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European Brown hare (*Lepus europaeus*) as an accidental host of *Trypanosoma pestanai*

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ABSTRACT

In Europe, trypanosomes are not considered a serious threat to humans or domestic animals; however, their potential ecological impact on wildlife remains largely unknown. This study investigates presence of trypanosomes in European brown hare (*Lepus europaeus*) in Czechia, where this species is an important game species and plays a significant role in ecosystems. Using nested PCR targeting 18S rDNA, trypanosome DNA was detected in two of 435 blood samples, representing the first confirmation of trypanosomes in the genus *Lepus*. Subsequent sequencing identified *Trypanosoma pestanai*, a species previously reported in the European badger (*Meles meles*). These findings suggest that *T. pestanai* may have a broader host range than previously recognized, emphasizing the need for further research into its ecological impact and its potential transmission dynamics in wildlife populations.

1. Introduction

The European brown hare (*Lepus europaeus*) is a common species in Czechia, thriving in diverse habitats such as farmlands, meadows, and forests. This mammal plays an important role in the ecosystem, serving as both a key herbivore and a crucial prey species for various predators. However, since the 1960s, its population has been in decline (Edwards et al., 2000). The primary drivers of this decline include changes in home range size due to reduced crop diversity and the expansion of large agricultural fields (Lundström-Gilliéron and Schlaepfer, 2003). Other contributing factors include an increasing number of predators, particularly the red fox (*Vulpes vulpes*) (Edwards et al., 2000), road mortality (Roedenbeck and Voser, 2008), the impact of agricultural machinery (Steen et al., 2012), and various diseases (Chroust et al., 2012; Pikula et al., 2004; Treml et al., 2007).

Trypanosomes are unicellular parasites with a worldwide distribution. They are transmitted by blood-sucking invertebrates (Hoberg et al., 2012) through various transmission routes, including injection via vector saliva, contaminative transmission through the vector's faeces, and ingestion of vectors (Haag et al., 1998). The pathogenesis and clinical signs of trypanosome infections are best documented in species with major health or economic impacts, notably *T. brucei* in livestock and *T. cruzi* in humans, while others are either non-pathogenic or have unknown impacts on their host (Magri et al., 2021). *Trypanosoma brucei* causes severe anemia, weight loss, abortion, cachexia, and often death if untreated (Smetko et al., 2015). *Trypanosoma cruzi*, the agent of Chagas disease, leads to chronic infection with potentially serious cardiac and gastrointestinal complications decades later (Nunes da Costa et al., 2021). *Trypanosoma brucei*, *T. congolense*, and *T. vivax* also infect African wildlife, where they cause variable symptoms, commonly fever, anemia, and lethargy (Kasozi et al., 2021). In contrast, non-pathogenic trypanosomes are typically found in European wild mammals (Magri et al., 2021).

Presence of trypanosomes has been confirmed in several wild

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mammals (Kostygov et al., 2021), including bats (Linhart et al., 2020), small rodents (Votýpka et al., 2022), and leporids (Leporidae), a family that includes rabbits and hares. While wild rabbits (*Oryctolagus cuniculus*) are commonly infected by *T. nabiasi* (Grewal, 1957; Díaz-Sáez et al., 2014; Merino-Espinosa et al., 2016), occurrence of trypanosomes in hares, including the European brown hare, remains largely unknown. The rabbit trypanosome belongs, together with a new unnamed *Trypanosoma* sp. from the Mongolian pika (*Ochotona pallasi*; Lagomorpha) (Wang et al., 2024) and trypanosomes of small rodents and insectivores, to the subgenus *Herpetosoma* (Votýpka et al., 2022; Babyesiza et al., 2024), which is highly host-specific and transmitted by fleas. The vector of *T. nabiasi*, for example, is the flea *Spilopsyllus cuniculi* (Brumpt, 1913), which has also been found in hares (Shepherd et al., 1977; Dik and Uslu, 2018).

Very little is known about the impact of trypanosomes on the health status of wild animals in Europe (Magri et al., 2021). While blood parasites rarely cause serious infections in wildlife populations, monitoring remains crucial, particularly given their potential zoonotic risk. Here, therefore, for the first time, we assess the presence of trypanosomes in the blood of European brown hares using nested PCR.

2. Material and methods

A total of 435 European brown hare blood samples were collected from animals killed during the 2018–2023 hunting seasons in Czechia: Borkovany hunting region (7 specimens), Bošovice (23), Drnholec (2), Kyjov (10), Letovice (3), Lovčičky (8), Opatovice (8), Rohatec - Hodonín (4), Tuřany (89), Velké Hostěrádky (5), Velké Pavlovice (149), Znojmo (3), Žichlínek (23), and several unspecified localities in southern Moravia (101).

DNA extraction followed the protocol described by Lesiczka et al. (2021), and the specific nested PCR protocol targeting 18S rDNA (∼2100 bp) was based on Seward et al. (2017). Amplicons were purified using the High Pure PCR Product Purification Kit (Roche, Switzerland) and sequenced commercially (SEQME, Czech Republic). Sequence editing and phylogenetic analysis were performed in the Geneious Prime program v. 2024 (Biomatters, Auckland, New Zealand) using the Geneious alignment algorithm with default settings, the resultant tree being created with the PhyML plugin.

3. Results and discussion

The nested PCR analysis revealed that two of the 435 brown hare blood samples were positive for *Trypanosoma*, with both yielding identical sequences (GenBank accession number PV085289.2) showing 100 % similarity to *Trypanosoma pestanai* (KY354582; Fig. 1). Both *T. pestanai*-positive hares were sampled at Tuřany (2 of 89 samples; 2.25 %). One of the two hares also proved positive for *Anaplasma phagocytophilum* (Lesiczka et al., 2021).

This is the first time trypanosomes have been directly detected in blood of European brown hares, and the detection of *T. pestanai* represents a significant advancement in our understanding of its host range. *Trypanosoma pestanai* is known to infect the European badger (*Meles meles*) and is the only trypanosome species known to infect European carnivores. First described in Portugal in 1905 (Bettencourt and França, 1905), it was later detected in France (Rioux et al., 1966), Great Britain (Ideozu et al., 2015), Ireland (McCarthy et al., 2009), and several other EU countries (Lindhorst et al., 2024). Infection, which is passed on through its vector, the badger flea (*Paraceras melis*) (Lizundia et al., 2011; Sgroi et al., 2021), is usually asymptomatic, with no association between parasitaemia and changes in the complete blood count (McCarthy et al., 2009).

Whereas *T. pestanai* has so far been detected only in badgers, with a single reported case of infection in a dog from Germany (Dyachenko et al., 2017), the flea *P. melis* has been relatively frequently found on the red fox, and occasionally on other hosts, such as the European mole (*Talpa europaea*), European fallow deer (*Dama dama*), or the lesser horseshoe bat (*Rhinolophus hipposideros*) (Ancillotto et al., 2014). Red foxes are frequently known to share burrows with badgers (Mori et al., 2014), which reflects not only their ecological interactions but also creates opportunities for the transfer of parasites like fleas.

Interestingly, the European brown hare does not typically use underground dens, preferring to remain partially hidden in depressions in the ground, known as “forms”. As such locations do not provide suitable environments for flea development, hares are generally free of fleas and acquire them only accidently (and for a limited time only) from other hosts or from their nests. The first report of European brown hares using badger burrows as resting sites comes from Italy (Pecorella and Mori, 2023). This type of behavior could explain the transmission of badger *T. pestanai* to an accidental host. The adult badger flea can survive up to 90 days after separation from its host; furthermore, *P. melis* congregates at the burrow entrance (Cox et al., 1999), meaning that a hare or other species does not necessarily have to enter the deeper parts of the burrow to catch the flea. Nevertheless, information regarding hares using badger dens as shelters is limited and it is unknown how frequently this behavior occurs across European hare populations.

While *P. melis* is the primary vector, detection of *T. pestanai* in ticks (*Ixodes ricinus* and *I. canisuga*) (Sgroi et al., 2021) suggests that alternative transmission pathways may exist, potentially broadening the host range due to the lower host specificity of ticks. Indeed, ticks are already considered potential vectors (Koual et al., 2023) for trypanosomes from the clade most closely related to *T. pestanai*.

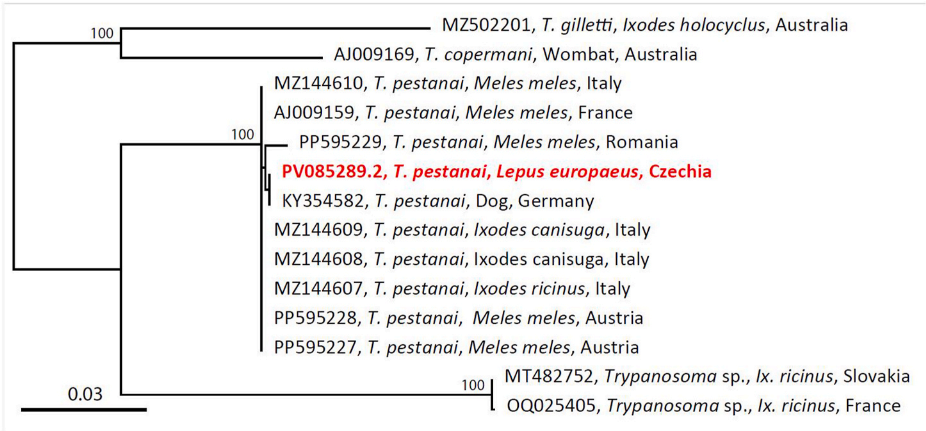

Fig. 1. Maximum likelihood phylogenetic reconstruction based on 18S rRNA of all available *Trypanosoma pestanai* sequences, including our sequence (PV085289.2) obtained from the blood of a European brown hare (*Lepus europaeus*) from Tuřany, Czechia. *Trypanosoma gilleti* and *T. copermani* were used as outgroups.

Developmental stages of some trypanosomes have been found in ticks, suggesting active development and potential for both transstadial and transovarial transmission (Kernif et al., 2024). For *Trypanosoma pestanai*, however, the role of ixodid ticks remains unclear, and it is unknown whether transmission is active or passive. Natural hosts of *Ixodes ricinus*, a known major vector of pathogens like *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum,* include European hares and badgers (Lesiczka et al., 2021; Butler and Roper, 1996; Ebani et al., 2016; Rizzoli et al., 2014). Coinfection with other tick-borne pathogens may affect the course of *T. pestanai* infection, as observed in *Babesia microti* and *Borrelia burgdorferi* coinfections (Diuk-Wasser et al., 2016).

While a pathogen's ability to infect multiple hosts can enhance its persistence, it can also involve ecological trade-offs, such as the dilution effect. In vector-borne systems, for example, greater host diversity may reduce transmission by limiting interactions between infected vectors and highly competent hosts, compared to scenarios with single host species (Schmidt and Ostfeld, 2001).

This potential host range expansion aligns with the concept of shared pathogen susceptibility among related species, such as hares and rabbits. The European brown hare and the rabbit are both vulnerable to Rabbit Hemorrhagic Disease Virus 2 (RHDV2) (Asin et al., 2024), which has even been detected in seven European badgers in Portugal (dos Santos et al., 2022). Given this, they may also share other pathogens, such as *Trypanosoma nabiasi* known from rabbits.

In many vector-borne diseases, climate change is a crucial factor, with rising temperatures expected to drive (re)emergence or persistence of pathogens (Biota, 2002). Climate shifts may influence not only the distribution of pathogens, hosts, and vectors, but also their behavior, potentially altering pathogen transmission by changing the timing and location of host-parasite interactions (Altizer et al., 2011).

Our finding of *T. pestanai* presence in the blood of two European brown hares could be an accidental finding, or it could indicate a more common, but as yet undocumented, occurrence. While nested PCR is a highly sensitive method, false-negative results can occur in cases of low parasitemia, which is typical of chronic infections (Desquesnes et al., 2022). As such, the actual prevalence of *T. pestanai* in the brown hare may be higher than currently recorded.

Co-infection with *A. phagocytophilum,* previously reported in dogs (Dyachenko et al., 2017), was recorded in one of the two *T. pestanai*-positive hares in our study. *Anaplasma phagocytophilum* infection is known to modulate the immune response to opportunistic infectious agents (Rikihisa, 2011), which might contribute to weakened immune system response to *T. pestanai* in hares. These findings suggest that further studies will be essential to improve our understanding of trypanosome species infecting European brown hares and other mammals (Magri et al., 2021).

Here, we report the first detection of *T. pestanai* in the European brown hare, expanding its known host range beyond that of the European badger. This finding suggests that the ecological niche of *T. pestanai* may be broader than previously recognized. While the badger flea, *P. melis*, is the confirmed vector, our findings raise the possibility that other vectors may also contribute to parasite transmission. Further research is needed to explore transmission dynamics, the role of vectors, and the potential pathological effects of *T. pestanai* on wildlife. Understanding its full host range and transmission routes will be essential for assessing its ecological impact and potential risks to both wildlife and domestic animals.

CRediT authorship contribution statement

Lucie Veitova: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Jiri Pikula:** Writing – review & editing, Resources. **Jan Votýpka:** Writing – review & editing, Formal analysis, Conceptualization. **Petr Linhart:** Writing – review & editing. **Vendula Kokesova:** Investigation. **Paulina Maria Lesiczka:** Investigation. **Vladimir Piacek:** Investigation. **Jana Sedlackova:** Writing – review & editing. **Anna Sindelarova:** Investigation. **Tomas Heger:** Investigation. **Hana Bandouchova:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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