zhang et al. (2019)	PCR	ITS	Domestic pigs	37	18	18	Unknown	7
Luo et al. (2019)	PCR	ITS	Domestic pigs	266	83	83	EbpC (58), Henan-IV (23), SCT01 (1) and SCT02 (1)	8
Zou et al. (2019)	Nested-PCR	ITS	Domestic pigs	345	41	41	EbpC (36), CHS12 (1), EbpD (1), PigEBITS5 (1), GB11 (1) and GB31 (1)	8
Zhou et al. (2020)	Nested-PCR	ITS	Domestic pigs	188	88	88	Unknown	8
Zhang et al. (2020)	Nested-PCR	ITS	Domestic pigs	725	177	177	EbpC (103), EbpA (40), FJF (3), CHNRR2 (1), KIN1 (1), CHG7 (1), CHS5(7), CM11 (10), FJS(1), CHG23(1), G(1), PigEBITS (1), and D(7)	9
Japan								
Abe and Kimata (2010)	PCR	ITS	Domestic pigs	30	10	10	EbpA (1), H (4), EBITS5 (1), D (1), EbpC (2) and unknown (1)	7
Malaysia								
Ruviniyia et al. (2020)	PCR	ITS	Domestic pigs	450	183	183	Unknown	9
Thailand								
Leelayoova et al. (2009)	PCR	ITS	Domestic pigs	268	42	42	E (12), O (8), H (1) and unknown (21)	8
Prasertbun et al. (2017)	Nested-PCR	ITS	Domestic pigs	210	59	59	H (5), O (30), D (5), EbpA (1), EbpC (4, and novel genotypes TMP6–11 to TMP1-5 (14)	8
Thathaisong et al. (2019)	Nested-PCR	SSU rRNA and ITS	Domestic pigs	244	36	36	Unknown	8
Sanyanusin et al. (2019)	Nested-PCR	ITS	Domestic pigs	102	16	16	H (4), E (4), TMP6 (2), TMP7 (1), TMP8 (1), TMP9 (1), TMP10(2) and TMP11 (1)	7
Europe								
Czech Republic								
Sak et al. (2008)	Nested-PCR	ITS	Domestic pigs	79	74	74	F (70), D (2) and Peru 9 (2)	7
Germany								
Dengjel et al. (2001)	Nested-PCR	ITS	Domestic pigs	50	5	5	F (3), G+H (1) and O (1)	7
Reetz et al. (2009)*	PCR	ITS	Domestic pigs	34	15	12	–	7

(Continues)

TABLE 1 (Continued)

Continent/ country/ reference	Diagnostic method	Gene	Animal	Sample size	Infected by microsporidia	*E. bieneusi*	*Enterocytozoon bieneusi* (genotypes [n; number])	QA
Slovakia								
Luptáková et al. (2010)*	PCR	SSU rRNA	Wild boars (*Sus scrofa*)	91	0		–	7
Valencáková et al. (2006)*	PCR	SSU rRNA	Domestic pigs	27	25		–	6
Different Area								
Němejc et al. (2014)*	PCR	ITS	Wild boars (*Sus scrofa*)	460	54	33	EbpA (12) and unknown (21)	8
Spain								
Galván-Díaz et al. (2014)	PCR	SSU-rRNA and ITS	Domestic pigs	34	15	7	Unknown	7
Dashti et al. (2020)**	PCR	ITS	Domestic pigs	186	42	42	EbpA (22), O (8), PigEb4 (3), PigSpEb1 (3), PigHN-II (2) and EbpA+PigEb4 (4)	8
Dashti et al. (2020)**	PCR	ITS	Wild boars (*Sus scrofa*)	142	3	3	EbpA (2) and PigSpEb1(1)	8
Africa								
Egypt								
Al-Herrawy (2016)*	PCR	SSU rRNA	Domestic pigs	96	15	12	–	7

*In these studies, *Enc. intestinalis* and *Enc. cuniculi* infection were surveyed.
**A study consists of two datasets.
QA, Quality assessment.
-Genotype EbpA is synonym of genotype F.
-Genotype Peru9 is synonym of genotype D.
-Genotype EbpC is synonym of genotype E.

2009) (Table 1). Among the *Enc. cuniculi* genotypes, the study of Reetz et al. (2009) shows all three positive samples of genotype III, but in the study of Němejc et al. (2014), equal proportions of genotypes I (n = 8 positive) and II (n = 8 positive) were found. In addition, two studies out of the total studies focused only on the detection of *Enc. intestinalis* among domestic pigs and wild boars (Luptáková et al., 2010, Valencáková et al., 2006).

A relatively weighted prevalence of microsporidia infection was obtained from both domestic pigs (37.6% [95% CI :30.8–44.9%] and wild boars (8.1% [95% CI :2.1–26.8%] (Supporting information Figures S1 and S2). Also, pooled prevalence rates were estimated for *E. bieneusi* in both domestic pigs (35% [95% CI :28.4–42.2%]) (Figure 2) and wild boars (10.1% [95% CI :1.7-42.4%] (Figure 3). The ITS gene was applied for molecular determination of *E. bieneusi* genotypes in most of the included studies, and genotypes EbpA (14 studies), EbpC and H (11 studies) and D (eight studies) in domestic pigs, and EbpA (three studies) in wild boars were most prevalent among all other genotypes (Table 1). Europe and America continents showed the highest total prevalence rates with 38.0% (95% CI: 12.6-72.3%) and 35.9% (95% CI: 8.0-78.4%), respectively, followed by Asia with 33.5% (95% CI: 26.8–40.9%), and Africa with 15.6% (95% CI: 9.6–24.63%) (Table 2). It is noteworthy that Table 2 demonstrates data on country-based prevalence of microsporidia infection. We found no significant association between gender and the microsporidia infection rate (OR = 1.91; 95% CI, 0.77–4.71%), although the prevalence was higher in males (27.7%; 95% CI: 11.2–53.8%) than in females (17.4%; 95% CI: 14.1–21.3%) animals (Table 3).

4 | DISCUSSION

The present study has shown a relatively high prevalence of microsporidia in domestic pigs (37.6%) compared to wild boars (8.1%). It should be noted, however, that studies on domestic pigs (30 datasets) were more numerous than on wild boars (four datasets). Most of the included studies used nested PCR with the ITS gene, which were able to identify different genotypes of *E. bieneusi* (Javanmard et al., 2018), whereas conventional PCR with SSU rRNA gene fails to identify genotypes (Mirjalali et al., 2014). In recent decades, genotyping of *E. bieneusi* using ITS gene has been known as a gold standard method, showing adequate information on pathogenicity and the source of the organism (Thellier & Breton, 2008). Currently, over 500 *E. bieneusi* genotypes have been identified in humans, animals and environmental samples (Li et al., 2019b). Reportedly, the EbpA, EbpC

Study name	Event rate	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Dengjel et al.	0.100	0.042	0.219	-4.661	0.000	2.78
Buckholt et al.	0.178	0.131	0.237	-8.314	0.000	3.55
Jeong et al.	0.142	0.113	0.176	-13.642	0.000	3.63
Sak et al.	0.937	0.857	0.973	5.832	0.000	2.81
Reetz et al.	0.353	0.213	0.524	-1.689	0.091	3.12
Leelayoova et al.	0.157	0.118	0.205	-10.015	0.000	3.58
Abe and Kimata	0.333	0.190	0.516	-1.790	0.074	3.03
Zhao et al.	0.895	0.815	0.942	6.401	0.000	3.19
Li et al.	0.451	0.362	0.544	-1.033	0.302	3.54
Fiuza et al.	0.593	0.490	0.689	1.772	0.076	3.49
Al-Herrawy and Gad	0.125	0.072	0.207	-6.305	0.000	3.26
Wan et al.	0.474	0.433	0.516	-1.222	0.222	3.69
Prasertbun et al.	0.281	0.224	0.346	-6.121	0.000	3.60
Wang et al.	0.455	0.423	0.488	-2.701	0.007	3.70
Shi et al.	0.233	0.168	0.313	-5.729	0.000	3.50
Wang et al.	0.789	0.754	0.821	12.745	0.000	3.67
Zou et al.	0.316	0.272	0.363	-7.157	0.000	3.66
Thathaisong et al.	0.148	0.108	0.198	-9.717	0.000	3.55
Li et al.	0.486	0.451	0.520	-0.813	0.416	3.70
Sanyanusin et al.	0.157	0.098	0.241	-6.177	0.000	3.35
Li et al.	0.542	0.514	0.570	2.895	0.004	3.71
Zhang et al.	0.486	0.332	0.644	-0.164	0.869	3.20
Luo et al.	0.312	0.259	0.370	-5.975	0.000	3.63
Zhou et al.	0.119	0.089	0.157	-12.042	0.000	3.58
Dashti et al.	0.226	0.171	0.291	-7.026	0.000	3.56
Galvan-Diaz et al.	0.206	0.101	0.373	-3.183	0.001	2.93
Zhou et al.	0.468	0.398	0.540	-0.875	0.382	3.61
Ruviniyia et al.	0.407	0.362	0.453	-3.936	0.000	3.68
Zhang et al.	0.244	0.214	0.277	-13.072	0.000	3.69
	0.350	0.284	0.422	-3.970	0.000	

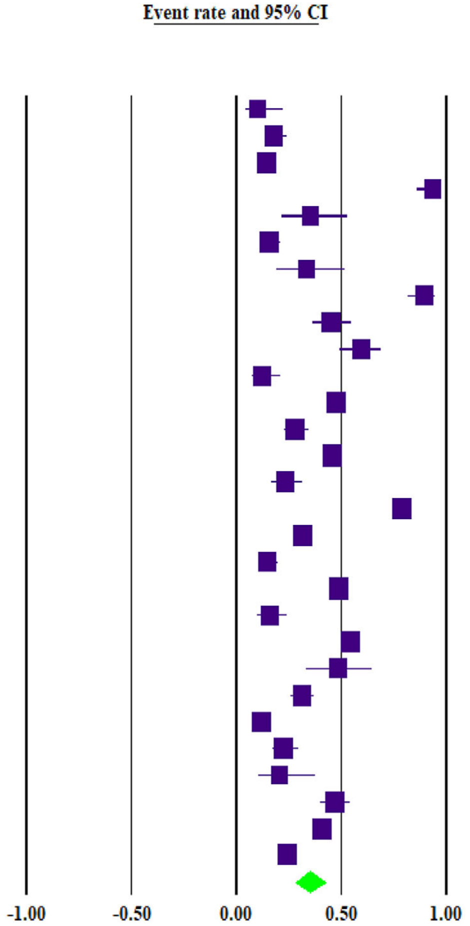

FIGURE 2 The pooled molecular prevalence of *E. bieneusi* infection in domestic pigs

Study name	Event rate	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Nemejc et al.	0.072	0.051	0.099	-14.170	0.000	34.42
Li et al.	0.412	0.362	0.464	-3.317	0.001	34.70
Dashti et al.	0.021	0.007	0.063	-6.573	0.000	30.88
	0.101	0.017	0.424	-2.280	0.023	

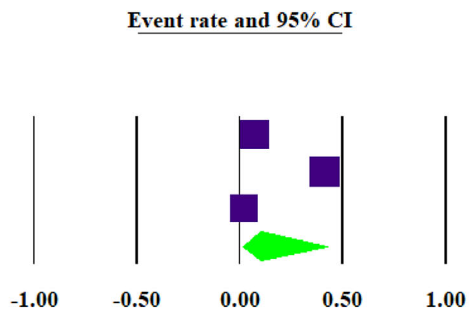

FIGURE 3 The pooled molecular prevalence of *E. bieneusi* infection in wild boars

and D of *E. bieneusi* have been the most prevalent genotypes among pigs and boars. As shown in Table 1, zoonotic transmission between pigs, boars and humans is possible for these genotypes, among which most of these genotypes have been reported in immunocompromised individuals (Thellier & Breton, 2008). Some of these genotypes have also been identified in immunocompetent individuals. For instance, genotype EbpA has been reported in immunocompetent individuals in the Czech Republic (Sak et al., 2011). Therefore, this shows the possible environmental transmission of infective spores between humans and these animals. Nevertheless, numerous samples from pigs, boars and humans should be genotyped to confirm the zoonotic transmission of the genotypes.

In the present study, China has the largest dataset (17 datasets) with a pooled prevalence of 39.2%, while only 11 other countries have been reported the microsporidia infection among domestic pigs and wild boars. However, little is known about microsporidia infections among pigs and boars in many parts of the world, especially in developing countries. As shown in Table 2, some countries have few studies that implicate the need for further studies and more attention to pigs and boars microsporidiosis in these countries. Because of the paucity of

TABLE 2 Sub-group analysis of continents, countries and animal type (domestic pigs and wild boars), based on molecular methods

Continent/ countries	Datasets (n)	Total samples (n)	Infected (n)	Pooled prevalence% (95% CI)	Heterogeneity			
					I^2	Q-value	*p*-value	t^2
Africa	1	96	15	15.6 (9.6–24.63)	0	0	1	0
Egypt	1	96	15	15.6 (9.6–24.63)	0	0	1	0
America	2	293	90	35.9 (8.0–78.4)	97.82	45.8	0	1.78
Brazil	1	91	54	59.3 (49.0–68.9)	0	0	1	0
USA	1	202	36	17.8 (13.1–23.7)	0	0	1	0
Asia	22	8438	3409	36.2 (29.1–44)	97.69	997.966	0	0.591
China	15	6662	2996	43.9 (35.5–52.8)	97.68	689.40	0	0.50
Thailand	4	824	153	18.2 (12.7–25.5)	81.84	16.52	0	0.15
South Korea	1	472	67	14.2 (11.3–17.6)	0	0	1	0
Malaysia	1	450	183	40.7 (36.2–45.3)	0	0	1	0
Japan	1	30	10	33.3 (19.0–51.6)	0	0	1	0
Europe	9	1103	233	28.7 (12.7–52.6)	96.164	156.398	0	3.45
Slovakia	2	118	25	22.3 (0.0–99.8)	95.74	23.46	0	28.64
Spain	3	362	60	15.8 (4.3–44.2)	0	0	1	0
Germany	2	84	20	23.3 (4.3–67.5)	91.12	11.258	0.001	1.752
Czech Republic	1	79	74	93.7 (85.7–97.3)	0	0	1	0
Different Area	1	460	54	11.7 (9.1–15.0)	0	0	1	0
Animal type								
Domestic pig	30	8880	3543	37.6 (30.8–44.9)	97.391	1111.47	0	0.654
Wild boar	4	1050	204	8.1 (2.1–26.8)	97.446	117.468	0	1.718

TABLE 3 Gender associated with microsporidia infection among domestic pigs and wild boars worldwide

Risk factors	Datasets no	Variables	Total samples	Infected samples	Pooled prevalence% (95% CI)	OR (95%CI)	OR heterogeneity (I^2)
Gender	4	**Male**	548	162	27.7 (11.2–53.8)	1.91 (0.77–4.71%)	89.504
		Female	703	123	17.4 (14.1–21.3)		

studies in some parts of the world, including America (two studies) and Africa (one study), data derived from these continents should be interpreted cautiously (Table 2).

There are some risk factors involved in the distribution of microsporidia, such as climatic conditions, type of animal husbandry and parasite control measures, etc. (Taghipour et al., 2020b, 2021b, 2021a). Traditional animal husbandry systems enable the access of pigs and boars to other domestic, wild and stray animals or close-contact with environmental sources (e.g., consumption of spore-contaminated water and food). Hence, different water resources, animals, and vegetations play a crucial role in maintaining the microsporidia in the environmental cycle. Therefore, pigs and boars may be considered as the major reservoir of microsporidia for transmission to humans.

Few studies estimated the prevalence based on animal gender (Table 3). In future studies, prevalence estimation is suggested by considering gender, age and different groupings (pre-weaned, post-weaned, growing and adult pigs).

The present study has some limitations and the results should be interpreted according to these limitations including lack of studies in many countries; low sample size in some studies; few studies on boars and absence of risk factors (e.g., gender and age) and clinical manifestation records (e.g., gastrointestinal disorders) in most studies. Therefore, it is noteworthy that the results may not precisely reflect the true prevalence rate, and the presented numbers are apparent prevalence rates. Nonetheless, we believed that what we had reported in the results is near to true prevalence of microsporidia in pigs and boars at a global scale.

5 | CONCLUSIONS

The present results highlight the role of domestic pigs and wild boars as reservoir hosts for human-infecting microsporidia. The results of this analysis could be used by veterinarians, public health authorities and medical practitioners to implement better preventive and treatment strategies against these pathogens. Also, the high-risk groups (i.e., immunocompromised individuals) must receive accurate and valid information about the risk of contact with the infected these animals. We recommend more research will be conducted to clarify the prevalence of microsporidiosis based on molecular methods, which would be a guide to the establishment of appropriate public health interventions.

AUTHOR CONTRIBUTIONS

All authors contributed to study design. AT and SB contributed to all parts of the study. AT, SK, SG and JS contributed to study implementation. AT and SB collaborated in the analysis and interpretation of data. AT, LZ and AA 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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CONFLICT OF INTEREST

The authors declare no conflict of interest..

ETHICAL APPROVAL

None required.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material.

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ORCID

Ali Taghipour https://orcid.org/0000-0002-6876-5902
Amir Abdoli https://orcid.org/0000-0003-4326-4586

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