Suitability of red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) as hosts of *Echinococcus multilocularis* based on egg production characteristics and literature data on the intestinal ecosystems of carnivores

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A B S T R A C T

Echinococcus multilocularis is the most important food-borne parasite in Europe. Its natural definitive host is the red fox (*Vulpes vulpes*) while other canid species play a secondary role in the maintenance of its endemics. However, recent studies call attention to the potential of golden jackal (*Canis aureus*) as a suitable definitive host for *E. multilocularis*. Our study aimed to evaluate the quantitative and qualitative egg production traits of adult *E. multilocularis* in different hosts as an indicator of reproductive success. Investigation of 111 and 82 parasites from 33 red foxes and 29 golden jackals, respectively, we ascertained that the proportion of worms with mature eggs was significantly lower in golden jackals than in red foxes. Those worms, which produced mature eggs in golden jackal hosts, originated from less crowded infrapopulations than their fox-originated counterparts. Other characteristics of the parasite's reproductive ability, such as the proportion of fertile worms, and mean egg production were similar in the two hosts. Comparing these findings to evolutionary data on different canid taxa, we hypothesised that the mutual presence of red fox and a differently evolved host of *E. multilocularis* might contribute to the formation of stable parasite circulation in these multi-host systems.

1. Introduction

Echinococcus multilocularis causes human alveolar echinococcosis (HAE), a disease, which represents one of the neglected diseases prioritised by the World Health Organization to eradicate or control (WHO, 2020). In Europe, this is the highest-ranked food-borne parasitic disease (van der Giessen et al., 2021) which leads on to the death of the patient without appropriate treatment (Brunetti et al., 2010).

Echinococcus mutilocularis can infect a considerably broad range of hosts. Maturation and egg production occur in canine and feline carnivores (Kapel et al., 2006; Thompson et al., 2006; Thompson, 2017), and more than 40 mammal species are identified as intermediate hosts (Gürler et al., 2023). The most important definitive host is proved to be the red fox (*Vulpes vulpes*), which disperse the parasite in all directions within the Holarctic realm from its place of origin, Beringia during the Pleistocene Epoch (Spotin et al., 2018; Massolo et al., 2022). Other canine hosts, such as the domestic dog (*Canis lupus familiaris*), grey wolf (*Canis lupus*), coyote (*Canis latrans*), racoon dog (*Nyctereus procionoides*), and golden jackal (*Canis aureus*), possess secondary role in the maintenance of *E. multilocularis* (Romig and Wassermann, 2024). However, human-mediated expansion of mesocarnivore populations can increase the risk of hyperendemic formation locally, as it is experienced in suburban coyotes around the city of Calgary, Canada (Romig and Wassermann, 2024), and in golden jackals in the Western Balkans region (Moloi et al., 2023).

In Hungary, the occurrence of *E. multilocularis* was confirmed in 2002 in the northeast region of the country by the border of Slovakia (Sréter et al., 2003). The first case in the golden jackal was detected more than a decade later in 2013, in the westernmost region of Hungary, Zala County (Széll et al., 2013). The potential of the golden jackal as a maintenance host of *E. multilocularis* has arisen in the Western Balkan distribution area of the parasite. In studies carried out in Serbia during 2014–2016 (Lalošević et al., 2016), Croatia (Sindičić et al., 2018), and Hungary during 2016–2020 (Balog et al., 2021), the prevalence of *E. multilocularis* in golden jackals proved very similar to the prevalence in the sympatric red fox populations.

The population expansion of the Balkan populations of the golden jackal began in the last decades of the 20th Century. Since the 1990s, the

existence of a resident population could have been proven in the South Transdanubia, the southwest region of Hungary (Tóth et al., 2009). The epidemiological role of the golden jackal in HAE was discussed in the study of Dezsényi et al. (2021) because an extraordinary case accumulation (18.75% of the total cases in Hungary) was detected in the South Transdanubia far from the previously known northeastern focus of the disease. Since the previous screening investigations in the local fox population did not show conspicuously high prevalence of the parasite (Casulli et al., 2010), the expanding golden jackal population arose as a probable risk factor in the background of human cases (Dezsényi et al., 2021).

The potential of the golden jackal as a maintenance host of *E. multilocularis* is an important research focus because the distribution area of this mesocarnivore grows rapidly (Lanszki et al., 2016), and thus they might take over from foxes in certain regions of Europe. The phylogenetic position of the golden jackal is closer to the grey wolf and domestic dog than to the red fox (Lindblad-Toh et al., 2005). Therefore, we hypothesised that the intestinal ecosystem of the golden jackal provides similar conditions for the development of *E. multilocularis* as those of dogs and wolves, thus reproduction performance of the parasite might be analogous throughout the genus *Canis*. Since golden jackals initiated a coevoluationary process with *E. multilocularis* later than red foxes, we presumed that competition between the host and its parasite might be stronger in golden jackals than in red foxes (Brockhurst et al., 2014). Based on these hypotheses, our study compared the crowding and egg production, as indicators of reproductive success, of *E. multilocularis* parasites isolated from hunter-harvested golden jackals and red foxes as the two clusters of *E. multilocularis* suprapopulation originated from the South Transdanubia Region of Hungary. The different conditions that exist in different host species were planned to be investigated from the parasites' perspective. The number of parasites in an individual host, the infrapopulation, represents not only a parasite load, but also a subset of a larger parasite population (Poulin, 2021). The terms "suprapopulation" and "infrapopulation" are defined by Margolis et al. (1982), Bush et al. (1997), and Kassai (1999). In this study, the term "suprapopulation", i.e. all individuals of a parasite species in all stages of development within all hosts in the investigated ecosystem of the South Transdanubia was applied. The term "infrapopulation" was used to define all individuals of a parasite species existing in an individual host. For the term "crowding", we applied the definition "the size of the infrapopulation, to which an individual parasite belongs" as described by Reicziegel et al. (2019).

2. Materials and methods

The study is based on the worms collected from red fox and golden jackal specimens investigated in previous studies, which were carried out in the same, South Transdanubian distribution area of the tapeworm *E. multilocularis* (Halász et al., 2021; Moloi et al., 2023). By this sampling method we could avoid the impact of different landscapes and climate on parasite reproduction. All specimens originated from carcasses, which were harvested by legal hunt in the framework of authorised wildlife management plans. None of the investigated carnivores were culled for scientific purposes. Exclusively sexually mature animals (above 12 months-old) were involved in this study. The evaluation of the genitals was completed with an age estimation based on the dentition and tooth wear (Lombaard, 1971; Anděra and Roulichová, 2007; Stoyanov, 2020).

The parasitological sampling was carried out by a multistage cluster sampling method, in which the primary units were the two clusters, fox- and jackal-originated worm specimens. The secondary units were infrapopulations of *E. multilocularis* living in a certain host individual, while the tertiary units were parasite individuals of the infrapopulation (Thrusfield and Brown, 2018). The crowding was determined by counting all parasites in the intestinal content. If the total number was more than 100, we made the counting on a subsample and calculated the size of the infrapopulation (Halász et al., 2021). Four cestodes were chosen from each intestinal content by unaided eyes to avoid any unintended preliminary judgement of the worm individuals. In the lack of optical support, selection of four specimens guaranteed that at least one unimpaired worm could be isolated from most of the investigated intestinal content. The apparently low number of specimens from a certain infrapopulation aimed to mitigate the effect of host individuals above host species.

The worms were made translucent with 20-min incubation in lactophenol for morphometric evaluation and egg counting, which was done at a magnification of 100–400×. After a morphological method based on the position of the genital pore on the last segment and the ratio of worm length to the terminal proglottids (Jones and Pybus, 2001), a species-specific PCR was carried out using the following forward and reverse primers: Cest1 (5′-TGC TGA TTT GTT AAA GTT AGT GAT C-3′) and Cest2 (5′-CAT AAA TCA ATG GAA ACA ACA ACA AG-3′) (Trachsel et al., 2007).

Egg counting and maturity determination were conducted after morphometric evaluation of the worms, whereas only the whole specimens classified as *E. multilocularis* were involved. If the number of eggs did not allow us to accurately determine them inside the worm's body, we opened the last segment by gently pressing the coverslip. We counted the released eggs outside the helminth body. We appraised the worm as immature if the posterior segment did not contain eggs (Fig. 1).

Our study's nomenclature was based on the international consensual terminology (Vuitton et al., 2020) except for the terms "mature egg" and "immature egg". Mature or thick-shelled eggs' criteria are the radially striated embryophore and the clearly visible oncosphere with the pairs of hooks in parallel alignment (Fig. 2). The eggs were evaluated as immature or thin-shelled, if radial striation and hook set of the oncospheres could not be identified (Fig. 3) (Jones et al., 1960; Alvarez Rojas et al., 2018). In an experimental study, Kamiya and Sato (1990) proved that thin-shelled eggs appear some days before the six-hooked oncosphere containing thick-shelled eggs during the developmental process.

Although the literature cited above (Vuitton et al., 2020) defines the fertile adult worm as "… *Echinococcus* spp. in the definitive host, the last segment of which contains eggs", we deemed distinguishing between thin-shelled and thick-shelled eggs. We supposed that the key to infection of intermediate hosts is the thick-shelled egg, whose peculiarities may have a greater survival potential (Świderski et al., 2017). Their presence may represent the possibility of transmitting the infection to intermediate hosts or even to humans (Thompson et al., 2006).

To characterise the effect of the two hosts on the parasites, we formed two clusters from the collected worms according to their host origin, i.e. golden jackal and red fox. Then we collected the following features of the certain parasite individuals: the presence of eggs in the uterus, the egg count in the uterus, the maturity of the eggs found, and
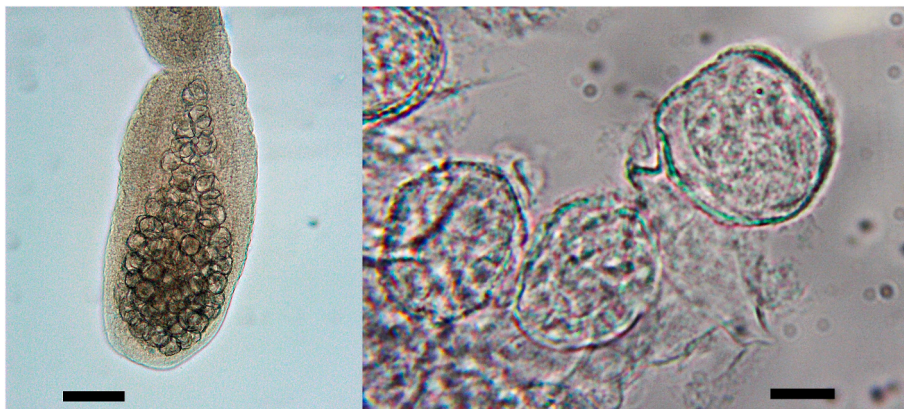

Fig. 1. Immature *E. multilocularis* without eggs in the last segment. *Scale-bar*: 100 μm.
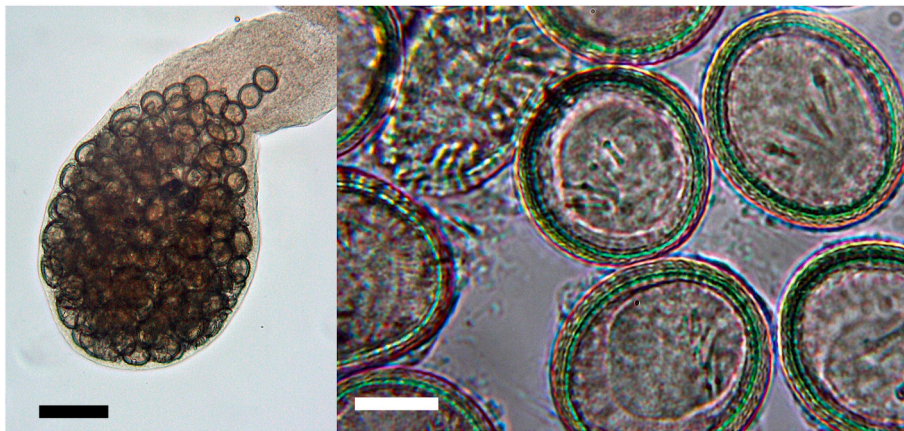
Fig. 2. Mature, thick-shelled eggs in *E. multilocularis* with striated embryophore and six-hooked oncosphere in the egg cavity of extracted eggs. *Scale-bars*: 100 μm (*left*) and 10 μm (*right*).

Fig. 3. Fertile *E. multilocularis* specimen with thin-shelled and translucent eggs without visible embryophore striae and oncosphere's hook set after extraction. *Scale-bars*: 100 μm (*left*) and 10 μm (*right*).

the crowding condition of the infrapopulation of origin. From these raw characteristics of the individual parasites, we calculated the following parameters for both host species: the proportion of fertile worms; the mean number of eggs per fertile worm; the proportion of worms with mature eggs; the mean number of mature eggs per worm; and the crowding conditions of worms with mature eggs.

For calculation, we used the online version of Quantitative Parasitology (Qpweb) software (Reiczigel et al., 2019). The association between the crowding (number of worms in a host) and the egg count was also calculated. Because the worm count values ranged from 4 to 15,000, they had a large variance and were widely dispersed from the mean. The logarithm of worm counts was used to evaluate the association between crowding and egg count to attenuate the variance. The relationship was investigated by a generalised linear model (GLM) approach with a Tweedie log function. For the calculation, we used the SPSS software (v25). For each statistical calculation, bootstrapping was used with 5000 iterations.

Parasitological data are included in Supplementary Table S1.

3. Results

The carcasses of infected definitive hosts (33 red foxes and 29 golden jackals) were investigated. The two clusters of the parasites that originated from the two different host species did not show significant differences in the proportion of fertile cestodes (Fisher's exact test, fox 86.5%, jackal 91.5%, $df = 191$, $P = 0.36$), and mean egg count per fertile worm (Bootstrap two-sample *t*-test, fox mean $\pm$ SD: 87.7 $\pm$ 62.72; jackal

mean $\pm$ SD: 76.7 $\pm$ 61.7, $df = 169$, $P = 0.25$).

On the other hand, the percentage of gravid worms with mature eggs was significantly higher in red fox specimens (Fisher's exact test, fox 94.8%, jackal 84%, $df = 169$, $P = 0.02$), although the mean number of mature eggs per worm was similar in the two investigated clusters (Bootstrap two-sample *t*-test, fox mean $\pm$ SD: 92.25 $\pm$ 62.2; jackal mean $\pm$ SD: 88 $\pm$ 60.7, $df = 152$, $P = 0.74$) (Table 1).

Crowding had a negative effect on the mean egg count of the infrapopulation ($\beta = -0.39$, $P < 0.001$) in both hosts. However, mature eggs developed in more crowded infrapopulations in the fox cluster than in the jackal cluster.

Table 1
Comparative analysis of worms and eggs of different status in red foxes and golden jackals.

	Red fox ($n = 33$)	Golden jackal ($n = 29$)	P-value
No. of worm specimens examined	111	82	
No. of worms with eggs	96 (86.5%)	75 (91.5%)	0.36
Mean no. of eggs per worm[a]	87.7	76.7	0.25
No. of worms with mature eggs	91 (82.0%)	63 (76.8%)	0.02
Mean no. of mature eggs per worm[b]	91.3	88	0.74
Crowding conditions of worms with mature eggs	669.6	231	0.01

[a] Immature parasites without egg production were excluded.
[b] Only worms with mature eggs were included in the calculation.

4. Discussion

The main finding of this study was that worms originating from jackals contained thick-shelled eggs in a significantly lower proportion than those originating from foxes, although the mean number of eggs per worm did not differ in the two host species. Considering that the investigated specimens originated from naturally infected wildlife, our study presents solely a snapshot of egg production performance of the parasite in different intestinal ecosystems. By our research design, we could not determine whether thin-shelled eggs originated from younger parasites or resulted from an impediment to egg development. For this reason, a significantly higher proportion of less developed eggs in golden jackals might have more explanations.

On the one hand, more thin-shelled eggs might mean a slower ontogeny of *E. multilocularis* in jackals. By random sampling, these developmental stages which need a longer period to pass through can be overrepresented. This explanation accords with experimental findings that the parasite's ontogeny in non-vulpine Canidae proved to be slower than in the red fox (Kapel et al., 2006; Al-Sabi et al., 2008; Thompson, 2017; Massolo et al., 2022).

On the other hand, it should not be excluded that some worms cannot complete egg development owing to the suboptimal conditions of their habitat. This phenomenon can be observed in feline definitive hosts, in which both parasite intensity and egg production capacity are lower than in canines (Kapel et al., 2006; Thompson, 2017). In our study, the proportion of gravid worms and fertile eggs did not differ in the two host species (Table 1). Therefore, definitive inhibition of parasite development in the golden jackal might be less likely.

A third possible cause of numerous thin-shelled eggs is multiple infections that resulted in more distinct classes of worms in the hosts' intestinal tract. However, Al-Sabi et al. (2008) experienced that in multiple infected hosts, most (74%) worms cannot produce eggs at all. In our study, over 80% of the parasite individuals produced eggs, of which over 80% proved to be fully mature in both hosts. Moreover, multiple infection is proved to be more characteristic for foxes than for other definitive hosts (Al-Sabi et al., 2008).

The suitability of a certain definitive host species depends on the microtopography of the small intestine, the composition of bile, biochemical and nutritive factors in the gut lumen, and the immune response ability of the local lymphatic tissue (Gregor et al., 2022). Protoscoleces that enter the small intestine are sensitive to bile acids. Although bile is essential for the initiation of larval development, glycine-conjugated deoxycholate, characteristic for herbivorous bile, possesses a strong lytic effect on the tegument of protoscoleces (Smyth, 1962).

Deoxycholate is a secondary bile acid, which is transformed from cholic acid by intestinal bacteria. From the intestinal lumen, deoxycholate is absorbed and transported back to the liver (Winston and Theriot, 2020; Doden et al., 2021). Hepatocytes sense secondary bile acids as endogenous toxins, thus tolerance to deoxycholate means a better tolerance to also exogenic toxins, such as plant secondary metabolites, which were evolutionary developed in herbs against herbivores. Thus, the interdependent co-evolution of plants, consumers, and the intestinal microbiota were shaping a delicate balance throughout the trophic webs (Gregor et al., 2022; Minard et al., 2022). The bile acid composition of different carnivore mammals is very similar, deoxycholate is conjugated with taurine, and a great proportion of bile acids are cholic acid in the form of sodium cholate or taurocholate, which are less erosive for the worms' tegument (Smyth, 1962).

Deoxycholate production depends on the host's microbiota, which is determined by the phylogenetic position of the host species and the diet of the individual (Wu et al., 2022). Although both red fox and golden jackal are omnivorous, as they consume less than 70% animal tissue in their diet, deoxycholate producing Firmicutes bacteria are less dominant in golden jackal's microbiota (Wu et al., 2022; Lapid et al., 2023) than in members of the genus *Vulpes* (Liu et al., 2020; Wu et al., 2022). On the

other hand, deoxycholate production in Felidae is very low contrary to the high Firmicutes content in their microbiota (Wu et al., 2022). This fact suggests finer interactions between the host, its microbiome and bile acids.

Among the biochemical and nutritional requirements of *Echinococcus* spp., glucose has a central role. Due to its obligate parasitic lifestyle, *E. multilocularis* can utilise completely digested nutrients. Carbohydrates are exploited in the form of glucose within an anaerobic environment. Studies on *Echinococcus* spp. adults revealed that lactate production and lactate dehydrogenase pathway of ATP synthesis characterise their metabolism (Constantine et al., 1998; Luo et al., 2022). Based on these research findings, we hypothesised that access to digested carbohydrates should be a limiting factor for adult parasites in the intestinal ecosystem.

In wild carnivores, enzymatic breakdown of polysaccharides is limited. The evolution of carnivory led to the contraction in gene families for metabolism of complex carbohydrates. Amylase enzyme coding AMY2B gene copy number decreased as a result of adaptation to predominant flesh consumption and low carbohydrate content of the diet. Due to the loss of AMY2B copies, the amylase enzyme activity is lower in carnivores than in herbivore mammals. Therefore, the oligosaccharide content achievable for brush border disaccharidases is also limited in a carnivore's small intestine (Abduriyim et al., 2019; Antkowiak et al., 2020).

Although most wild canid species possess only one copy of AMY2B, they can adapt to increased starch content of their food contrary to cats that are hypercarnivores depending on animal-originated diet (Shrestha et al., 2011; Antkowiak et al., 2020). Golden jackals and red foxes are both opportunistic feeders; carnivorous components of their diet are between 50 and 70% (Lanszki et al., 2016; Lange et al., 2021). Lanszki et al. (2016) pointed out a peculiar distinction between red foxes and golden jackals in utilisation of plant materials. Foxes consume three times more fruits than jackals. Wild fruit carbohydrates contain 20–60% fructose (Barros et al., 2010). Fructose content of the chyme partially inhibits the glucose uptake of the enterocytes (Koepsell, 2020; Song et al., 2023), which provides accessible glucose for *Echinococcus* spp. adults located between intestinal villi in intimate connection with the brush border and its disaccharidase enzymes (McManus, 2009). The positive effect of the fructose-supplemented diet of the host on taeniids was studied in *Hymenolepis* spp. The research demonstrated that fructose alone cannot support the growth and egg production of the parasites but improves the positive effect of a starch-rich diet (Read et al., 1958).

Besides fructose, wild berries also contain phenolic compounds (Barros et al., 2010). These secondary plant metabolites reduce the gene expression level of sugar transporters of the enterocytes and, therefore, inhibit glucose uptake by the host (Barreto-Peixoto et al., 2023). This effect increases the glucose concentration of the intestinal fluid, though little is known about the influence of phenolic compounds on the survival of *E. multilocularis*. Studies on *E. granulosus* protoscoleces (Ali et al., 2020) and *Raillietina echinobothrida* (Giri and Roy, 2014) proved that secondary plant metabolites can damage the tegument of cestodes.

Considering that carnivores diverged from their plant-eating ancestors, their detoxification ability is limited, thus they hardly tolerate high levels of phenolic compounds (Shrestha et al., 2011; Yoshimura et al., 2021). Between different carnivore taxa, detoxification capacity of the liver varies, which is well demonstrated by different levels of contraction and pseudogenization in the UDP-glucuronosyltransferase (UGT) gene family. These genes code the major phenol-detoxification enzymes of the liver (Shrestha et al., 2011; Kim et al., 2016). To the best of our knowledge, UGT copy number variation in golden jackals has not been investigated yet. Kondo et al. (2022) compared evolutionary features of UGT genes in carnivore taxa. They ascertained gene expansion in domestic dogs and fox species but not in wolves and felids. The authors regarded UGT gene expansion as an adaptation to a degree of herbivory.

Scientific literature on intestinal ecosystems of golden jackal and red fox revealed some variations. Diet analysis demonstrated a preference for fruits in foxes (Lanszki et al., 2016). Studies on components of

intestinal microbiota proved that golden jackal harbours less Firmicutes bacteria than vulpine carnivores (Wu et al., 2022; Lapid et al., 2023). These data can support the hypothesis that in natural conditions, golden jackal's intestinal fluid contains low concentration of potentially toxic compounds, which can harm *E. multilocularis*. On the other hand, a small proportion of carbohydrate consumption (Lanszki et al., 2016; Lange et al., 2021) and presumably low amylase activity (Abduriyim et al., 2019) in the golden jackal provides less accessible glucose for the parasite.

The theory of low carbohydrate level was supported by the finding that in golden jackals, crowding caused conspicuous impact on egg maturation. In golden jackal specimens, mature egg producing individuals were obtained from less crowded (almost third in size) infrapopulations than those of fox origin. This finding suggests that in the golden jackal's intestinal ecosystem, an intense competition takes place for resources.

Comparing our research findings with literature data, we concluded that the golden jackal's intestinal ecosystem cannot provide a huge amount of nutrients for *E. multilocularis*. Nevertheless, the jackal's gut would carry a lower risk of intoxication for the parasite. In the fox's intestinal ecosystem, the parallel presence of higher amounts of accessible glucose and higher risk of toxic compounds might force the rapid maturation of the worms, which could result in shorter longevity and extensive egg production for a short time period. This hypothesis agrees with the experiences gained by experimental infection of different carnivore taxa with *E. multilocularis*. In these experiments, the researcher determined the shortest, though the most extensive, egg production in foxes (Thompson and Eckert, 1982; Kapel et al., 2006; Thompson et al., 2006; Al-Sabi et al., 2008).

Based on these findings, we supposed that the mutual presence of red fox and golden jackal in a habitat can enhance the public health risk of HAE. Foxes can contribute to the egg load of the environment during the period from late spring to early autumn, which is both the main season for wild berry ripening and the breeding of the intermediate hosts of *E. multilocularis*. During this period, foxes consume a great proportion of fruits (Lanszki et al., 2016). Fruit components can increase both sugar and phenolic compound concentrations in the intestinal fluid, which might result in maturation and egg production with high peaks but in short phases.

The ecosystem in the golden jackal host may provide a less turbulent environment for the parasites. In these conditions, the parasites can grow and mature less rapidly but can count on a longer lifespan. As a result, egg maturation drags but lasts for long, contributing to a steady egg shedding almost all year round. This hypothetical egg-producing characteristic of jackal's *E. multilocularis* might mitigate the impact of climate warming by sustaining transmission during the initiation and the termination phase of the rodents' breeding season.

Long-lasting carrying of the parasite might ensure the maintenance of the populations between periods of extensive transmission between definitive and intermediate hosts. This might compensate for the short lifespan of the intermediate hosts of *E. multilocularis*, thus golden jackals might serve as bridge hosts in multi-host ecosystems. However, their bridge effect is not as remarkable as that of ungulate intermediate hosts of *E. granulosus* (Massolo et al., 2022). The survival of adult *E. multilocularis* in the golden jackal is to be determined to assess the potential bridge effect of this host species.

Thompson et al. (2006) suggested that short life span of *E. multilocularis* in foxes increases the possibility of reinfection and, thus sexual breeding. This phenomenon contributes to the maintenance of genetic diversity in *E. multilocularis* populations (Thompson et al., 2006). Based on another theory, the combination of continuous inbreeding and rare outcrossing guarantees heterosis in a population (Chelo et al., 2019). The golden jackal, presumably, is an appropriate reservoir host for inbred genetic lines of *E. multilocularis*, which might produce heterozygous offspring as a result of outcrossing with another inbred line when they meet each other in the intestinal tract of a fox host.

In other aspects of suitability, we could not determine the difference between the golden jackal and the red fox. Previous studies in the same suprapopulation also supported the similarity of the two hosts as both the prevalence and the mean intensity of *E. multilocularis* infection were comparable in red foxes and golden jackals (Moloi et al., 2023). Moreover, in the present study we found that crowding increased the proportion of less matured eggs in *E. multilocularis*; this phenomenon was identified in both hosts. This finding also supported the hypothesis that ongoing infection might reduce the possibility of reinfection (Thompson et al., 2006). Limitation of egg production in a crowded parasite population might be a symptom of competition for sources, especially for carbohydrates. On the other hand, this self-limitation of larger infrapopulations might contribute to maximizing the genetic diversity of the whole *E. multilocularis* population in a certain habitat.

Though it is based on solely deduction, we hypothesise that alternation of sexually and asexually reproduced generations of *E. multilocularis* forces a continual coevolution with a multi-host system to maintain a constant level of genetic diversity, thus virulence. This might result in a pattern, which is observable in the chase Red Queen dynamic where coevolutionary warfare constantly changes. The variation in the selective effect of different types of hosts can cause oscillation in the gene frequency, thus the relative breeding success of different generations (Brockhurst et al., 2014; Strotz et al., 2018). Previous studies in the same carnivore communities suggested that golden jackal might generate a slightly higher risk for HAE than red fox (Halász et al., 2021; Moloi et al., 2023). Regarding the recent findings, we concluded that the common presence of two, dissimilarly adapted, host species in a multi-host system might stabilise endemic focuses of *E. multilocularis* even in suboptimal environmental conditions.

However, our study has some significant shortcomings. The size of an infrapopulation was determined by counting all parasites without sorting them by intestinal section of origin. Therefore, the local effect of crowding could not be determined, though the aggregated distribution of the *E. multilocularis* adults is proved (Umhang et al., 2011). Our hypothesis was that the summarised parasite number could provide enough information about the living conditions (nutrient sources, damaging effects) of the concerned infrapopulations. However, by this method we might have lost valuable information on the driving forces of parasite establishment and egg production in different segments of the small intestine.

We did not investigate the diet composition of the host individuals. Our hypothesis was that the nutrient supply throughout the whole parasite development affected the egg production process. Therefore, we did not analyse the momentary stomach content and scat composition. Instead, we used summarised data on the local (Lanszki et al., 2016) and the European (Lange et al., 2021) wild canid populations' seasonal feed preference.

Diet composition investigations proved that microtine species, mostly *Microtus arvalis*, are a very important component in the diet of both the red fox and the golden jackal all year round (Lanszki et al., 2016; Lange et al., 2021). The seasonal pattern of rodent consumption differs by region, though most studies agree that the proportion of rodents in the foxes' summer diet decreases with a parallel increase in plant material (Weber et al., 1993; Burlet et al., 2011; Lanszki et al., 2016; Lange et al., 2021). Such a remarkable seasonal change in the diet of the golden jackals cannot be observed (Lanszki et al., 2016).

These research findings seemed to support our deduction that the carbohydrate rich summer diet of foxes can enhance the rapid maturation and extensive egg production of the fox-associated parasite cluster. This hypothesis is also supported by the studies of Morishima et al. (1999) and Citterio et al. (2021), who found high summer prevalence of *E. multilocularis* in foxes. However, Fischer et al. (2005) did not prove a difference between the seasonal *E. multilocularis* prevalence in foxes, while Hofer et al. (2000), Lewis et al. (2014) and Woolsey et al. (2015) found that winter prevalence was statistically higher than that of any

other season. These latter findings contradict our hypothesis that foxes are more important hosts during the breeding season of small rodents, while jackals become important during the cold season. Thus, further research is needed to determine the seasonal pattern of egg shedding by the two hosts within the South Transdanubian *E. multilocularis* suprapopulation.

We did not investigate the biomass of available intermediate host species. Instead, we used the data of a survey based on barn owl (*Tyto alba*) pellet analysis (Purger, 2014) confirming that within the investigated region, the dominant rodent species is the common vole (*Microtus arvalis*), while the historical intermediate host, the water vole (*Arvicola amphibius*) (Weber et al., 1993; Burlet et al., 2011; Lewis et al., 2014) is very rare. A synchronous population expansion of the common vole is experienced all over Europe (Jacob et al., 2020), which could lead to the dominance of this species as an intermediate host of *E. multilocularis* even in the historical distribution area of the parasite (Beerli et al., 2017).

Finally, apart from crowding, we did not collect any further information on the parasitological status of the investigated host individuals. Since very few parasites were collected from each animal, neither the average egg count per worm in a host nor the faecal egg count were determined. Our main goal was to evaluate the differences between the jackal and fox clusters of an *E. multilocularis* suprapopulation. We hypothesised that the simplest way to neutralise the effect of an individual host was to choose the fewest parasite items from an infrapopulation. We considered that faecal egg count characterised rather the infrapopulation than the individual, thus the cluster. For a better understanding of the influence of crowding on reproduction, in future research, it is suggested to compare highly crowded infrapopulations to each other by collecting, counting, and examining parasites from separated segments of the small intestine. Additionally, faecal egg count produced by a thoroughly investigated infrapopulation could also allow a deeper insight into the reproduction biology of *E. multilocularis* in its natural populations.

5. Conclusions

By investigation of egg production performance of *E. multilocularis* adults that originated from different hosts, we proved that the parasite produced more thin-shelled eggs and crowding caused more harm to the reproduction in golden jackals than in red foxes. Comparing these findings with the results of other studies dealing with different aspects of intestinal ecosystems of golden jackal and red fox, we supposed that golden jackal might increase the breeding success of *E. multilocularis* in habitats shared with red foxes. This study investigated naturally infected, hunter-harvested specimens, therefore most of our conclusions are based on a speculative approach, thus our research generated more questions than answers. Our deductive reasoning highlighted the potential future research directions, such as determination of *E. multilocularis* adults' life-span in the golden jackal, the effect of diet components on the survival of adult parasites, the role of herbivore characteristics in host-parasite coevolution, and the dynamics of *E. multilocularis* reproduction in other multi-host systems.

CRediT authorship contribution statement

Sibusiso Moloi: Investigation, Data curation, Project administration, Writing – original draft. **Tibor Halász:** Investigation, Resources. **Ágnes Csivincsik:** Methodology, Writing – review & editing, Supervision. **Gábor Nagy:** Conceptualization, Investigation, Formal analysis, Writing – original draft, Supervision.

Ethical approval

All animals of this study originated from carcasses, which were harvested in the framework of a legal hunting management programme.

None of the investigated carnivores were culled for scientific purposes.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary files (see also https://zenodo.org/uploads/13993516).

Declaration of competing interests

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2024.100225.

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