Variations in small mammal helminths structure during host population peak and decline periods and according to locality

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ABSTRACT

Our results indicated that 64 % (38/59) of small mammals (*Apodemus flavicolis, A. sylvaticus, Myodes glareolus, Microtus agrestris, M. arvalis*, *Sorex araneus* and *S. minutus*) studied in 2015 were infected with *Syphacia*, *Trichuris*, *Aonchotheca*, Heligmosomidae and tapeworms, and 64 % (27/42) of animals investigated in 2018 were infected with *Syphacia*, *Trichuris*, *Aonchotheca,* Heligmosomidae and tapeworms. In 2018, prevalence of infection was 83 % (15/18) in autumn in NW Bohemia and 50 % (12/24) in autumn 2018 in South-East Bohemia. Regarding locality, total prevalence of infection and species richness were higher in North-West Bohemia. Total prevalence of infection according to season was 72 % (18/25) in spring and 62 % (47/76) in autumn. In NW Bohemia in 2015, 72 % (18/25) of animals were infected in spring and 59 % (20/34) in autumn. Statistically significant differences in parasite infection of Murinae and Arvicolinae were evident when comparing years and seasons.

INTRODUCTION

Humans and their expanding civilizations affect the very nature of host-parasite coevolutionary dynamics, climate change and biotic invasion. Moreover, landscape modification influences the biology of hosts and their parasites (Thompson 2005; Morand et al. 2006).

Intestinal helmiths can influence survival, behavior, reproduction, mate choice and energy demands. Parasites can have detrimental effects on fecundity and survival in small mammal populations, and if parasites reduce host fecundity or survival, it is then theoretically possible that they may play a role in regulating host numbers (Tompkins & Begon, 1999). Scott (1987) described the regulation of mouse colony abundance by *Heligmosomoides polygyrus*. *Capillaria hepatica* also reduces natality and survival in laboratory mice (Singleton & Spratt 1986; Morand et al. 2006).

Deter et al. (2007) reported that *Microtus arvalis* females parasitized by *Trichuris arvicolidae* gave birth to fewer pups than did non-parasitized females. *T. arvicolidae* infection had a significant effect on individual mass at birth, with pups from parasitized females having significantly lower mass than pups from non-parasitized females.

The structure of helminths in wild rodents in Europe was documented by Behnke et al. (2001), Haukisalmi et al. (2006) and Milazzo et al. (2003). It is evident that both external (year, season, etc.) and internal (age, sex, etc.) factors influence helminthfauna (Abu-Madi et al. 2000; Behnke et al. 2001). Surprisingly, it was determined that internal factors have a less significant impact in shaping the representation of helminthofauna than do external factors (Behnke et al. 2001; Milazzo et al. 2003), and it was further proven that the sex of the host rarely plays a role in parasite communities. However, there are reports of helminths that are influenced by the sex of the host (Behnke et al. 2001). Parasitic diseases are increasingly becoming recognized as a major factor affecting host behavior, survival strategies and population dynamics (Behnke et al. 2001). The aim of this study was as follows: (1) to assess the prevalence of intestinal helminth infection among small rodents (Murinae: *Apodemus flavicollis*, *A. sylvaticus* and *Arvicolinae: Microtus arvalis, M. agrestis*, *Myodes glareolus*) in selected areas of the Czech Republic; (2) to compare gastrointestinal helminth species richness between these two rodents; and (3) to evaluate the effects of an internal factor (sex).

MATERIAL AND METHODS

Study sites

1. Krušné Hory Mts. (NW Bohemia)

The study site was located in a forested area with coniferous and deciduous trees, which is also rich with herbal plants. Spruce trees are the most predominant in the area. The area also includes meadows, water dam and ponds

2. Southeastern region of the CR (Křižanovská vrchovina)

This study site is in an area that comprises forests and meadows. A forested area in this region is characterized by a predominance of deciduous and coniferous trees.

Collection of rodents

We used snapping traps baited with roasted wick. The wicks were taken from kerosene lamps (the wick was cut into squares the size of 1 to 3 cm^2) and soaked in fat with flour. The traps were placed in a network of points spaced 10 m apart (2 traps at each point).

Helminthological autopsy and diagnostics

The biological material was stored at – 20 °C until the autopsy. Autopsies were carried out in June 2018 and June 2019 on rodents caught in 2015 and 2018 respectively. The autopsy began with the opening of the abdominal cavity, followed by the removal of the internal organs (liver and digestive tract). All individual parts of the gastrointestinal tract were separated and assessed in a petri dish. The organs were then diluted, washed and blended.

Samples were then taken and examined (binocular magnifier, microscope). Helminths found in the digestive tract were fixed in a 4% formaldehyde or 96% ethanol solution.

Statistical analysis

Results were analyzed using Statistica 6.1. The rates of prevalence were compared using non-parametric tests. Prevalence between seasons and years (spring 2015/autumn 2015/autumn 2018); all small mammals examined at study; infection of Murinae/Arvicolinae rodents were compared using χ^2 test.

RESULTS

Results from NW Bohemia (Krušné Hory) spring 2015

A total of 25 rodents were investigated: 14 Murinae (12 *Apodemus flavicolis* and 2 *A. sylvaticus*) and 11 Arvicolinae (*Myodes glareolus*). Overall prevalence of infection was 72 % (18/25). There was a prevalence of infection among Murinae (*Apodemus flavicolis* and *sylvaticus*) and Arvicolinae (*Myodes glareolus*) of 71 % (10/14; Fig. 1) 73 % (8/11; Fig. 1) respectively.

Murinae was infected with nematoda (*Syphacia*, *Trichuris*, *Aonchotheca* [*Capillaria*] and Heligmosomidae); Arvicolinae, on the other hand, was infected with nematoda (*Syphacia*, *Aonchotheca* [*Capillaria*] and Heligmosomidae).

Results from NW Bohemia (Krušné Hory) autumn 2015

A total of 34 rodents were investigated: 15 Murinae (*Apodemus flavicolis*) and 19 Arvicolinae (16 *Myodes glareolus* and 3 *Microtus Arvalis*). Overall prevalence of infection was 59 % (20/34). Prevalences of infection for Murinae (*Apodemus flavicolis*) were 73 % (11/15; Fig. 1) and Arvicolinae (*Myodes glareolus* and *Microtus agrestis*) 47 (9/19; Fig. 1) respectively.

Murinae was infected with nematoda (*Syphacia*, *Ttrichuris* (Fig. 3, 4) and Heligmosomidae), as well as with cestoda in one rodent;

Arvicolinae, on the other hand, was mostly infected with nematoda (Heligmosomidae, *Syphacia* and *Trichuris*), as well as with cestoda in four rodents.

The overal prevalence of parasite infection among rodents in 2015 (spring/autumn) was 64 % (38/59).

Results from NW Bohemia (Krušné Hory) autumn 2018

A total of 18 rodents were investigated: 3 Murinae (*Apodemus flavicolis*), 5 Arvicolinae (3 *Myodes glareolus* and 2 *Microtus agrestis*) and 10 Soricidae (8 *Sorex araneus* and 2 *Sorex minutus*). Overall prevalence of infection was 83 % (15/18). Prevalences of infection for Murinae (*Apodemus flavicolis*), Arvicolinae (*Myodes glareolus* and *Microtus agrestis*), and Soricidae (*Sorex araneus* and *Sorex minutus*) were 33 % (1/3; Fig. 1), 80 % (4/5; Fig. 1) and 100 % (10/10; Fig. 1) respectively.

Murinae were infected with nematoda (*Aonchotheca* [*Capillaria*]); Arvicolinae, were infected with nematoda (Heligmosomidae, *Syphacia* and *Trichuris*), as well as with cestoda in one rodent; Sorcidae were infected with nematoda (*Syphacia*), as well as with cestoda in nine rodents.

The southeastern region of the CR (Křižanovská vrchovina)

A total of 24 rodents were investigated: 16 Murinae (*Apodemus flavicolis*) and 8 Arvicolinae (*Microtus arvalis*). Overall prevalence of infection was 50 % (12/24). Prevalences of infection for Murinae (*Apodemus flavicollis*) and Arvicolinae (*Microtus arvalis*) were 31 % (5/16; Fig. 1) and 88 % (7/8; Fig. 1) respectively.

Murinae was infected with nematoda (*Syphacia*), as well as with cestode in two rodents. Arvicolinae, on the other hand, was infected with nematoda (*Syphacia* (Fig. 2)*, Trichuris* and Heligmosomidae (Fig. 5).

The overal prevalence of parasite infection anong rodents in autumn 2018 was 64 % (27/42).

There was no statistically significant difference between years and seassons (spring 2015, autumn 2015 and autumn 2018; p=1,0000). There was however, a statistically significant difference between the Murinae examined at study years and seasons (spring 2015/autumn 2015/autumn 2018) (p=0,3865). There is also a statistically significant difference between the Arvicolinae examined at study years and seassons (p=0,479). There was no statistically significant difference between prevalence of all small mammals examined at study years and seassons (spring 2015/ autumn 2015/autumn 2018) (p=1,0000).

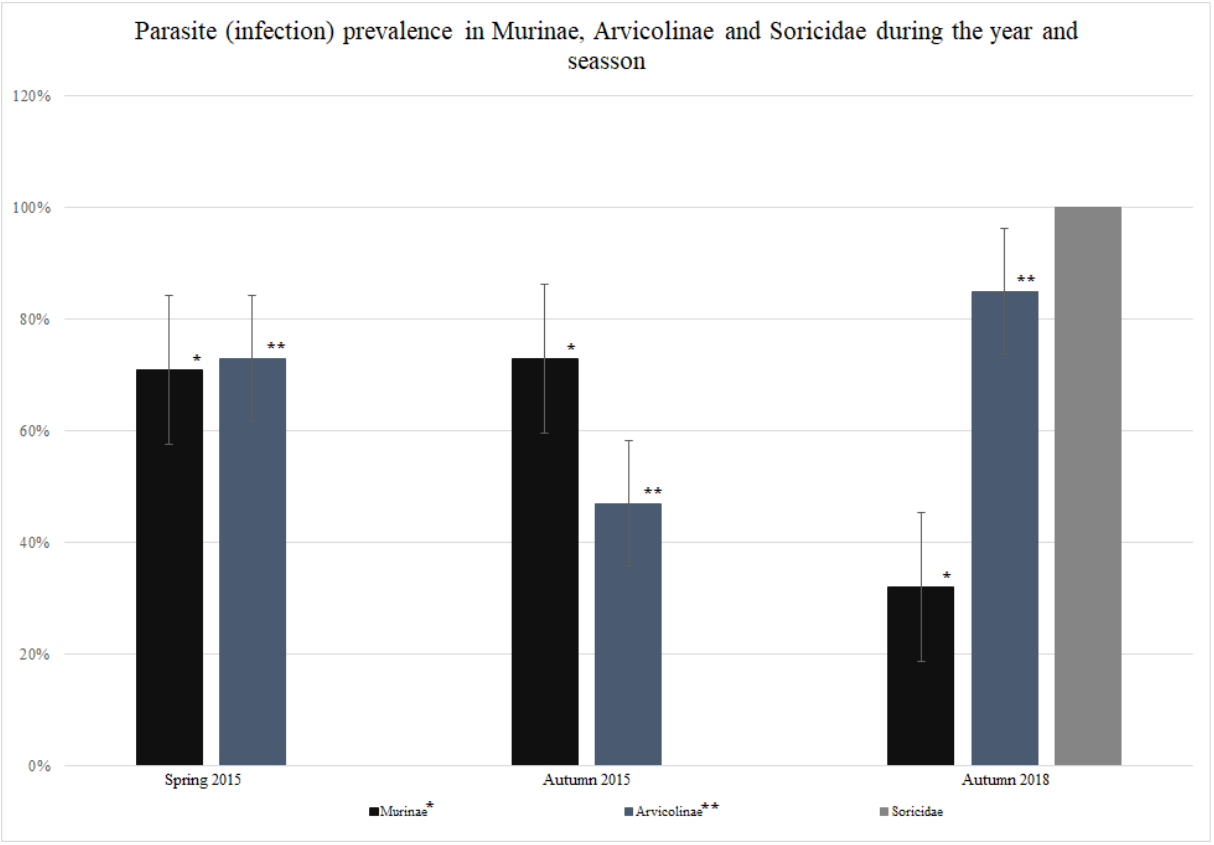

Figure 1 Parasite (infection) prevalence in Murinae (*Apodemus flavicollis* and *A. sylvaticus*), Arvicolinae (*Myodes glareolus*, *Microtus arvalis* and *M. agrestis*) and Soricidae (*Sorex araneus* and *S. minutus*) according to study periods (spring 2015, autumn 2015 and autumn 2018). There was no statistically significant difference between years and seassons (spring 2015, autumn 2015 and autumn 2018) (p=1,0000). There was no statistically significant difference between prevalence of all small mammals examined at study years and seassons (p=1,0000). There was a statistically significant difference in prevalence of infection among Murinae between spring and autumn as well as between 2015 and 2018 (p=0,3865*). There was also a statistically significant difference in prevalence of infection among Arvicolinae (p=0,479**) between spring and autumn as well as between 2015 and 2018.

Table 1 Parasite (infection) prevalence in Murinae (*Apodemus*), Arvicolinae (*Myodes*, *Microtus*) and Soricidae (*Sorex*) according to year, season and locality

Host	**Season/year**	**Prevalence (%)**	**Parasites**	**Habitat**
Murinae (*Apodemus*)	Spring/2015	71 (10/14)	*Syphacia*, Heligmosomidae, *Aonchotheca* (*Capillaria*)	NW*
Murinae (*Apodemus*)	Autumn/2015	73 (11/15)	*Syphacia*, Heligmosomidae, *Trichuris*, cestoda	NW*
Murinae (*Apodemus*)	Autumn/2018	32 (6/19)	*Syphacia*, *Aonchotheca* (*Capillaria*), cestoda	NW* (1/3), SE** (5/16)
Arvicolinae (*Myodes*)	Spring/2015	73 (8/11)	*Syphacia*, Heligmosomidae, *Aonchotheca* (*Capillaria*)	NW*
Arvicolinae (*Myodes*, *Microtus*)	Autumn/2015	47 (9/19)	*Syphacia*, Trichuris, Heligmosomidae, cestoda	NW*

Arvicolinae (*Myodes*, *Microtus*)	Autumn/2018	85 (11/13)	*Syphacia*, Heligmosomidae, *Trichuris*, cestoda	NW* (4/5), SE** (7/8)
Soricidae (*Sorex*)	Autumn/2018	100 (10/10)	*Syphacia*, cestoda	NW*

** NW = North-west Bohemia*
***SE = South-east Bohemia*

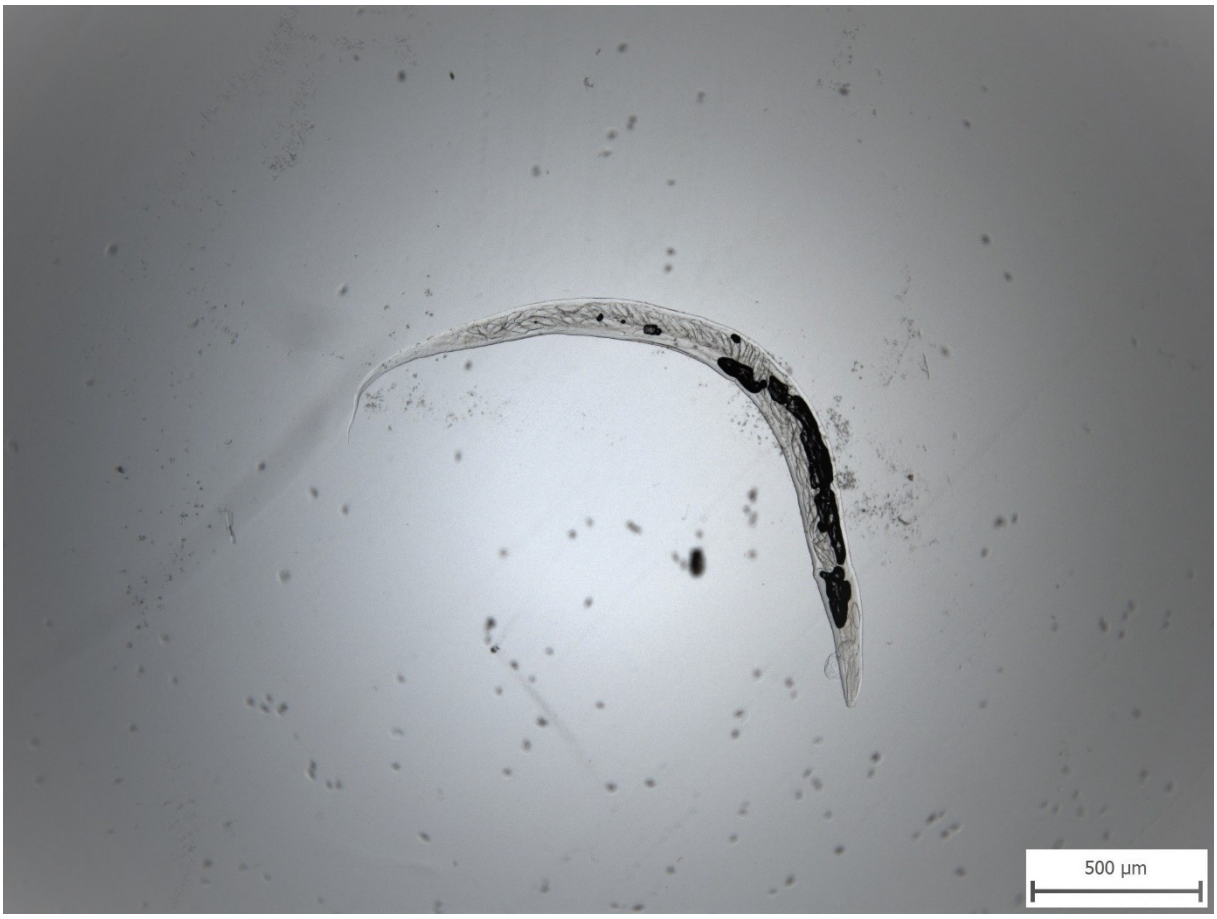

Figure 2 Pinworm female (*Syphacia* sp.; lenght: 4336 µm) with eggs from *Microtus arvalis* (captured in southeast Bohemia in autumn 2018)

Figure 3 *Trichuris* sp. female (lenght: 19762 µm) from *Apodemus flavicollis* (captured in northwest Bohemia in spring 2015)

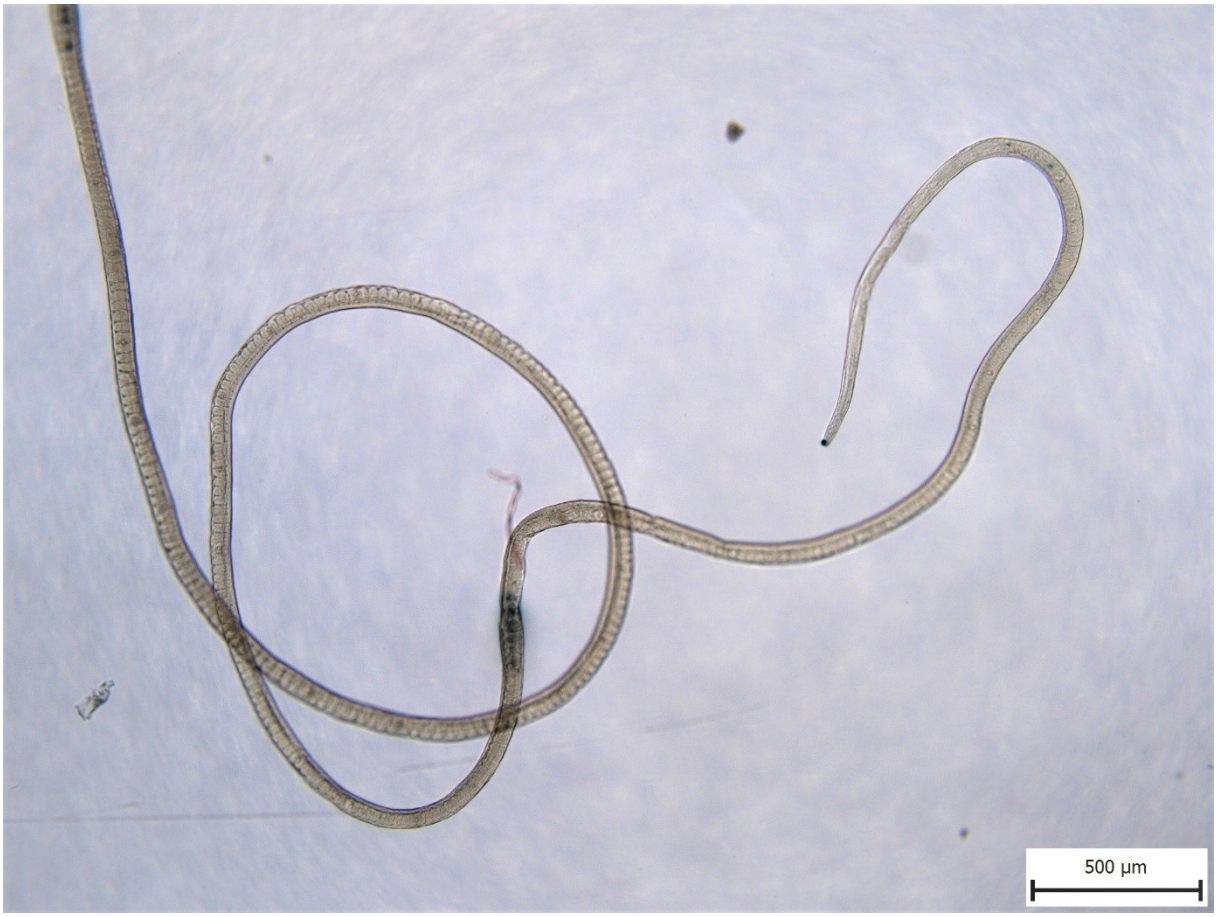

Figure 4 Anterior part (lenght: 9686 µm) of *Trichuris* sp. female from *Apodemus flavicollis* (captured in northwest Bohemia in spring 2015)

Figure 5 Anterior part of a Heligmosomidae nematode female (length: 4193 µm) from *Microtus arvalis* (captured in southeast Bohemia in autumn 2018)

DISCUSSION

Rodents used in this study were captured in two locations (habitats). The first of these was exposed to a direct emission burden (NW Bohemia - Krušné hory), while the second was exposed to very little direct industrial emission burden (SE Bohemia - Křižanovská vrchovina). It has been shown that hosts are more seriously infected by helminths in areas affected by industry (Jankovská et al., 2005).

We found mostly nematode parasites as well as one tapeworm in the adult stage. Behnke et al. (2001) reported similar results in Poland, and Klimpel et al. (2007) observed only nematodes in Germany.

Our observations in SE Bohemia (Křižanovská vrchovina) indicated that gastrointestinal helminth species richness was higher in voles (*Microtus arvalis*), which were infected by 3 nematodes (*Trichuris, Syphacia* sp., Heligmosomidae). A majority of the yellow-necked mice (*Apodemus flavicollis*) were infected by a single nematode species (*Syphacia* sp.); one yellow-necked mouse (*Apodemus flavicollis*) was infected by an adult tapeworm. Klimpel et al. (2007) studied parasite fauna of the bank vole (*Myodes/Clethrionomys glareolus*) in an urban region of Germany and found that these bank voles were infected exclusively by nematodes (*Aonchotheca* [*Capillaria*], *Heligmosomum costellatum,* and *Heligmosomoides polygyrus* as adults in the digestive tract; *Pelodera* syn. *Rhabditis strongyloides* as third larval stages in the skin of the rodents). With the exception of *Pelodera strongyloides,* we observed the same nematodes in digestive tracts. The most abundant nematodes in our study were pinworms (*Syphacia)* (Fig. 2).

We captured rodents in SE Bohemia (Křižanovská vrchovina) in the autumn of 2018 and in NW Bohemia (Krušné hory) in the spring and autumn of 2015 as well as in the autumn of 2018. Abu-Madi et al. (2000) monitored seasonal and site-specific variations in the component community structure of intestinal helminths of *Apodemus sylvaticus* from three contrasting habitats in southeast England, and these authors reported that the highest rates of parasitic infection appeared in autumn.

When we compared 2015 with 2018, we found a parasite prevalence of 71 % among *Apodemus* and 73 % among *Myodes/Microtus* in the spring of 2015; for autumn of the same year (2015), parasite prevalences was 73 % and 47 % among *Apodemus* and *Myodes/Microtus* respectively (Fig. 1, Tab. 1); for 2018, parasite prevalence was 32 % among *Apodemus*, 85 % among *Microtus/Myodes* and 100% among *Sorex* in autumn). The role of habitat and season was also monitored by Pakdeenarong et al. (2013). These authors reported significantly higher rates of

individual helminth infection during the wet season. Habitat significantly influenced individual helminth species richness and individual helminth abundance, with a decrease in individual helminth species richness and individual helminth abundance from forest habitat to agricultural and human settlement habitats. In our study, there were no statistically significant differences between prevalence of infection in NW Bohemia and that of SE Bohemia.

Ferrari et al. (2004) monitored the role played by host sex in parasite dynamics. They conducted field experiments on the yellow-necked mouse (*Apodemus flavicollis*) and its dominant nematode parasite, *Heligmosomoides polygyrus,* and they determined that males are responsible for spreading infection within the host population, while females are more likely to play a relatively trivial role.

Parasite prevalence seems to be dependent on the population parameters of the host species (Janova et al. 2010). Those authors (Janova et al., 2010) monitored the prevalence of *Heligmosomum costellatum* in a common vole population in southern Moravia, Czech Republic. They reported that 27.6 % of 503 common voles tested positive for *H. costellatum,* and the most infected common vole groups consisted of older, heavier and already reproducing females captured from April to August.

The numbers of infected males and females in our experiment were similar. Of the 5 infected *Apodemus flavicollis,* 3 were male and 2 were female; of the 7 infected *Microtus arvalis,* 4 were male and 3 were female. *Trichuris* sp. (figs. 3, 4) were found in two *Microtus arvalis* females. Deter et al. (2007) reported that *Microtus arvalis* females parasitized by *Trichuris arvicolidae* gave birth to fewer pups than did non-parasitized females. *T. arvicolidae* infection had a significant effect on individual mass at birth, with pups from parasitized females having significantly lower body mass than pups from non-parasitized females.

Therefore, certain intestinal helminths may serve as biological control systems in countering overpopulation among common voles (*Microtus arvalis*) and other rodents.

CONCLUSION

The impact of parasitism on host population dynamics is determined in part by the number of parasites present during host population fluctuations. Vole populations fluctuate over several years, it allowing these responses to be studied during the phase of population growth, population abundance and population decline.

In our studied locations (habitat) of study, the various populations were found to be at different states. In NW Bohemia the population was larger than that in SE Bohemia, but the difference was not statistically significant. Levels/prevalences of parasite infection were similar at both localities/habitat.

The effect of locality/habitat: Prevalence of infection was 83 % (15/18) in NW Bohemia (autumn 2018) and 50 % (12/24) in SE Bohemia (autumn 2018); there were no statistically significant differences between prevalence of infection in NW Bohemia and that of SE Bohemia.

The effect of season: In NW Bohemia in 2015 72 % (18/25) of animals were infected in spring and 59 % (20/34) in autumn. There were a statistically significant differences between prevalence of infection among both Murinae (p=0,3865) and Arvicolinae (p=0,479).

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TABLE AND FIGURE LEGEND

1. **Figure 1** Parasite (infection) prevalence in Murinae (*Apodemus flavicollis* and *sylvaticus*), Arvicolinae (*Myodes glareolus*, *Microtus arvalis* and *agrestis*) and Soricidae (*Soerax araneus* and *minutus*) according to season and year (spring 2015, autumn 2015 and 2018). There is no statistically significant difference between our 3 study periods (spring 2015, autumn 2015 and 2018) (p=1,0000). There is a statistically significant difference between the 3 study periods with respect to prevalence of infection among Murinae (p=0,3865). There is a statistically significant difference between the 3 study periods with respect to prevalence of infection among Arvicolinae (p=0,479). There is no statistically significant difference between the 3 study periods with respect to prevalence of infection among all small mammals (p=1,0000).

2. **Figure 2** Pinworm female (*Syphacia* sp.; lenght: 4336 μm) with eggs from *Microtus arvalis* (captured in southeast Bohemia in autumn 2018)

3. **Figure 3** *Trichuris* sp. (length: 19762 μm) from *Apodemus flavicollis* (captured in northwest Bohemia in spring 2015)

4. **Figure 4** Anterior part (length: 9686 μm) of *Trichuris* sp. female from *Apodemus flavicollis* (captured in northwest Bohemia in spring 2015)

5. **Figure 5** Anterior part of a Heligmosomatidae nematode female (length: 4193 μm) from *Microtus Arvalis* (captured in southeast Bohemia in autumn 2018)

6. **Table 1** Parasite (infection) prevalence among Apodemus, Myodes, Microtus and Sorex according to year, season and locality