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A Record of Microsporidian Pathogen of the European wasp, Vespula vulgaris Linnaeus, 1758 (Hymenoptera: Vespidae) in Turkey

Avrupa Yaban Arısı Vespula vulgaris Linnaeus, 1758 (Hymenoptera: Vespidae)'in Türkiye'de Microsporidian Patojeni Kaydı

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Abstract

Microsporidia are common enigmatic pathogens of hymenopterans. Although these species are more concerned with Apidae (especially honeybees), they are also known to infect members of Vespidae. Apart from these species, many defined and undefined microsporidia infections were detected infecting Vespidae individuals in the literature. Especially *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae) infected different microsporidian species like a *Nosema bombi*, *Vavraia culicis*, *Nosema vespula*, etc. Molecular identification-based microsporidian records in predator species such as *V. vulgaris* are highly suspicious. In such predator insects, microsporidian infections should be supported by characteristic visuals of the pathogen's life cycle. With this perspective this study is the first and only study that presents the life-cycle stages and spore morphometrics data of a microsporidium isolated from *V. vulgaris*. *V. vulgaris* samples were collected from July to September 2021 in Trabzon, Turkey. During the observations, 415 samples were examined, and five of them were infection positive (microsporidiosis prevalence 1.20%). Infection was found mostly in the midgut of the host, and infection was mostly chronic. Fresh mature spores were oval in shape and measured 4.57±0.54 (3.26–5.95; n=200) µm in length and 2.43±0.33 (1.43–3.35; n=200) µm in width. The current microsporidium has a *Nosema*-like disporoblastic merogony and sporogony.

Keywords: Hymenoptera, Microsporidium, *Nosema*, Pathogen, Wasp, *Vespula vulgaris*

Özet

Microsporidia, hymenopteranların yaygın gizemli patojenleridir. Bu türler daha çok Apidae (özellikle bal arıları) ile ilgili olsa da Vespidae üyelerini de enfekte ettikleri bilinmektedir. Bu türlerin dışında literatürde Vespidae bireylerini enfekte eden tanımlanmış ve tanımlanmamış birçok microsporidia enfeksiyonu tespit edilmiştir. Özellikle *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae), *Nosema bombi*, *Vavraia culicis*, *Nosema vespula*, vb. gibi farklı mikrosporidian türleri ile enfekte olmuştur. *V. vulgaris* gibi yırtıcı türlerdeki moleküler tanımlamaya dayalı mikrosporidian kayıtları oldukça şüphelidir. Bu tür yırtıcı böceklerde mikrosporidia enfeksiyonları, patojenin yaşam döngüsünün karakteristik görselleriyle desteklenmesi gerekir. Bu bakış açısıyla bu çalışma, *V. vulgaris*'ten izole edilen bir microsporidium'un yaşam döngüsü aşamalarını ve spor morfometrik verilerini sunan ilk ve tek çalışmadır. *V. vulgaris* örnekleri Temmuz-Eylül 2021 tarihleri arasında Trabzon ilinde toplandı. Gözlemler sırasında 415 örnek incelendi ve bunlardan 5 örnekte enfeksiyon pozitif çıktı (mikrosporidiosis oranı %1.20). Enfeksiyon çoğunlukla konağın orta bağırsağında bulundu ve çoğunlukla kronikti. Taze olgun sporlar oval şekilliydi ve 4.57±0.54 (3.26–5.95; n=200) µm uzunluğunda ve 2.43±0.33 (1.43–3.35; n=200) µm genişliğinde ölçüldü. Mevcut microsporidium, Nosema benzeri bir dissporoblastik merogoni ve sporogoniye sahiptir.

Anahtar Kelimeler: Hymenoptera, Microsporidium, *Nosema*, Patojen, Yaban arısı, *Vespula vulgaris*

1. INTRODUCTION

Vespidae is one of the most significant insect families with more than 5000 species globally. This group, which has a cosmopolitan distribution on earth, spreads in Turkey with 298 records, 65 of which are endemic subspecies and species (Yıldırım & Gusenleitner, 2012; Yıldırım & Bekircan, 2020). These species consist of social and solitary forms. *Vespula vulgaris* Linnaeus, 1758 (Vespidae: Hymenoptera), a common wasp or European wasp, is a eusocial vespid that fed its larvae with masticated insect parts or glandular secretions by adult females (Goulet & Huber, 1993). Although this omnivorous wasp is native to the Euroasia, it is located in Argentina, Australia, and New Zealand and is listed as the world's worst invasive species (Gruber et al., 2019). This invasive species not only poses a significant danger to native species but also can sting humans to attack or in defense. With this behavior, exposure to stings is a real medical concern since some people can die from anaphylactic shock during their outdoor activities (Boeve et al., 2014). Due to these aforementioned, *V. vulgaris* is considered in the pest category.

Struggling with the *V. vulgaris* and other social wasps is generally done with chemical control methods like insecticides in bait formulation or direct insecticide application into nests (Rose et al., 1999). Although chemical control is an effective and fast-paced control method in

the short term, it has disadvantages like insecticide use being expensive, labour-intensive, and potentially hazardous to non-target organisms. In addition, it should not be forgotten that this species, defined as a pest during its invasive periods, plays an important ecological role and acts as a regulator species on the other pest insect populations in nature, such as preying on flies and caterpillars (Boeve et al., 2014). For this reason, it is necessary to be more careful when chemical control of this species, and different control methods should be developed.

There is increasing awareness of the microbial control of pests worldwide, especially in the last decades. For this reason, the studies to determine the microbial communities and their effects on the fitness and performance of pests are increasing day by day. Similarly, this study tries to determine the natural pathogen and parasites of *V. vulgaris* and is the first attempt to assess the natural pathogens of Turkey's wild *V. vulgaris* populations.

2. MATERIALS and METHODS

Vespula vulgaris individuals were collected from July to September 2021 in Trabzon, Turkey. For the collection process, common plastic bottle yellowjacket traps were used (Erdoğan & Dodoloğlu, 2013). In these traps, fresh meat was used as bait not to catch the honey bees. Traps were placed in different locations far from each other to catch adult samples belonging to different nests. These traps were checked weekly, and the baits in the traps were replaced with new ones. The captured samples were labeled and brought to the laboratory as soon as possible after the necessary macroscopic examinations were made. In microscopic observations to determine the pathogens and parasites, wet smears were prepared using Ringer's solution dissected individuals and examined under the light microscope (Baki & Bekircan, 2018; Bekircan, 2020; Bekircan & Tosun, 2021). In order to determine the life cycles of possible pathogens, infection-positive smears were stained using the Giemsa stain protocol, which is frequently used in insect pathology (Yıldırım & Bekircan, 2020; Yıldırım et al., 2022). Zeiss AXIO microscope equipped with an Axicam ERc5s digital camera was used for photographing the infection-positive samples, and the ZEN 2.3 Blue Edition imaging software was used for measurement and analysis.

3. RESULTS and DISCUSSION

During the field study, 415 samples were collected from five different traps. In the light microscopic observations, microsporidiosis was determined in only five of the 415 samples, and with this result, infection prevalence was calculated as 1.20%. The infection was detected

in a very limited region of the midgut of infected hosts. The number of mature spores, the first and most important finding of microsporidiosis, was very few (Figure 1). Fresh mature spores were oval in shape and measured 4.57±0.54 (3.26-5.95; n=200) µm in length and 2.43±0.33 (1.43-3.35; n=200) µm in width.

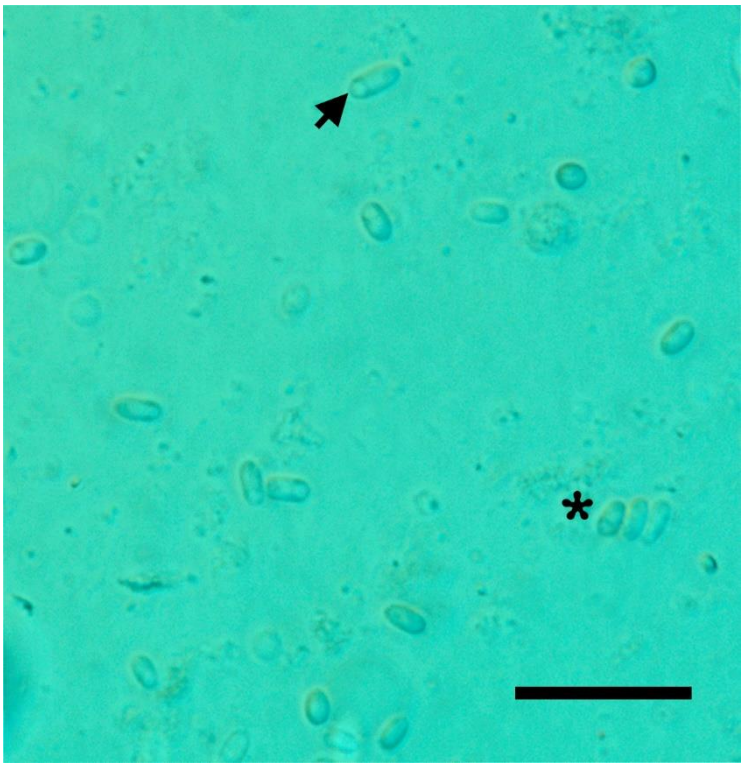

Figure 1. The light micrograph of the fresh oval spores. While the arrow indicates the mature spore's posterior vacuole, the asterisk shows chain formation with binary fission (Unite bar=20 µm).

Various life cycle stages were observed in Giemsa stained microscopic smears. Meronts were spherical or ovoid in shape. While the spherical meronts were measured 2.46-2.72 × 2.72-2.90 (n=8) µm, ovoid meronts were measured as a 3.08-3.34×2.20-2.55 (n=6) µm (Figure 2a). Similarly, sporonts were spherical and elongated oblong and measured 3.34-4.22 × 2.64-3.60 (n=6) µm and 3.52-5.45 × 1.84-2.64 (n=5) µm, respectively (Figure 2b). Sporoblasts, which mature into a spore and originate from the divided diplokaryotic sporonts, were elongated in shape. And sporoblasts were measured 5.01-7.56 × 2.99-3.43 (n=4) µm (Figure 2c). Finally, mature Giemsa stained spores were measured again (3.90±0.55 (2.99–5.10; n=30) µm in length and 2.14±0.40 (1.49–2.90; n=30) µm in width) (Figure 2d).

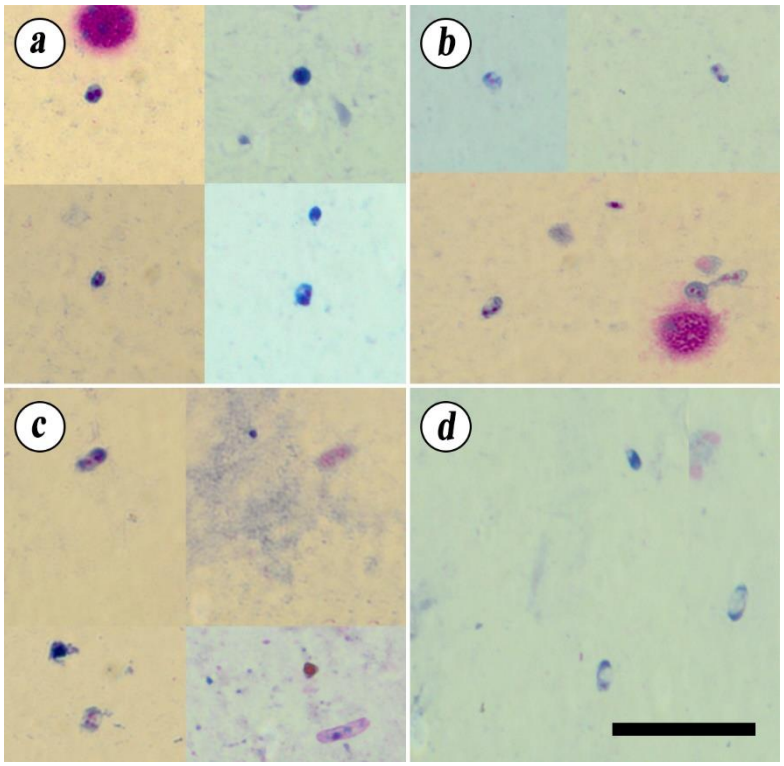

Figure 2. Light micrographs of the Giemsa stained life-cycle stages. **a**: Spherical and oval binucleate meront (midgut); **b**: Binucleate oval sporont (midgut); **c**: Sporoblast (midgut); **d**: Mature oval spores (midgut) (Unite bar=20 µm).

The approach to using pathogens and parasites is widely agreeable worldwide for pest control, especially in the last quarter. Therefore, the number and scope of studies on detecting and identifying pathogens and parasites are increasing day by day (Felden et al., 2020; Lester et al., 2015; Rose et al., 1999). Similarly, this research is the first and only study conducted to determine natural pathogens and parasites of European wasps, *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae) in Turkey. The genus *Vespula* is exposed to various diseases and pathogens like other hymenopterans. To date, nearly 50 fungi, 12 bacteria, seven nematodes, four protozoans, and two virus species have been detected from different individuals of this genus (Felden et al., 2020).

Microsporidia are common enigmatic pathogens of hymenopterans. The most reputed microsporidia species infecting hymenopterans are *Vairimorpha* (*Nosema*) *ceranae* and *Vairimorpha* (*Nosema*) *apis* (Graystock et al., 2013; Plischuk et al., 2009; Tosun & Bekircan, 2021). Although these species are more concerned with Apidae (especially honeybees), they are also known to infect members of Vespidae (Gabín-García et al., 2021; Lester et al., 2014; Rose et al., 1999). Apart from these species, many defined and undefined microsporidia infections were detected infecting Vespidae individuals in the literature. Especially *V. vulgaris*

infected different microsporidian species like a *Nosema bombi*, *Vavraia culicis*, *Nosema vespula*, etc. (Felden et al., 2020; Lester et al., 2014; Lester et al., 2015; Quinn et al., 2018).

Modern microsporidian taxonomy is built on classical morphological approaches (spore shape, structure, size, host type, etc.) and molecular-based methods (Vega & Kaya, 2012; Weiss & Becnel, 2014). However, in microsporidian pathogens isolated from *V. vulgaris*, there is almost no description based on this basis. For instance, a base sequence uploaded to NCBI GenBank by Da Silva et al. in 1994 entered the literature as *Nosema vespula* (Accession no: U11047) and is widely used in molecular comparisons for species identification (Bekircan et al., 2016; Biganski et al., 2020; Vossbrinck & Debrunner-Vossbrinck, 2005). However, there is no study prepared with taxonomic characters (like spore structure or life-cycle) proving that this mysterious record is indeed a microsporidian infection. The records of microsporidiosis detected in these studies only on a molecular basis are suspicious because microsporidium spores and DNAs that settle in the predator's digestive tract after consuming an insect with microsporidiosis by predator insects such as *V. vulgaris* may cause false infection positivity in these molecular analyses. The study of Quinn et al. in 2018 with metatranscriptomic analysis on *V. vulgaris* revealed results confirming this claim. *Vavraia culicis* (Weiser, 1947), a microsporidium infecting mosquitoes, was detected in the *V. vulgaris* samples analyzed in this study and in the same publication, the authors stated that this would not be possible (Quinn et al., 2018). Therefore, microscopic findings such as mature spore structure and life-cycle stages should be detected before molecular analysis, especially in studies to identify microsporidian pathogens of predatory insects.

The current study is the first and only study that presents the life-cycle stages and spore morphometrics data of microsporidium isolated from *V. vulgaris*. Therefore, it was impossible to compare the current microsporidium with the other isolates previously isolated from *V. vulgaris* in the literature in terms of the classical morphological approach (based on nuclear arrangement, spore shape, structure, and size, etc.). It would not be unreasonable to compare the current microsporidium with *V. ceranae*, *V. apis*, and *Nosema bombi*, which were detected by molecular techniques in *V. vulgaris* and caused infection in other Hymenoptera individuals according to these taxonomical characters. The spore shape and dimensions traditionally have been significant taxonomic characteristics used in Microsporidia taxonomy (Canning & Vávra, 2000; Sprague et al., 1992). The spore dimension of the current microsporidium (4.57±0.54 × 2.43±0.33 µm) clearly differentiates from the *V. apis*. The spore dimensions of *V. apis*, *V. ceranae*, and *Nosema bombi* are 5-7 × 3-4 µm, 4.7 × 2.7 µm, and 4.20-5.39 × 2.13-3.50 µm,

respectively (Fries, 1993; Fries et al., 1996; McIvor & Malone, 1995; Zander, 1909). In addition, the current microsporidium (in midgut) differs from *Nosema bombi* (in Malpighian tubules) with the infection site, which is another important taxonomic characteristic (Sprague et al., 1992). The current microsporidium is similar to *V. ceranae* in terms of spore morphology and infection site. However, the type of infection they develop in their hosts is quite different from each other. While *V. ceranae* creates systemic infection in its hosts, the current microsporidium creates a chronic infection in *V. vulgaris* (Chen et al., 2009). Unfortunately, this feature of the current microsporidium has prevented adequate sample availability for us to carry out the detailed molecular and electron microscopical studies necessary for species identification.

The current microsporidium's life-cycle type, detected in the examinations of Giemsa stained preparations, is disporoblastic development (diplokaryotic merogony and sporogony) as in the Nosema genus (Nägeli, 1857, Weiss & Becnel, 2014). Although the life cycle stages and types are similar to that of the genus *Nosema*, this claim is contradictory since we could not support it from a molecular point of view. However, there are records of Nosema-like spores detected in the genus *Vespula* in the literature, which could not be amplified the SSU-rRNA locus by the primers (Gabín-García et al., 2021). Because of all these shortcomings, it would be more appropriate to express the microsporidium detected in this study as *Microsporidium sp*. in order not to cause systematic confusion.

4. CONCLUSION

This manuscript is the first and only research to reveal a microsporidium species' life cycle and spore morphology that causes infection in *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae).

DECLARATIONS

The authors declare that they have no conflicts of interest.

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