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Microsporidiosis in Iran: A systematic review and meta-analysis

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ABSTRACT

Objective: To examine all evidence about Microsporidia infection in vertebrate/invertebrate hosts and Iranian populations distributed in different regions of the country.**Methods:** All published articles up to December 2015, including descriptive and cross-sectional studies related to the prevalence and genotyping of Microsporidia infection in Iran, was considered in this systematic review. The meta-analysis was done using the random-effects model and Stats Direct statistical software. MEGA 5.05 software and maximum likelihood algorithm with Kimura 2-parameter model were used for phylogenetic analysis.**Results:** Of the 1152 investigated studies, 33 eligible studies reported a prevalence of Microsporidia infection in vertebrate and invertebrate hosts. According to this systematic review, the overall prevalence rate of Microsporidia infection in immunocompromised patients in Iran was 8.18%. Furthermore, the overall prevalence rate of Microsporidia infection in immunocompromised patients with chronic diarrhoea, patients with non-diarrhoea, gastroenteritis, and patients with CD4 (<200 cells/ μ L) was 15.4%, 4.1%, 0.5%, and 12.9% respectively. The highest prevalence rate of human and animal Microsporidia was estimated in Kerman (29%) and Khuzestan (26.5%). The overall prevalence rate of Microsporidia infection in honeybees using the random-effects model was 40%. Furthermore, the highest prevalence rate of noseamosis was described in East Azerbaijan (48.2%). The most Microsporidia isolates from immunocompromised patients and pigeons in Iran belonged to genotypes D ($n = 16$; 50%) and E ($n = 6$; 20.6%) of *Enterocytozoon bienersi*.**Conclusions:** This study may be the first systematic review and meta-analysis that provides a broad outlook on the prevalence of microsporidiosis in Iran. It is necessary to investigate Microsporidia infection in vertebrate and invertebrate hosts and environmental resources in Iran.

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1. Introduction

Microsporidia are opportunistic pathogens that infect a wide range of vertebrate and invertebrate hosts [1,2]. These obligate intracellular eukaryotes consist of more than 170 genera and 1300 species [3,4]. The life cycle of Microsporidia species that infect all major animal groups is direct and simple [2]. *Enterocytozoon bieneusi* (*E. bieneusi*) is a zoonotic pathogen and animals contribute considerably to infections found in watersheds. Owing to the unpredictably high level of infections in calves, cats, dogs, and wild mammals (beavers, foxes, otters, raccoons, and muskrats), watersheds have been considered a reservoir of human infections [5,6]. *Nosema apis* (*N. apis*) and *Nosema ceranae* (*N. ceranae*) are the two causes of nosemosis in honeybees, which reduce both the honey yield and the population of honeybees [7,8]. Microsporidia as a single-celled organism, may cause infection in immune-competent population (travellers, persons wearing contact lenses, children, elderly, and undernourished people) and immunocompromised patients (HIV-positive, organ transplant recipients, cancer patients undergoing chemotherapy) [2,9]. The prevalence rate of human microsporidiosis range is between 0% and 50%, but its prevalence in HIV positive patients lies between 1.5% and 50% depending on the geographical region [10,11]. The most prevalent signs of microsporidiosis are fever, weight loss, as well as chronic and self-limiting diarrhoea in immunocompromised and healthy people, respectively [12]. At least 15 species are recognized to be pathogenic for human including among others *E. bieneusi*, *Encephalitozoon cucinuli* (*E. cucinuli*), *Encephalitozoon intestinalis* (*E. intestinalis*) and *Encephalitozoon hellem* (*E. hellem*) [13]. *E. bieneusi* can cause infections like cholangitis, cholecystitis, malabsorption, bronchitis, rhinitis, pneumonia [14]. Disseminated microsporidiosis is caused by a number of *Encephalitozoon* species (*E. cucinuli*, *E. hellem*, and *E. intestinalis*), *Pleistophora* and *Trachipleistophora* [10]. The other species of human Microsporidia include *Vittaforma cornea*, *Nosema ocularum*, *Brachiola algerae* which lead to keratoconjunctivitis [10]. *Nosema* infections have significant effects on honeybees including dysentery, shortened life periods of honeybees, and decrease in colony size [15,16]. The most effective drugs for treating microsporidiosis in humans are albendazole and fumagillin for keratoconjunctivitis. In contrast to *E. bieneusi*, albendazole is more effective against *Encephalitozoon* species, such as *E. intestinalis* [17,18]. The diverse modes of transmission are as follows: faecal–oral or oral–oral route, inhalation or ingestion of Microsporidia spores in contaminated water or food [5]. In addition, human to human transmission is proved in some studies [5]. Risk factors related to microsporidiosis include the status of the immune system (immunosuppression), swimming in a polluted pool, eating raw meat, being stung by a bee or wasp, dealing with recreational water sources and animals (zoonotic role) [5]. The numbers of Microsporidia genotypes, based on the internal transcribed spacer (ITS) nucleotide sequence of the ribosomal RNA gene, have augmented. Formerly, over 93 *E. bieneusi* genotypes had been published in GenBank. Some genotypes are host-specific, while others may infect a range of host species. Also, genetic characterization of Microsporidia species leads to a better understanding of the route(s) of transmission and the causes involved in the transmission cycle [19,20]. Microsporidial infections in human are detected by serology,

light microscopy using specific staining techniques, electron microscopy, and PCR methods [10]. To this end, this study carried out a systematic review with meta-analysis of Microsporidia studies in vertebrate and invertebrate hosts and Iranian populations distributed in different regions of the country. The present study may be the first meta-analysis that provides overall results based on available molecular and staining methods. According to this systematic review, not only we can create awareness about Microsporidia prevalence in various regions of Iran, but we will also be able to implement better preventive and treatment strategies.

2. Materials and methods

2.1. Search strategy

Pubmed, Science Direct, Scopus, Proquest, and Google scholar were used for searching English articles. SID, Magiran, Iran Medex, and Iran Doc were used for looking up articles in Persian. Both English and Persian language articles have been included in this study. After the search of the above databases, manual searches were conducted. All published articles up to December 2015 were chosen. Keywords used for searches were Microsporidiosis, Microsporidia, *Microsporidium*, Microspora, *Nosema*, *E. bieneusi*, *Encephalitozoon* spp., Iran, Human, Immunocompromised patients, Animal, Epidemiology, and Prevalence.

2.2. Study selection

The inclusion criteria were: all published articles up to December 2015, including descriptive, cross-sectional, case-control and epidemiology studies, and articles published in English and Persian. The studies with the reported overall prevalence rates for Microsporidia and Microsporidiosis were selected. Exclusion criteria were: articles that used other diagnostic methods, except staining and molecular techniques, articles written in a language other than English and Persian, unscientific publication about Microsporidia infection (abstracts, national conference proceedings) and duplicate studies with overlapping data. The suitability of all studies was considered by three different authors. Any disagreement was resolved by discussion among the authors. After selecting articles, the authors recorded the following information in a standard data extraction form. A flow diagram of the study design process has been shown in Figure 1.

2.3. Data extraction and analysis

After precise extraction of information, the results were categorized in a table composed of parts of province, year of publication, total individuals or participants, positive cases, gender, age, diagnostic methods, and genus of the organism involved. Fact estimates and 95% confidence intervals (CI) of prevalence of all involved studies were assessed. The total prevalence and group-specific prevalence were considered by age groups (>20, <20 years), gender (male and female). A forest plot was used to show the heterogeneity among the studies. It showed proportions of individual studies and total prevalence. The statistical techniques, I^2 and Cochran Q tests (P -value < 0.05) were used to compute the differences. For the purpose of meta-analysis, a random-effects model was used. The

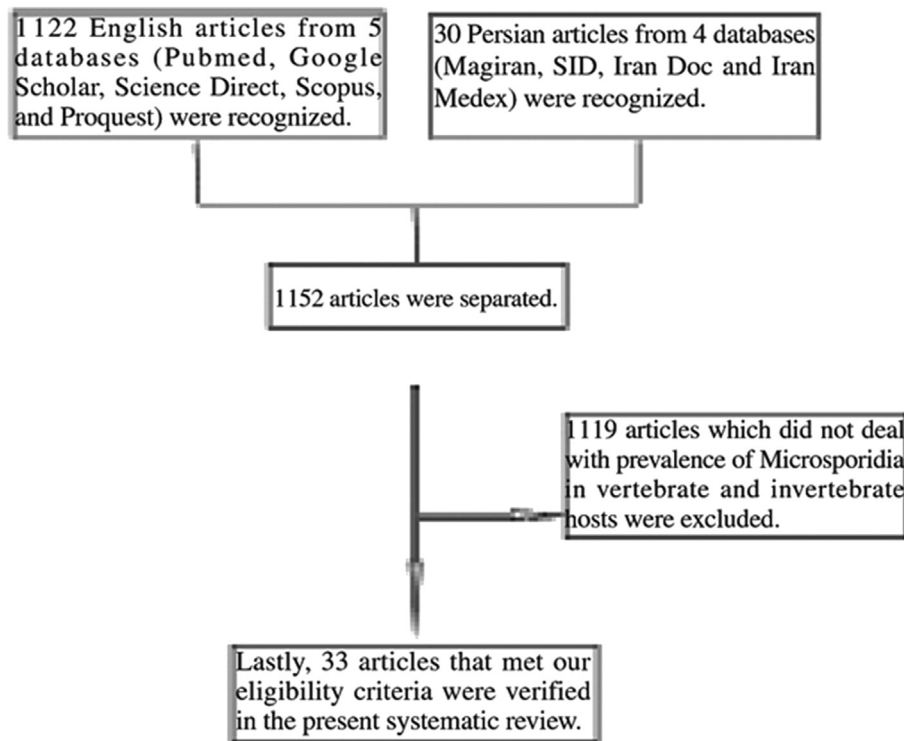


Figure 1. Flow diagram describing the study design process.

meta-analysis was completed with the trial version of Stats Direct statistical software. To demonstrate the taxonomic status of Microsporidia (genera and species) and their genotypes, the sequences were directly retrieved from GenBank database in FASTA format. Contigs (overlapped sequences) from all samples were aligned and edited at consensus positions and analysed using Sequencher Tmv.4.1.4 Software for PC (Gene Codes Corporation). MEGA 5.05 software and maximum likelihood algorithm with Kimura 2-parameter model were used to construct a phylogenetic tree. The accuracy of the phylogenetic tree was evaluated by 1000 bootstrap re-samplings.

3. Results

3.1. Prevalence of Microsporidia in human

Out of 1152 studies from the literature search, 33 records were suitable for inclusion in the systematic review and meta-analysis. Tables 1 and 2 showed the results of the literature search. There was a broad difference in the prevalence estimates among the various studies and the Q statistic was ($Q = 79.33$, $df = 5$, $P < 0.0001$, $I^2 = 93.7%$) in immunocompromised persons. Also, the overall prevalence rate of Microsporidia infection in immunocompromised patients in Iran using the random-effects model in meta-analysis was 8.18% (95%CI = 2.6%–16.4%) (Figure 2). Furthermore, the overall prevalence rate of Microsporidia infection in immunocompromised patients with chronic diarrhoea, immunocompromised patients with non-diarrhoea, gastroenteritis and patients with CD4 (<200 cells/ μ L) was 15.4% (11.5%–20.3%), 4.1% (2.4%–7.0%), 0.5% (0.3%–0.7%), and 12.9% (10.0%–16.4%), respectively (Table 2). The prevalence rates among immunocompromised

patients with chronic diarrhoea were significantly different from patients without diarrhoea ($P < 0.001$). In the present study, the most important examined factors were age and gender. There was no significant difference in Microsporidia prevalence between gender ($P > 0.05$), but there was a significant difference between <20 and >20 age groups (Table 2). The human prevalence rate varied from 0% in the Guilan to 29% in the Kerman province.

3.2. Prevalence of Microsporidia in honeybees

Table 3 showed the results of the literature search. The overall prevalence rate of Microsporidia infection in honeybees in Iran using the random-effects model in meta-analysis and the Q statistic was 40% (95%CI = 23%–60%) and ($Q = 231.93$, $df = 6$, $P < 0.0001$; $I^2 = 97.4%$) respectively (Figure 3). According to staining method positivity, the highest prevalence rate of nosemosis was estimated in East Azerbaijan (48.2%) (Table 3).

3.3. Prevalence of Microsporidia in other vertebrate and invertebrate animal groups

Table 4 shows the results of the literature search. The highest prevalence rate of animal Microsporidia was described in Khuzestan (26.5%). But, in two studies from Lorestan and Bushehr, Microsporidia infection rate was reported to as zero. We could not estimate the overall prevalence rate in the other vertebrate and invertebrate animal groups using the random-effects model in the meta-analysis because there were not enough similar articles to analyze and it has not been widely studied in Iran.

Table 1

Baseline characteristics of included human studies.

| Province | Year | Participation | | | Positive cases | | | | Method | | Genus | Ref |
|--|------|-------------------|------|--------|-----------------------|-----------|------------|-------------------------|--------|----------|--|------|
| | | Total individuals | Male | Female | Total individuals (%) | Male (%) | Female (%) | Age | PCR | Staining | | |
| Healthy people | | | | | | | | | | | | |
| East Azerbaijan | 2012 | 1825 | NA | NA | 4 (0.21) | NA | NA | – | 0 | 4 | – | [39] |
| Chaharmahal & Bakhtiari | 2013 | 65 | 47 | 18 | 12 (18.5) | NA | NA | Age range (17–60 years) | 12 | – | – | [40] |
| Tehran | 2014 | 178 | NA | NA | 7 (3.9) | NA | NA | Age range (12–68 years) | 7 | – | <i>E. intestinalis</i> <i>E. bienewsi</i> | [41] |
| Tehran | 2015 | 91 | 53 | 38 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 17–90 years | 0 | 0 | – | [42] |
| Isfahan | 2015 | 100 | 20 | 80 | 0 (0.0) | 0 (0.0) | 0 (0.0) | – | – | 0 | – | [43] |
| Kerman | 2015 | 100 | NA | NA | 29 (29) | NA | NA | – | – | 29 | – | [44] |
| Patients with gastroenteritis | | | | | | | | | | | | |
| Guilan | 2009 | 617 | 350 | 267 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0–over 30 years | – | 0 | – | [45] |
| Tehran | 2014 | 175 | NA | NA | 18 (10.3) | NA | NA | – | 18 | 18 | – | [46] |
| Tehran, Khorasan, Razavi, Mazandaran, Kurdistan, Qazvin, East Azerbaijan, Guilan | 2015 | 4200 | NA | NA | 4 (0.09) | NA | NA | 0–over 51 years | – | 4 | – | [47] |
| Immunocompromised patients | | | | | | | | | | | | |
| Tehran | 2012 | 71 | NA | NA | 13 (18.3) | NA | NA | – | 16 | 13 | <i>E. intestinalis</i> <i>E. bienewsi</i> | [48] |
| Fars | 2013 | 356 | 273 | 83 | 8 (2.24) | NA | NA | 10–69 years | 8 | 8 | <i>E. bienewsi</i> | [38] |
| Fars | 2013 | 44 | 23 | 21 | 3 (6.8) | 3 (13.04) | 0 (0.0) | 18 months–10 years | 3 | 3 | <i>E. bienewsi</i> | [49] |
| Hamedan | 2013 | 180 | 94 | 86 | 1 (0.55) | 0 (0.0) | 1 (1.16) | 17–71 years | – | 1 | – | [50] |
| Tehran | 2014 | 81 | 58 | 23 | 25 (30.9) | 17 (29.3) | 8 (34.8) | 20–65 years | 25 | 25 | <i>E. bienewsi</i> | [51] |
| Tehran | 2014 | 258 | NA | NA | 11 (4.3) | NA | NA | – | 11 | – | <i>E. intestinalis</i> <i>E. bienewsi</i> | [41] |
| Iran ^a | 2015 | 329 | NA | NA | 14 (4.25) | 8 | 6 | – | 14 | 14 | <i>E. bienewsi</i> | [20] |
| Patients diabetes | | | | | | | | | | | | |
| Isfahan | 2015 | 100 | 20 | 80 | 2 (2) | NA | NA | – | – | 2 | – | [43] |

^a Tehran and other provinces of the country.**Table 2**

Factors related to positivity for Microsporidia in Iranian human population.

| Factor | Total individuals | Positive cases | Overall prevalence (%) (95%CI) | P-values | Ref |
|---------------------|-------------------|----------------|--------------------------------|----------|---------------|
| Gender | | | | | |
| Male | 578 | 20 | 3.5 (2.3–5.3) | 0.17 | [42,45,49–51] |
| Female | 435 | 9 | 2.1 (1.1–3.9) | | |
| Age | | | | | |
| <20 | 617 | 0 | 0.0 | <0.001 | [42,45,51] |
| >20 | 172 | 25 | 1.5 (1.0–2.1) | | |
| Stool appearance | | | | | |
| Chronic diarrhoea | 260 | 40 | 15.4 (11.5–20.3) | <0.001 | [38,48,49,51] |
| Non diarrhoea | 292 | 12 | 4.1 (2.4–7.0) | | |
| Gastroenteritis | 5027 | 23 | 0.5 (0.3–0.7) | – | [45–47,50] |
| CD4 (<200 cells/μL) | 420 | 54 | 12.9 (10.0–16.4) | – | [20,38,48,51] |

3.4. Species and genotypes of Microsporidia

E. bienewsi is the most prevalent Microsporidia species in the included animal and human studies (Tables 1, 4 and 5). Based on molecular method positivity, *N. ceranae* was the most common *Nosema* species found to infect honeybees in Iran until now (Table 3). The most of Microsporidia isolates from immunocompromised patients and pigeons in Iran belonged to

genotypes D ($n = 16$; 50%) and E ($n = 6$; 20.6%) of *E. bienewsi*. Furthermore, genotypes L, M, J, and (genotypes D and K) of *E. bienewsi* displayed low-frequency rates (Table 5). A phylogenetic analysis revealed that the *E. bienewsi*, *E. intestinalis*, *E. hellem*, and *E. cuniculi* were grouped in their specific complex. All of *E. cuniculi*, obtained in this study were grouped in one clade with the highest bootstrap value (Figure 4).

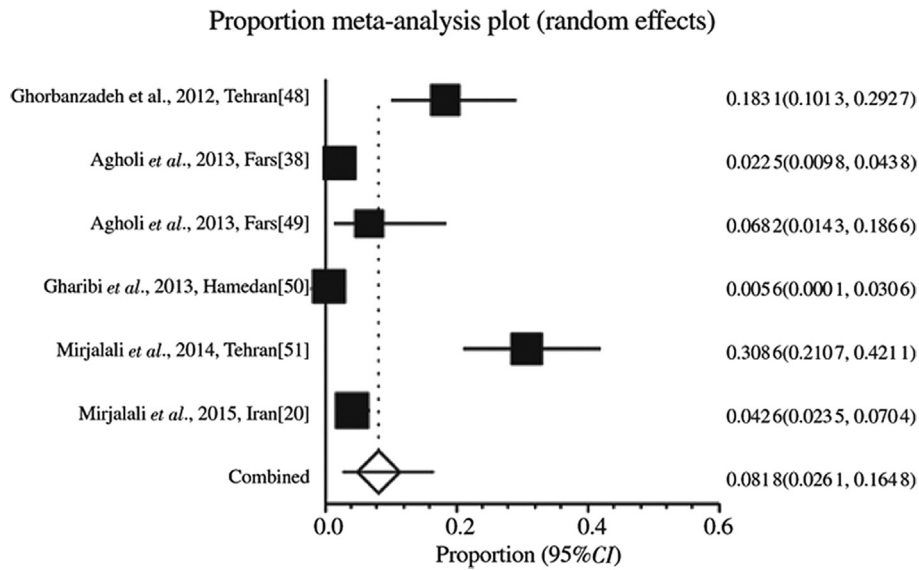


Figure 2. Forest plot diagram of 6 studies showing *Microsporidia* infection in Iranian immunocompromised population (first author, year and province of study) according to staining method positivity.

Table 3

Baseline characteristics of included honeybee studies.

| Province | Year | Number of cases | Invertebrate host | Positive cases | | Genus | Ref |
|-----------------|------|-----------------|------------------------|----------------|-----|-------------------|------|
| | | | | Staining | PCR | | |
| East Azerbaijan | 2008 | 150 (Hives) | Honeybees | 129 | – | <i>Nosema</i> | [52] |
| East Azerbaijan | 2009 | 294 (Colonies) | Honeybees | 72 | – | <i>Nosema</i> | [34] |
| West Azerbaijan | 2009 | 20 (Apiaries) | Adult honeybees | 6 | – | <i>N. apis</i> | [35] |
| East Azerbaijan | 2010 | 215 (Honeybees) | Honeybees | 79 | – | <i>N. apis</i> | [53] |
| Mazandaran | 2011 | 30 (Honeybees) | Adult worker honeybees | 6 | 6 | <i>N. ceranae</i> | [32] |
| North Khorasan | 2012 | 54 (Apiaries) | Adult honeybees | 12 | – | <i>N. apis</i> | [31] |
| East Azerbaijan | 2013 | 387 (Honeybees) | Honeybees | 225 | 260 | <i>N. ceranae</i> | [16] |

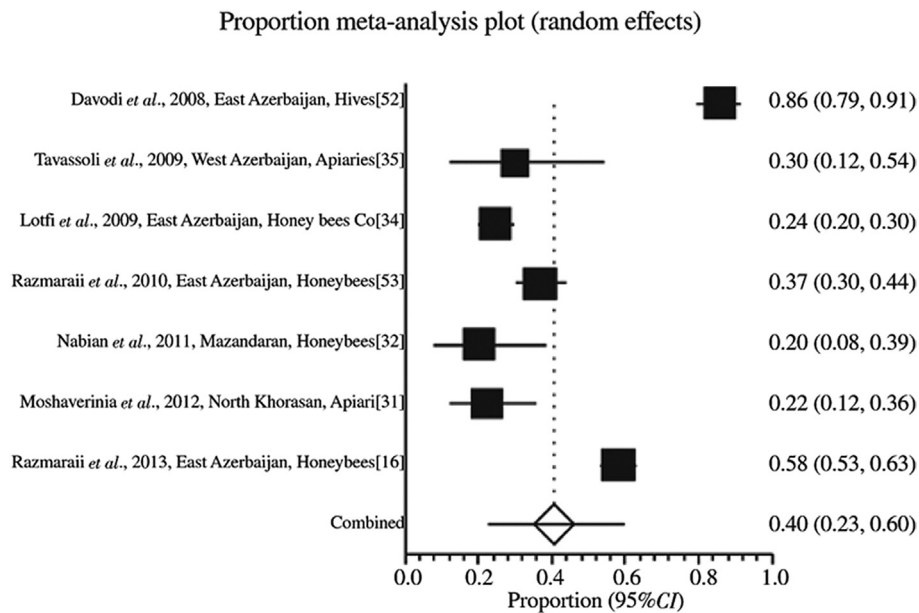


Figure 3. Forest plot diagram of 7 studies showing *Nosema* infection rate in honeybees (first author, year and province of study) according to the staining method.

Table 4

Baseline characteristics of included other (vertebrate and invertebrate) animal studies.

| Province | Year | Number of cases | Animal host | Positive cases | | Genus | Age | Ref |
|-----------------|------|-----------------|--|----------------|-----|---|--|------|
| | | | | Staining | PCR | | | |
| Khuzestan | 2009 | 50 | Lizard fish | 22 | – | <i>Glugea</i> | – | [54] |
| Tehran | 2012 | 126 | Slaughtered cattle | – | 19 | <i>E. bienersi</i> | – | [55] |
| Isfahan | 2012 | 140 | Dog, cat | 34 | 34 | [<i>E. bienersi</i> (8/100), <i>E. intestinalis</i> (5/100), <i>E. cuniculi</i> (18/100)] of dogs, [<i>E. bienersi</i> (3/40)] of cats | Mean age: dogs (4 years), cats (1.5 years) | [56] |
| Tehran | 2012 | 300 | Red fish (<i>Carassius auratus</i>) | 1 | – | Microsporidia spp. | – | [57] |
| Tehran | 2013 | 147 | Free-ranging and captive pigeons | – | 31 | <i>E. bienersi</i> , <i>E. intestinalis</i> , <i>E. hellem</i> , <i>E. cuniculi</i> | – | [9] |
| Lorestan | 2014 | 451 | Birds | 0 | – | – | – | [58] |
| Bushehr | 2015 | 100 | Marine shrimps | 0 | – | – | – | [59] |
| Khuzestan | 2015 | 312 | Free-ranging and captive pigeons | 74 | 71 | 29 (<i>E. bienersi</i> , <i>E. intestinalis</i>), 8 (<i>E. cuniculi</i>), 1 (<i>E. hellem</i>), 11 (<i>E. bienersi</i> , <i>E. cuniculi</i>), 1 (<i>E. bienersi</i> , <i>E. hellem</i>) | – | [60] |
| Ardabil, Tehran | 2015 | 142 | Animals with close-contact to human (mice, rabbits, ownership dogs, cats, sheep, dairy cattle) | 15 | 15 | <i>E. bienersi</i> , <i>E. cuniculi</i> | – | [61] |

Table 5Overview of *E. bienersi* and *Encephalitozoon* isolates included in the studies.

| Province | Host | Genus & species | Genotype | Accession number | Ref |
|-------------------|---------------------------|------------------------|----------|------------------|------|
| Fars | HIV+/AIDS | <i>E. bienersi</i> | D & K | – | [38] |
| Tehran | Pigeon | <i>E. bienersi</i> | D | JF776168 | [9] |
| | Pigeon | <i>E. bienersi</i> | M | JF776169 | |
| | Pigeon | <i>E. bienersi</i> | J | JF776170 | |
| | Pigeon | <i>E. intestinalis</i> | – | JF792394 | |
| | Pigeon | <i>E. hellem</i> | I | JF792395 | |
| | Pigeon | <i>E. hellem</i> | III | JF792396 | |
| | Pigeon | <i>E. cuniculi</i> | I | JF792397 | |
| | Pigeon | <i>E. cuniculi</i> | II | JF792398 | |
| Fars | Children liver transplant | <i>E. bienersi</i> | D | – | [49] |
| Tehran | HIV+/AIDS | <i>E. bienersi</i> | – | KF875441 | [51] |
| | HIV+/AIDS | <i>E. bienersi</i> | – | KF875442 | |
| | HIV+/AIDS | <i>E. bienersi</i> | – | KF875443 | |
| | HIV+/AIDS | <i>E. bienersi</i> | – | KF875444 | |
| | HIV+/AIDS | <i>E. bienersi</i> | – | KF875445 | |
| Iran ^a | Breast cancer | <i>E. bienersi</i> | E | KJ700424 | [20] |
| | Acute myeloid lymphoma | <i>E. bienersi</i> | D | KJ700425 | |
| | Gastric cancer | <i>E. bienersi</i> | E | KJ700426 | |
| | Gastric cancer | <i>E. bienersi</i> | D | KJ700427 | |
| | HIV+/AIDS | <i>E. bienersi</i> | E | KJ700428 | |
| | HIV+/AIDS | <i>E. bienersi</i> | D | KJ700429 | |
| | HIV+/AIDS | <i>E. bienersi</i> | D | KJ700430 | |
| | HIV+/AIDS | <i>E. bienersi</i> | D | KJ700431 | |
| | HIV+/AIDS | <i>E. bienersi</i> | E | KJ700432 | |
| | HIV+/AIDS | <i>E. bienersi</i> | E | KJ700433 | |
| | Liver transplantation | <i>E. bienersi</i> | D | KJ700434 | |
| | Heart transplantation | <i>E. bienersi</i> | D | KJ700435 | |
| | Kidney transplantation | <i>E. bienersi</i> | D | KJ700436 | |
| | Liver transplantation | <i>E. bienersi</i> | D | KJ700437 | |
| Ardabil, Tehran | Cattle | <i>E. bienersi</i> | – | KJ414443 | [61] |
| | Sheep | <i>E. bienersi</i> | – | KJ414444 | |
| | Mouse | <i>E. cuniculi</i> | – | KJ414445 | |
| | Cat | <i>E. bienersi</i> | – | KJ414446 | |
| | Cat | <i>E. bienersi</i> | – | KJ414447 | |
| | Dog | <i>E. bienersi</i> | – | KJ414448 | |
| | Rabbit | <i>E. bienersi</i> | – | KJ414449 | |
| | Rabbit | <i>E. cuniculi</i> | – | KJ414450 | |
| | Mouse | <i>E. cuniculi</i> | – | KJ414451 | |
| | Mouse | <i>E. cuniculi</i> | – | KJ414452 | |
| Khuzestan | Pigeon | <i>E. bienersi</i> | D | AB897488 | [60] |
| | Pigeon | <i>E. bienersi</i> | D1 | AB897489 | |
| | Pigeon | <i>E. bienersi</i> | D2 | AB897490 | |
| | Pigeon | <i>E. bienersi</i> | D3 | AB897491 | |

Table 5 (continued)

| Province | Host | Genus & species | Genotype | Accession number | Ref |
|----------|--------|------------------------|----------|------------------|-----|
| | Pigeon | <i>E. bieneusi</i> | L | AB897493 | |
| | Pigeon | <i>E. bieneusi</i> | L.a | AB897494 | |
| | Pigeon | <i>E. bieneusi</i> | L.1 | AB897495 | |
| | Pigeon | <i>E. bieneusi</i> | L.2 | AB897496 | |
| | Pigeon | <i>E. bieneusi</i> | M | AB897497 | |
| | Pigeon | <i>E. bieneusi</i> | M1b | AB897498 | |
| | Pigeon | <i>E. bieneusi</i> | E1 | AB897499 | |
| | Pigeon | <i>E. intestinalis</i> | – | AB897501 | |
| | Pigeon | <i>E. hellem</i> | – | AB897502 | |
| | Pigeon | <i>E. hellem</i> | – | AB897503 | |
| | Pigeon | <i>E. hellem</i> | – | AB897505 | |
| | Pigeon | <i>E. cuculi</i> | – | AB897507 | |

^a Tehran and other provinces of the country.

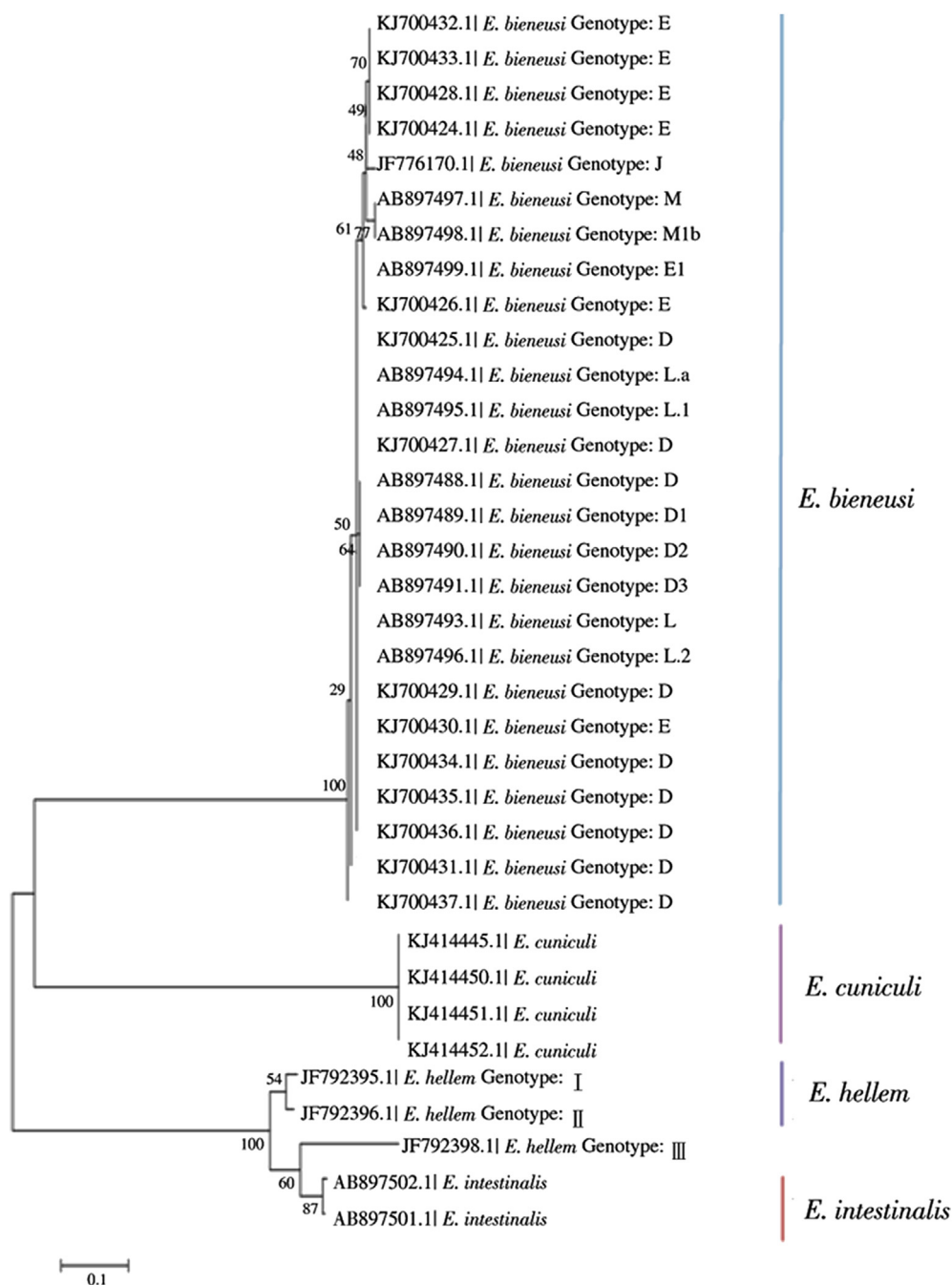


Figure 4. Phylogenetic analysis of SSU-ITS nucleotide sequences of *E. bieneusi* and *Encephalitozoon* spp. isolates recovered from human population and animals (vertebrate hosts) in Iran.

The tree was constructed by using the Kimura 2-parameter model in MEGA software version 5.05. The numbers above the branches indicated the percentage of bootstrap re-sampling.

4. Discussion

Few studies are available of Microsporidia prevalence among healthy individuals and immunocompromised patients in Iran. The present systematic review evaluates Microsporidia infection rate in vertebrate and invertebrate hosts using the collected data from various regions of Iran. This study was done using nine databases, and 33 records published up to December 2015 that show the prevalence rate of microsporidiosis in Iran. Microsporidiosis is recognized as an opportunistic infection that occurs worldwide. However, mainly due to differences in diagnostic methods, the prevalence rates of infection varies [11]. Most studies about the prevalence of Microsporidia have been conducted in developed countries where laboratory tools are easily accessible compared to those in developing countries. Microsporidia prevalence rate from HIV-seropositive patients in developed countries have been found to be between 2% and 78% [1]. In developing countries, *E. bienersi's* prevalence rate has been noted to be 2.5%–51% for HIV-seropositive adult patients with diarrhoea and 4.6% for patients without diarrhoea. In contrast, *Encephalitozoon* spp. infection has a very low rate [1]. HIV positive patients with microsporidiosis were mostly reported to be from Southeast Asia (India, Thailand), the Middle-East (Turkey), Europe, Africa (Tunisia, Mali, Uganda, Senegal, Zimbabwe), and Latin America (Brazil, Peru) [10]. In this study, the overall prevalence rate of Microsporidia infection in immunocompromised patients in Iran using the random-effects model in the meta-analysis was 8.18% (95%CI = 2.6%–16.4%). Furthermore, the overall prevalence rate of Microsporidia infection in immunocompromised patients with chronic diarrhoea, immunocompromised patients with non-diarrhoea, gastroenteritis, and patients with CD4⁺ (<200 cells/μL) was 15.4% (11.5%–20.3%), 4.1% (2.4%–7.0%), 0.5% (0.3%–0.7%), and 12.9% (10.0%–16.4%) respectively. Immunodeficiency, particularly that related to HIV positive patients (CD4⁺ T cells ≤ 50 cells per micro litre blood) and children are all considered risk factors for intestinal microsporidiosis [21]. Therefore, Microsporidia infection in children with gastrointestinal complaints in Iran should be assessed. Poor hygiene and a developing immune system in very young children pose a higher risk for microsporidian infection. In comparison with this study about the prevalence of this pathogen in patients with low CD4⁺ count, the prevalence of microsporidiosis in patients with high CD4⁺ count has also been described [22]. The results of this study showed a significantly high percentage of total Microsporidia infection among patients with chronic diarrhoea than those without diarrhoea ($P < 0.001$). It is not clear if diarrhoea in immunocompromised patients can be attributed to this pathogen. It is better to examine this likelihood and relate it with pathogens isolated from patients with diarrhoea. Age and gender were the most important factors in the present study. The overall rate of Microsporidia infection in the Iranian population was 1.5% in >20 years age group. This indicates the need for further study on children and people aged less than 20 years old. In our study, more male patients were infected with Microsporidia than female patients, but there was no significant difference in occurrence between genders in the statistical analysis. In Turkey, a study of 93 patients with cancer and undergoing chemotherapy and 30 healthy volunteers showed an infection rate of 69.9% (65 cases) and 16.7% (five cases), respectively. From 70 positive cases, 44 and 26 cases corresponded to males and females respectively. Of the patients

with diarrhoea, 68.6% (35/51) were infected with Microsporidia [23]. In another study from Pakistan, the prevalence rate of Microsporidia infection in patients with chronic diarrhoea and hepatocellular carcinoma (HCC) and healthy groups was found to be 3% by staining and 4% using PCR method. The patients were 222 (67%) males and 108 (33%) females with a mean age of 41 ± 14 years (range 15–83 years) [24]. In Iraq a total of 58 children with malignancy and 107 healthy children, 10.3% of patients were infected. None of the healthy children were infected [25]. As observed in this study, the difference between the presence of *Microsporidium* and diarrhoea was statistically significant ($P < 0.001$). The highest rate of infection was observed in male patients, while the prevalence was in disagreement with the result of these studies. The prevalence of *E. bienersi* in children (mostly HIV⁺) have been reported from African countries like Nigeria (0.8%), South Africa (4.5%), Uganda (32.9%), and Zimbabwe up to 50% [21]. In our study, the human Microsporidia prevalence rate varied from 0% in Guilan to 29% in the Kerman province. The first case of ocular microsporidiosis was detected in an 11-year-old Tamil boy with stromal keratitis in 1973 [26]. Moreover, a case series of five patients with microsporidial stromal keratitis was reported in South India [27]. Increasing worldwide reports should bring about more studies to identify the possibility of ocular microsporidiosis in Iran, especially in people who wear contact lenses. Some of the studies indicated that humans are more susceptible to microsporidiosis in winter than spring because of decreasing immunity and resistance during winter [28].

Microsporidia is also found in various animals (vertebrate and invertebrate), but the importance of zoonotic infection especially in developing countries has not been studied. In the present study, the infection rate of *E. bienersi* in dairy cattle, beef, and water buffaloes was 4.9%, 5.4%, and 2.2% respectively and the overall infection rate was 4.4% [29]. In our study, the highest prevalence rate of animal Microsporidia was described in Khuzestan (26.5%). But, in two studies from Lorestan and Bushehr, Microsporidia infection rate was reported to as zero. With increasing global reports, it would be wise to examine microsporidial infection in different groups of animals in Iran.

Microsporidia have been recognized to be the main cause of disease in insects (silk worms and honeybees), fish and mammals [30]. *N. apis* and *N. ceranae* are the only causes of nosemosis in honeybees, which reduce both the honey yield and the population of honeybees [7,8]. The results of studies indicated that cold winter, high rainfall, poor controlling of the apiaries especially in winter, other parasitic diseases and agriculture pesticide are factors for the rising vulnerability of honeybees to *Nosema* [31]. So, it is necessary to be aware of the disease signs in order to be ready for infection control. Microscopic examination is the most common technique for diagnosis of *Nosema* spores in all of the studies in Iran, but it seems that in mixed infections caused by *N. ceranae* and *N. apis* in honeybees, molecular techniques will be required for the discrimination of two Microsporidia species from each other, because the spores of these species could be differentiated morphologically only by expert researchers. The first molecular investigation of *N. ceranae* in honeybees in Iran was done by Nabain et al. [32,33]. Previous reports of the distribution of nosemosis in Iran supposed that only one *Nosema* species, *N. apis*, infected honeybees. But in recent studies, *N. ceranae* has been found to be the most dominant

species in Iran [16,32]. In our study, the overall prevalence rate of *Nosema* is 40% (23%–60%) in honeybees. According to staining method positivity, the highest prevalence rate of nosemosis was estimated in East Azerbaijan (48.2%). Most studies showed that *N. ceranae* is more pathogenic and prevalent *Nosema* species which is similar to the studies conducted in Iran [33]. According to the results from different studies, the highest infection rate was reported in spring and the lowest in the summer [31,34]. Nosemosis is a threat to the beekeeping industry. For this reason, a mixture of fumagillin treatment and sterilization of devices (heat or acetic acid) is used for controlling nosemosis [35]. However, recent studies have shown that fumagillin changed the structural and metabolic proteins in honeybee midgut tissues that may aggravate *N. ceranae* infection [36].

Using light microscopy after specific staining is proper as a routine test, but it cannot distinguish species of Microsporidia. Molecular methods have led to more reliable results compared to others techniques (staining, immunological, etc.). In spite of PCR, the staining method is simple, cheap, and more practical for early diagnosis. Cell culture is another method that assists the investigation of Microsporidia biology and allows for easy assessment of drugs. But, the most common microsporidial species infecting humans, *E. bienersi*, has not been cultured in long-term cultures [37]. Over 93 genotypes of *E. bienersi* have been recognized in humans and vertebrate animals. Genotype D and E have been reported as the most prevalent in some studies [19,20]. Genotype D was not only derived from human population, but also isolated from animals such as pig, cattle, horse, dog, beaver, fox, and raccoon [38]. In our study, most Microsporidia isolates from immunocompromised patients and pigeons in Iran belonged to genotypes D and E of *E. bienersi*. Understanding the genetic differences of Microsporidia strains among the population will be worthwhile for determining the source, transmission, and pathogenesis of this microorganism.

In conclusions, this study may be the first systematic review that provides a broad outlook of the prevalence of microsporidiosis in Iran. It is necessary to examine Microsporidia infection in vertebrate and invertebrate hosts and environmental resources in Iran. There is no doubt that advances in diagnostic methods and molecular epidemiology will be helpful for our knowledge about the mode of transmission and risk factors related to microsporidiosis.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

- [1] Sak B, Brady D, Pelikanova M, Kvetonova D, Rost M, Kostka M, et al. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *J Clin Microbiol* 2011; **49**(3): 1064-1070.
- [2] Didier E, Wossbrinck C, Stowall M, Green L, Bowers L, Fredenburg A, et al. Diagnosis and epidemiology of microsporidian infections in humans. *Southeast Asian J Trop Med Public Health* 2004; **35**: 65-81.
- [3] Meyers WM, Firpo A, Wear DJ. *Topics on the pathology of protozoan and invasive arthropod diseases*. Washington D.C.: Armed Forces Institute of Pathology Press; 2011, p. 1-24.
- [4] Ramanan P, Pritt BS. Extraintestinal microsporidiosis. *J Clin Microbiol* 2014; **52**(11): 3839-3844.
- [5] Dengjel B, Zahler M, Hermanns W, Heinritz K, Spillmann T, Thomschke A, et al. Zoonotic potential of *Enterocytozoon bienersi*. *J Clin Microbiol* 2001; **39**(12): 4495-4499.
- [6] Rinder H, Thomschke A, Dengjel B, Gothe R, Loscher T, Zahler M. Close genotypic relationship between *Enterocytozoon bienersi* from humans and pigs and first detection in cattle. *J Parasitol* 2000; **86**(1): 185-188.
- [7] Abedi V, Safaei H. A survey of *Nosema apis* infection in apiaries of North Khorasan province, Iran. *Iran J Vet Sci Technol* 2014; **4**(2): 25-30.
- [8] Modirrousta H, Moharrami M, Mansouri M. Retrospective study of the *Nosema ceranae* infection of honey bee colonies in Iran (2004–2013). *Arch Razi Inst* 2014; **69**(2): 197-200.
- [9] Pirestani M, Sadraei J, Forouzandeh M. Molecular characterization and genotyping of human related microsporidia in free-ranging and captive pigeons of Tehran, Iran. *Infect Genet Evol* 2013; **20**: 495-499.
- [10] Anane S, Attouchi H. Microsporidiosis: epidemiology, clinical data and therapy. *Gastroenterol Clin Biol* 2010; **34**(8): 450-464.
- [11] Didier ES, Weiss LM. Microsporidiosis: current status. *Curr Opin Infect Dis* 2006; **19**(5): 485-492.
- [12] Desportes I, Charpentier YL, Galian A, Bernard F, Cochand-Priollet B, Lavergne A, et al. Occurrence of a new microsporidian: *Enterocytozoon bienersi* ng. n. sp., in the enterocytes of a human patient with AIDS. *J Protozool* 1985; **32**(2): 250-254.
- [13] Didier ES. Microsporidiosis: an emerging and opportunistic infection in humans and animals. *Acta Trop* 2005; **94**: 61-76.
- [14] Kotler DP, Orenstein JM. Clinical syndromes associated with microsporidiosis. *Adv Parasitol* 1998; **40**: 321-349.
- [15] Chen Y, Evans JD, Zhou L, Boncristiani H, Kimura K, Xiao T, et al. Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. *J Invertebr Pathol* 2009; **101**(3): 204-209.
- [16] Razmaraii N, Sadegh-Eteghad S, Babaei H, Paykari H, Esmailinia K, Frogly L. Molecular identification of *Nosema species* in East Azerbaijan province. *Iran Arch Razi Inst* 2013; **68**(1): 23-27.
- [17] Blanshard C, Ellis DS, Tovey DG, Dowell S, Gazzard BG. Treatment of intestinal microsporidiosis with albendazole in patients with AIDS. *Aids* 1992; **6**(3): 311-314.
- [18] Molina JM, Tourneur M, Sarfati C, Chevret S, de Gouvello A, Gobert JG, et al. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med* 2002; **346**(25): 1963-1969.
- [19] Santin M, Fayer R. Microsporidiosis: *Enterocytozoon bienersi* in domesticated and wild animals. *Res Vet Sci* 2011; **90**(3): 363-371.
- [20] Mirjalali H, Mirhendi H, Meamar AR, Mohebbi M, Askari Z, Mirsamadi ES, et al. Genotyping and molecular analysis of *Enterocytozoon bienersi* isolated from immunocompromised patients in Iran. *Infect Genet Evol* 2015; **36**: 244-249.
- [21] Lobo ML, Xiao L, Antunes F, Matos O. Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and-negative patients. *Int J Parasitol* 2012; **42**(2): 197-205.
- [22] Sowerby T, Contreas C, Berlin O, Donovan J. Microsporidiosis in patients with relatively preserved CD4 counts. *Aids* 1995; **9**(8): 975.
- [23] Hamamci B, Cetinkaya U, Berk V, Kaynar L, Kuk S. Prevalence of *Encephalitozoon intestinalis* and *Enterocytozoon bienersi* in cancer patients under chemotherapy. *Mikrobiyol Bul* 2015; **49**(1): 105-113.
- [24] Yakoub J, Abbas Z, Beg MA, Jafri W, Naz S, Khalid A, et al. Microsporidial infections due to *Encephalitozoon intestinalis* in

- non-HIV-infected patients with chronic diarrhoea. *Epidemiol Infect* 2012; **140**(10): 1773-1779.
- [25] Nadham Kadham M, Maysloon AAS. Microsporidiosis among children with malignant diseases in Basrah, Iraq. *Pak J Med Sci* 2012; **28**(4): 621-624.
- [26] Ashton N, Wirasinha PA. Encephalitozoonosis (nosematosis) of the cornea. *Br J Ophthalmol* 1973; **57**(9): 669-674.
- [27] Vemuganti GK, Garg P, Sharma S, Joseph J, Gopinathan U, Singh S. Is microsporidial keratitis an emerging cause of stromal keratitis? A case series study. *BMC Ophthalmol* 2005; **5**: 19.
- [28] Liu H, Shen Y, Yin J, Yuan Z, Jiang Y, Xu Y, et al. Prevalence and genetic characterization of *Cryptosporidium*, *Enterocytozoon*, *Giardia* and *Cyclospora* in diarrheal outpatients in China. *BMC Infect Dis* 2014; **14**: 25.
- [29] Ma JB, Li P, Zhao XP, Xu HL, Wu WX, Wang YF, et al. Occurrence and molecular characterization of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in dairy cattle, beef cattle and water buffaloes in China. *Vet Parasitol* 2015; **207**(3-4): 220-227.
- [30] Franzen C. Microsporidia: a review of 150 years of research. *Open Parasitol J* 2008; **2**: 1-34.
- [31] Moshaverinia A, Abedi V, Safaei H. A survey of *Nosema apis* infection in apiaries of North Khorasan province, Iran. *Iran J Vet Sci Technol* 2012; **4**(2): 25-30.
- [32] Nabian S, Ahmadi K, Shirazi MN, Sadeghian AG. First detection of *Nosema ceranae*, a microsporidian protozoa of European honeybees (*Apis mellifera*) in Iran. *Iran J Parasitol* 2011; **6**(3): 89.
- [33] Utuk AE, Piskin FC, Girisgin AO, Selcuk O, Aydin L. Microscopic and molecular detection of *Nosema* spp. in honeybees of Turkey. *Apidologie* 2015; **47**(2): 267-271.
- [34] Lotfi A, Jamshidi R, Aghdam Shahryar H, Yousefkhani M. The prevalence of nosemosis in honey bee colonies in Arasbaran region (Northwestern Iran). *Am Eurasian J Agri Enviro Sci* 2009; **5**(2): 255-257.
- [35] Tavassoli M, Eiganinejad S, Alizadeh-Asl S. A survey on *Nosema apis* infection in apiaries of Urmia, North-West of Iran. *Iran J Vet Sci Technol* 2009; **1**(1): 35-40.
- [36] Huang WF, Solter LF, Yau PM, Imai BS. *Nosema ceranae* escapes fumagillin control in honey bees. *PLoS Pathog* 2013; **9**(3): e1003185.
- [37] Garcia LS. Laboratory identification of the microsporidia. *J Clin Microbiol* 2002; **40**(6): 1892-1901.
- [38] Agholi M, Hatam GR, Motazedian MH. HIV/AIDS-associated opportunistic protozoal diarrhea. *AIDS Res Hum Retroviruses* 2013; **29**(1): 35-41.
- [39] Shahbazi A, Gholami S, Mirsamadi N, Nokhahi I, Ghazanchaii A, Kumar D, et al. Detection of *Cyclospora*, Microsporidia and *Cryptosporidium* by direct microscopy and PCR in stools specimens in Northwest, Iran. *Res Opin Anim Vet Sci* 2012; **2**(5): 352-359.
- [40] Khalili B, Imani R, Boostani S. Intestinal parasitic infections in chronic psychiatric patients in Sina Hospital Shahre-Kord. *Iran Jundishapur J Microbiol* 2013; **6**(3): 252-255.
- [41] Tabatabaie F, Tafreshi ZA, Shahmohammad N, Pirestani M. Molecular detection of microsporidiosis in various samples of Iranian immunocompromised patients. *J Parasit Dis* 2015; **39**(4): 634-638.
- [42] Ghamiloui M, Valadkhani Z, Rahimi F. A study of microsporidiosis in corneal scrapings of keratitis patients referring to Farabi Eye Hospital, Tehran, Iran in 2013–14. *Curr Med Mycol* 2015; **1**(3): 39-44.
- [43] Mohtashampour M, Hoseini SGG, Pestehchian N, Yousefi H, Fallah E, Hazratian T. Intestinal parasitic infections in patients with diabetes mellitus: a case-control study. *J Anal Res Clin Med* 2015; **3**(3): 157-163.
- [44] Ghaderipour M, Rezaeian M, Khanaliha Kh, Barkhori M, Mirjalali H. Human intestinal microsporidial infection in some urban areas in Jiroft, Kerman province in 2015. *Iran J Parasitol* 2015; **10**(Suppl. 1): 269.
- [45] Vahabzadeh H, Nahrevanian H, Assmar M, Zahraei M, Habibzadeh M, Mafi M. Prevalence of sporozoan and parasitic enteropathogen protozoans in patients with gastroenteritis in Gilan. *J Biol Sci* 2009; **3**(2): 81-90.
- [46] Khanaliha K, Mirjalali H, Mohebbali M, Tarighi F, Rezaeian M. Comparison of three staining methods for the detection of intestinal *Microspora* spp. *Iran J Parasitol* 2014; **9**(4): 445-451.
- [47] Nahrevanian H, Assmar M, Zahraei SM, Mafi M, Masoumi Asl H, Ghasemi FS. Sporozoan protozoa and enteroparasites in the gastroenteric patients referring to the healthcare centers of seven provinces of Iran. *Int J Enteric Pathog* 2015; **3**(3): e27087.
- [48] Ghorbanzadeh B, Sadraie J, Emadi Kuchak H. Diagnosis of *Cryptosporidium* and intestinal microsporidia in HIV/AIDS patients with staining and PCR methods on 16srRNA gene. *Arak Med Univ J* 2012; **15**(7): 37-47.
- [49] Agholi M, Hatam GR, Motazedian MH. Microsporidia and coccidia as causes of persistence diarrhea among liver transplant children: incidence rate and species/genotypes. *Pediatr Infect Dis J* 2013; **32**(2): 185-187.
- [50] Gharibi Z, Daadras F, HosseinMaghsood A, Fallah M, Saedijam M. The identification of intestinal microsporidia by trichrome staining and calcofluor white methods among kidney-transplanted patients in Hamadan. *Med Lab J* 2013; **7**(4): 47-52.
- [51] Mirjalali H, Mohebbali M, Mirhendi H, Gholami R, Keshavarz H, Meamar AR, et al. Emerging intestinal Microsporidia infection in HIV+/AIDS patients in Iran: microscopic and molecular detection. *Iran J Parasitol* 2014; **9**(2): 149-154.
- [52] Davodi J, Naderi A, Mohammadpour F, Golzaradabi S, Faramarzi A. Study of infection rate of suburb bee hives to parasites *Nosma apis*, *Varroa* spp. and *Acarapis woodi* in Miyaneh. *Agroecol J* 2008; **4**(13): 39-43.
- [53] Razmaraii N, Karimi H. A survey of *Nosema* of honey bees (*Apis mellifera*) in East Azarbaijan province of Iran. *J Anim Vet Adv* 2010; **9**(5): 879-882.
- [54] Peyghan R, Nabavi L, Jamshidi K, Akbari S. Microsporidian infection in lizardfish, *Saurida undosquamis* of Persian Gulf. *Iran J Vet Res* 2009; **10**(2): 180-185.
- [55] Pirestani M, Sadraei J, Forouzandeh M. Molecular characterization of zoonotic isolates of *Enterocytozoon bieneusi* in Iran. *Feyz J Kashan Univ Med Sci* 2012; **16**(1): 51-57.
- [56] Jamshidi S, Tabrizi AS, Bahrami M, Momtaz H. Microsporidia in household dogs and cats in Iran; a zoonotic concern. *Vet Parasitol* 2012; **185**(2): 121-123.
- [57] Bahri A, Mokhayer B, Khoshkhoo Z, Asadzadeh Am. Investigating the parasitic infections in gold fish (*Carassius Auratus*) native to Iran in aquariums in spring season in 2011 (Non-imported) in Tehran province. *J Aquat Anim Fish* 2012; **3**(11): 1-7.
- [58] Badparva E, Ezatpour B, Azami M, Badparva M. First report of birds infection by intestinal parasites in Khorramabad, west Iran. *J Parasit Dis* 2015; **39**(4): 720-724.
- [59] Sadeghi RL, Golchin Am, Azhdari S, Farzam M, Mohammadi S, Farokhnia F, et al. Investigation on microsporidian infection prevalence rate in marine shrimps in coastal city of Bushehr. *Univ Res J Med Sci* 2015; **2**(3): 16-20.
- [60] Tavalla M, Kazemi E. Prevalence of *Microsporidia* spp. in pigeons in a south region of Iran, Ahvaz. *Iran J Parasitol* 2015; **10**(Suppl. 1): 43.
- [61] Askari Z, Mirjalali H, Mohebbali M, Zarei Z, Shojaei S, Rezaeian T, et al. Molecular detection and identification of zoonotic microsporidia spore in fecal samples of some animals with close-contact to human. *Iran J Parasitol* 2015; **10**(3): 381-388.