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Molecular Prevalence and Genotype Distribution of *Enterocytozoon bieneusi* in Different Hosts in Türkiye: A Systematic Review and Meta-Analysis

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Abstract

Enterocytozoon bieneusi is one of the most common zoonotic pathogens, infecting a wide range of hosts and has also been reported worldwide. In this study, we conducted a systematic review and meta-analysis showing the prevalence and genotypic characterization of *E. bieneusi* in Türkiye. Four databases [PubMed, Web of Science, Google Scholar, and Turkish Academic Network and Information Center (Ulakbim)] were used for the search, and a total of 15 studies were included. These studies were distributed across eleven provinces and ten hosts, including humans, livestock, pets, and milk samples from cattle, sheep, and water buffalo. A total of 3427 samples were examined, and the pooled prevalence of *E. bieneusi* was calculated to be 9.4% (95% CI, 5.5–15.5%). Most samples in the dataset were from humans ($n = 723$). ERUSS1 ($n = 73$) was the dominant genotype among the identified *E. bieneusi* genotypes. The five genotypes (D, Type IV, YNDCEB-90, Peru 6, and ERUNT1) were assigned to Group 1, which is considered zoonotic and poses a major public health threat to humans, and the remaining genotypes ($n = 20$) were assigned to Group 2, known as a ruminant-specific group. No genotypic characterization of human samples was performed. We recommend additional studies to identify *E. bieneusi* genotypes in humans and in unstudied areas as target regions to improve our understanding of the epidemiology of *E. bieneusi* in Türkiye.

Keywords: *Enterocytozoon bieneusi*, meta-analysis, prevalence, systematic review, Türkiye

What is already known on this topic?

- *To date, E. bieneusi has been identified in humans, domestic and wild animals, and some insect species from many parts of the world.*

What this study adds on this topic?

- *This study presents a systematic review of the data concerning host distribution, prevalence, geographical regions, and genotypic characteristics of E. bieneusi isolates derived from previous research in Türkiye up to this point.*
- *It is suggested to better understand the epidemiology and zoonotic potential of E. bieneusi in Türkiye by focusing on new hosts or regions that have not been studied.*

Introduction

Microsporidia are obligate intracellular parasites that infect both vertebrate and invertebrate hosts and are found worldwide (Pan et al., 2018; Han et al., 2020). They have been identified in different environmental ecosystems (Cali & Takvorian, 2004). To date, there are more than 1700 described species belonging to at least 200 genera (Han et al., 2021). Also, 17 microsporidian species in nine genera are known as human pathogens, especially in immunosuppressed individuals or asymptomatic immunocompetent individuals (Anane & Attouchi, 2010; Pan et al., 2018). *Enterocytozoon bieneusi* Desportes, 1985 and *Encephalitozoon* spp. are the most common species among microsporidians, and *E. bieneusi* is the most identified species from different hosts (Pekmezci et al., 2019; Wang et al., 2018).

So far, about 500 distinct *E. bieneusi* genotypes belonging to 11 different phylogenetic groups have been identified based on analyzing the nucleotide variations in the internal transcribed spacer (ITS) region of the rRNA gene (Li et al., 2019a; 2019b). Of the 500 genotypes, 142 are from humans and 49 from both humans and animals (Zheng et al., 2020). The phylogenetic analysis of these genotypes revealed 11 main groups that differ in their host specificity and zoonotic characteristics. Most zoonotic genotypes have been assigned to Group 1 and Group 2. Group 1 has the largest number of genotypes, of which genotypes D, EbpC, EbpA, and Type IV have been identified across the widest host and geographic range. This group is characterized as human pathogenic group, while Group 2 is considered ruminant-specific (Li et al., 2019a). Although the host specificity of these groups is limited, some genotypes, such as C and B, which belong to Group 1, have only been detected in humans in different countries (Li et al., 2020). In recent years, some Group 2 genotypes, such as BEB4, BEB6, J, and I, have been detected in different

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hosts, including humans (Liu et al., 2022), drawing attention to the zoonotic transmission of *E. bieneusi*. Besides, Groups 3–11 exhibit strong host specificity (Li et al., 2019a).

From a "One Health" perspective, which considers the interconnected health and welfare of humans, animals, and environments (Monath et al., 2010), understanding the genotype diversity, host distribution, and geographical spread of *E. bieneusi* is crucial for both public health and animal breeding. Consequently, we conducted a systematic review and meta-analysis to assess the molecular prevalence, genetic diversity, and host distribution of *E. bieneusi* in Türkiye.

Materials and Methods

Information Sources and Systematic Search

The present study followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statements (Moher et al., 2009). Literature searches for published studies on the molecular prevalence and genotypic characterization of *E. bieneusi* in different hosts in Türkiye were retrieved from four databases: PubMed, Web of Science, Google Scholar, and Turkish Academic Network and Information Center (Ulakbim), searching up to March 2024. The search process was carried out using MeSH terms in combination: ("Microsporidium" OR "Microsporidia" OR "*Enterocytozoon*" OR "*Enterocytozoon bieneusi*") AND ("Prevalence" OR "Molecular Prevalence" OR "Genotype") AND ("Turkey" OR "Türkiye"). Moreover, manual searches were done as a supplement.

Inclusion Criteria, Study Selection and Data Extraction

Initially, articles were screened by their title and abstract, and the chosen ones were then imported into the EndNote software (Thomson Reuters, New York, USA). One conference proceeding was searched manually and included (Çifci et al., 2023). Duplicate research was checked and deleted. The inclusion criteria are as follows: (1) studies focusing on the prevalence and genotyping of *E. bieneusi* in Türkiye, (2) studies written in English and/or Turkish, (3) studies published up to March 2024, (4) studies that reported the precise total sample size and number of positive samples, and (5) use of different molecular methods. The exclusion criteria of studies were as follows: (1) not in English or Turkish language, (2) data error, no data, or data duplication, (3) studies without *E. bieneusi*, (4) studies that do not explicitly give total sample size and/or positive cases and/or prevalence values, and (5) all types of review articles, case reports, and case series. In the following step, the data from the finalized studies included the first author's last name, publication year, country, diagnostic method, type of source, total sample size tested, and the number of positive samples isolated.

Data Synthesis and Statistical Analysis

For each selected study, the random-effects model, which allows for a distribution of true effect sizes among studies, was used to calculate the point estimates and their respective 95% CIs. Forest plot analysis was carried out to reveal possible heterogeneity among included studies. The heterogeneity between studies was computed via the I^2 index. An I^2 value at >80% was defined as high heterogeneity (Taghipour et al., 2020; 2021a). All analytical functions were applied using comprehensive meta-analysis software (version 2, BIOSTAT, Englewood, NJ, USA) (Borenstein, 2022; Nasiri et al., 2015).

Results

A total of 620 records were initially determined from four databases. According to the exclusion criteria, 606 studies were excluded. One conference proceeding was manually detected and included in the dataset. Finally, 15 studies were conducted for a meta-analysis (Figure 1, Tables 1 and 2). These studies were distributed across 11 provinces and ten hosts, including sheep, humans, cattle, chickens, cats, water buffalo, budgerigars, pigeons, horses, and donkeys, as well as milk from cattle, sheep, and water buffalo (Figure 2). A total of 3427 samples were tested, and the pooled prevalence of *E. bieneusi* in different hosts and milk samples was calculated at 9.4% (95% CI, 5.5–15.5%; I^2, 96.30%) (Figure 3). Most studies focused on animal hosts ($n = 11$), and all but one study (Yildirim et al., 2020a) examined stool samples. The largest number of samples in the dataset belonged to humans ($n = 723$) (Table 1). ERUSS1 ($n = 73$) was the dominant genotype among the identified *E. bieneusi* genotypes. It was found that Kayseri had the highest number of studies ($n = 6$) among all provinces (Tables 1 and 2). Except for three studies (Aydemir et al., 2023; Hamamcı et al., 2015; Oğuz Kaya et al., 2018), the other studies determined the genotypic characterization of *E. bieneusi*. The five genotypes (D, Type IV, YNDCEB-90, Peru 6, and ERUNT1) were assigned to Group 1, considered zoonotic and posing a major threat to humans (Li et al., 2019c), and the remaining genotypes ($n = 20$) were assigned to Group 2, a ruminant-specific group, possessing increasing zoonotic concern as its host specificity is not so strong (Li et al., 2019b; Wang et al., 2013). The genotypes of Group 1 were identified in animal hosts such as cats, donkeys, water buffalo, pigeons, and chickens (Table 2).

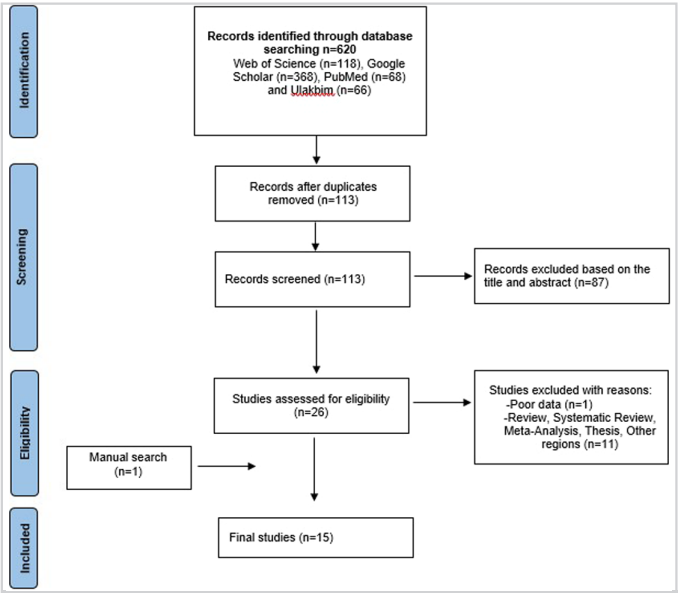

Figure 1.
PRISMA Flow Diagram Describing Included/Excluded Studies.

Discussion

A better understanding of the epidemiology, transmission dynamics, and host range of microsporidiosis will contribute to the development of new strategies for disease prevention or control (Li et al., 2014). In the last decade, the development of novel or current diagnostic methods has contributed to the epidemiologic knowledge of microsporidia (Taghipour et al., 2024). Advances in molecular diagnostic methods have provided valuable information on the phylogenetic relationships, genotypic variations, host range, and

Table 1.
Characteristics of the Included Studies on *Enterocytozoon bieneusi* in Human

Province	Host/ Source	Type of Sample	Diagnostic Methods	Sample Size	Positive Sample	Prevalence (%)	Detected Genotypes	References
Muş, Bitlis	Human	Stool	*IFA-MAbs, **RT-PCR	200	46	23.0	–	Aydemir et al. (2023)
Kayseri	Human	Stool	IFA-MAbs	200	8	4.0	–	Çetinkaya et al. (2015)
Kayseri	Human	Stool	IFA-MAbs	123	9	7.3	–	Hamamcı et al. (2015)
Ankara	Human	Stool	Uvitex 2B/ Calcofluor white and Nested PCR	200	7	3.5	–	Oğuz Kaya et al. (2018)

*Immunofluorescence Assay Using Monoclonal Antibodies, **Reverse Transcription-Polymerase Chain Reaction

specificity of microsporidia, as well as their zoonotic potential (Li et al., 2020). *Enterocytozoon bieneusi* is the most important and common species of microsporidia (Matos et al., 2012; Wang et al., 2024). With this study, we reviewed the molecular prevalence and genotype distribution of *E. bieneusi* in various hosts in Türkiye. Finally, 15 studies were documented in four databases. PCR-based molecular diagnostic methods were used in most of the studies in the dataset (n = 13). In two different studies, IFA-Mabs and RT-PCR, as well as Uvitex 2B and Nested PCR, methods were used together (Aydemir et al., 2023; Oğuz Kaya et al., 2018). In the human studies, fluorescence microscopy was used as a primary diagnostic method, and in none of these studies was the genotypic characterization of the positive samples performed. There is indeed a gap at this point. Genotypic characterization of positive samples from humans is crucial for revealing the route of transmission of *E. bieneusi* genotypes identified between animals and humans in our country. The dataset findings indicated that 25.0% of the genotypes belonged to Group 1. Except for genotype YNDCEB-90, genotypes D, Type IV, Peru 6, and ERUNT1 were found in cats, pigeons, and chickens, which are closely related to humans. Similarly, these genotypes have previously been detected in humans and non-human primates, cattle, horses, dogs, cats, etc., in different countries (Santín et al., 2005; 2010; Wang et al., 2018 Li et al, 2018;. The BEB6 (n = 38) and J (n = 3) genotypes were identified in sheep, horses, cattle, and water buffalo and included in Group 2. These genotypes have zoonotic significance and are found in both domestic animals and humans

Table 2.
Characteristics of the Included Studies on *Enterocytozoon bieneusi* in Animal Hosts

Province	Host/Source	Type of Sample	Diagnostic Methods	Sample Size	Positive Sample	Prevalence (%)	Detected Genotypes	References
Van	Sheep	Stool	Nested PCR	200	16	8.0	BEB6 (n = 16)	Apaydın et al. (2023)
Sivas	Cattle	Stool	Nested PCR	150	29	19.3	N (n = 2), ERUSS1 (n = 24), ERUSS2 (n = 1), ERUSS3 (n = 1), and ERUSS4 (n = 1)	Bilgin et al. (2020)
Kayseri, Nevşehir and Kırşehir	Chicken	Stool	Nested PCR	300	22	7.3	ERUNT1 (n = 21), ERUSS1 (n = 1)	Ercan et al. (2020)
İzmir	Cat	Stool	*RT-PCR, Nested PCR	339	170	50.1	D (n = 3), Type IV (n = 44)	Erkunt Alak et al. (2023) and Surgeç et al. (2023)
Kayseri, Samsun, and Sivas	Water buffalo	Stool	Nested PCR	300	8	2.6	YNDCEB-90 (n = 5) and J (n = 3)	Onder et al. (2022)
Samsun	Pigeon	Stool	Nested PCR	250	35	14.0	Peru 6 (n = 35)	Pekmezci et al. (2021a)
Samsun	Budgerigar	Stool	Nested PCR	143	5	3.5	N (n = 2), TURKM1 (n = 3)	Pekmezci et al. (2021b)
Kayseri, Nevşehir	Cattle, sheep, and water buffalo	Milk	Nested PCR	450	46	10.1	ERUSS1 (n = 24), BEB6 (n = 14), TREb1 (n = 1), TREb2 (n = 2), TREb3 (n = 2), TREb4 (n = 1), TREb5 (n = 1), and TREb6 (n = 1)	Yildirim et al. (2020a)
Kayseri, Nevşehir	Horse	Stool	Nested PCR	300	56	18.7	ERUSS1 (n = 24), BEB6 (n = 8), ERUH2 (n = 6), ERUH3 (n = 5), ERUH4 (n = 4), ERUH5 (n = 6), ERUH6 (n = 3), and ERUH7 (n = 2)	Yildirim et al. (2020b)
Aksaray	Donkey	Stool	Nested PCR	200	12	6.0	YNDCEB-90 (n = 12)	Çifci et al. (2023)
Samsun	Cat	Stool	Nested PCR	72	4	5.5	D (n = 2), Type IV (n = 2)	Pekmezci et al. (2019)

*Reverse Transcription-Polymerase Chain Reaction

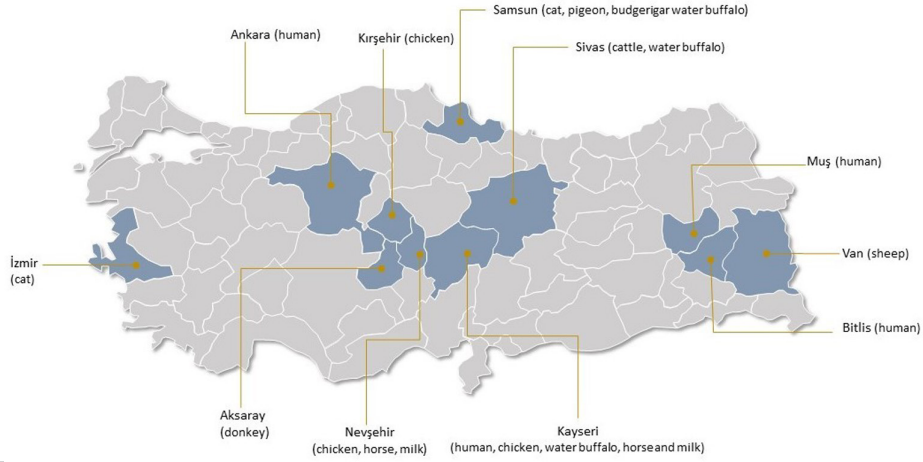

Figure 2.
The Sampling Localities and Hosts Distribution of *Enterocytozoon bieneusi* in Türkiye.

(Li et al., 2022; Wang et al., 2013; Zhang et al., 2018; 2021). The ERUSS1 genotype ($n = 73$) is the dominant genotype with a broad spectrum of host variations, including cattle, chickens, horses, sheep, and water buffalo. The ERUSS1, BEB6, and TREb1-6 genotypes were detected from milk samples that were possibly contaminated by the environment of dairy farms or infected dairy animals (Yildirim et al., 2020a).

The overall prevalence of *E. bieneusi* in humans was determined to be 7.4% (95% CI: 2.2–20.2%; I^2, 94.0%), while the prevalence in animals in our data set was 10.2% (95% CI, 5.4–18.5%; I^2, 97.0%). In a similar study, Wang et al. (2024) reported a lower global prevalence of *E. bieneusi,* 6.59% (95% CI: 4.97–8.68; I^2, 97.0%), in humans. Qin et al. (2022) investigated the global prevalence of *E. bieneusi* in cattle from 11 different countries and reported lower and higher rates of 5.0% (95% CI, 1.0–11.9%) and 17.4% (95% CI, 14.1–21.1%), respectively. Likewise, the pooled prevalence of *E. bieneusi* in cattle was calculated to be 13.9% (95% CI: 11.4–16.8%) (Taghipour et al., 2022). The global prevalence of *E. bieneusi* was figured out to be 17.4% (95% CI: 11.8–25%) for sheep, 16.3% (95% CI: 11.3–22.8%) for goats, and 7.4% (95% CI: 5.1–10.5%) for cats (Taghipour et al., 2021a, Taghipour et al., 2021b). Taghipour et al. (2024) point out that a high degree of heterogeneity is often observed in systematic reviews and meta-analyses. The heterogeneity of the present study was high (I^2, 96.30%), which confirms this assumption.

The prevalence of *E. bieneusi* was found in both humans and animals in 11 provinces in four different geographical regions of Türkiye. Samples from humans, cats, cattle, and water buffaloes were examined from two different provinces, while the others were tested in a single province. The most studied region is Central Anatolia. No study was found in the northwestern, northeastern, and southeastern regions of Türkiye, the land border regions of the country. The identification of existing or novel genotypes in these regions, where both wildlife and human migration movements have been recorded, will make an important contribution to understanding the epidemiology of *E. bieneusi*.

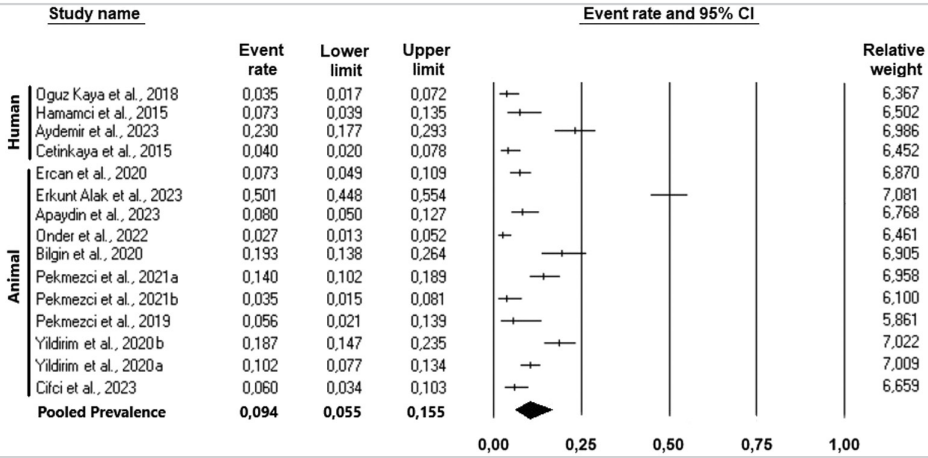

Figure 3.
The Pooled Prevalence of *Enterocytozoon bieneusi* in Different Hosts in Türkiye Based on the Random-Effects Model.

Conclusion and Recommendations

The lack of genotypic characterization of *E. bieneusi* in human samples, as well as the clustering of studies in certain regions and the lack of data from other regions, were the main limitations of this study. In conclusion, the study has contributed to knowledge, especially in understanding the molecular epidemiology of *E. bieneusi*, an opportunistic pathogen in humans and several hosts. The detection of zoonotic genotypes in livestock, pets, and milk samples should be considered particularly by breeders, owners, veterinarians, and those working in the dairy industry. We propose further studies to identify *E. bieneusi* genotypes in humans and in unstudied areas as target regions to improve our understanding of the epidemiology of *E. bieneusi* in Türkiye.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.E., A.Y.; Design – N.E., A.Y.; Supervision – N.E., A.Y.; Materials – N.E.; Data Collection and/or Processing – N.E., A.Y.; Analysis and/or Interpretation – N.E., A.Y.; Literature Search – N.E.; Writing Manuscript – N.E.; Critical Review – N.E., A.Y.

Declaration of Interests: The authors have no conflict of interest to declare.

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