Prevalence and parasite load of nematodes and trematodes in an invasive slug and its susceptibility to a slug parasitic nematode compared to native gastropods

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**ABSTRACT**

The invasive slug *Arion vulgaris* (Gastropoda: Arionidae) is an agricultural pest and serious nuisance in gardens of Central and Northern Europe. To investigate if the success of *A. vulgaris* in Norway can be attributed to a release from parasites, we compared the prevalence and parasite load of nematodes and trematodes in *A. vulgaris* to that of three native gastropod species, *A. circumscriptus*, *A. fasciatus* and *Arianta arbustorum*, in SE Norway. We found *A. vulgaris* to have the highest prevalence of both parasite groups (49% nematodes, 76% trematodes), which does not support the parasite release hypothesis, but rather points to *A. vulgaris* as a potentially important intermediate host of these parasites. For trematodes the number of individuals (parasite load) did not differ among host species; for nematodes it was higher in *A. vulgaris* than *A. fasciatus*. To further compare the parasite susceptibility of the surveyed gastropods, we exposed *A. vulgaris*, *A. fasciatus*, and *A. arbustorum* to a slug parasitic nematode, *Phasmarhabditis hermaphrodita*, in the laboratory. This nematode is commercially available and widely used to control *A. vulgaris*. The non-target species *A. fasciatus* was most affected, with 100% infection, 60% mortality and significant feeding inhibition. *A. vulgaris* was also 100% infected, but suffered only 20% mortality and little feeding inhibition. The load of *P. hermaphrodita* in infected specimens was not significantly different for the two *Arion* species (median: 22.5 and 45, respectively). Only 35% of *A. arbustorum* snails were infected, none died, and parasite load was very low (median: 2). However, they showed a near complete feeding inhibition at highest nematode dose, and avoided nematode-infested soil. Our results indicate that *A. vulgaris* may be less susceptible to *P. hermaphrodita* than the native *A. fasciatus*, and that non-target effects of applying this nematode in fields and gardens should be further investigated.

1. Introduction

Most terrestrial gastropods (slugs and snails) are beneficial decomposers feeding on decaying organic matter, but some are true herbivores and can cause serious damage in horticultural as well as cereal crops (Barker, 2002; Cameron, 2016; Kerney and Cameron, 1979). In the last few decades, the invasive slug, *Arion vulgaris* Moquin-Tandon, 1855, has been a significant nuisance in ornamentals and vegetables of domestic gardens across Europe (Hatteland et al., 2013a; Kozłowski and Kozłowski, 2011; Speiser and Kistler, 2002). It was reported in Central Europe in the late 1950s (Schmid 1970, as cited in Pfenninger et al., 2014) and first recorded in Norway in 1988 (von Proschwitz and Winge, 1994). It has since spread rapidly with unintended human help through transport of soil and plants (Andersen et al., 2012; Hatteland et al., 2013a; Kozłowski, 2007). In Scandinavia the high nuisance factor, due to sheer numbers and relatively large size (up to 18 cm long), has attracted media attention every slug season as well as frustrated gardeners (e.g. Dommerud, 2019; Holtung, 2019; Njie, 2015). There is also evidence that *A. vulgaris* may spread plant and animal pathogens and reduce quality of animal feed (silage) (Gismervik et al., 2014, 2015; Telfer et al., 2015).

Natural enemies of terrestrial gastropods, reviewed by Barker (2004), include a large spectrum from mammals, birds and amphibians, to trematodes, nematodes, spiders and mites. Here we briefly summarize what is known of nematodes and trematodes in terrestrial gastropods (slugs and snails).

Nematodes associated with slugs and snails include parasitic (definitive host) and paratenic (for transport) species as well as those using...
slugs and snails as intermediate hosts, such as the heartworm (Angiostrongylus vasorum) (Baillie, 1866)) (Ferdushy et al., 2009; Lange et al., 2018; Morand et al., 2004). A review on nematode-terrestrial gastropod associations is given by Grewal et al. (2003a). One of the first systematic surveys on nematodes in slugs was reported by Mengert (1953); in recent years various surveys around the world have increased the knowledge on nematodes associated with molluscs (e.g. Singh et al., 2019). Sudhaus (2018) gave an interesting account on dispersion of rabditid nematodes in the guts of slugs and snails. Ross et al. (2010) studied the role of nematode parasite release for slugs introduced to the USA from Europe. They found a nematode prevalence of 16.4% in native slugs collected in the UK, which was significantly higher than the 5.4% in the introduced slugs collected in the USA. They also found nematodes in 93% of UK collection sites, and only in 34% of US sites. In a first survey conducted in Norway to investigate naturally occurring nematodes in native and invasive slugs, Ross et al. (2015) in contrast found that nematode diversity, prevalence and intensity was highest in the invasive A. vulgaris.

A well known and widely distributed nematode of slugs is the facultative parasite Phasmarhabditis hermaphrodita (A. Schneider, 1859), the only species formulated into a biological control agent, Nemaslug® (A. Schneider, 1859), Tandonia sowerbyi (Hazay, 1880) and Carinarion sp., although they were also infected. Other gastropods like hedgehogs, avid eaters of slugs. Foster et al. (1958a,b) went on to study the ecology of the same parasites, postulating that the final host were small mammals like hedgehogs, avid eaters of slugs.

In the current study we compared parasite load and prevalence of nematodes and trematodes in the invasive A. vulgaris to that of three gastropod species native to Norway (Arion (Carinaria) circumscriptus) (Johnston, 1828, A. (Carinaria) fasciatus Nilsson, 1823 and Arianta arbustorum (Linnaeus, 1758), our main aim being to investigate whether A. vulgaris experiences parasite release (as described in Torchin et al., 2003). To further compare the parasite susceptibility of the surveyed gastropods and detect non-target effects of P. hermaphrodita, we exposed the invasive A. vulgaris and the two non-target species A. fasciatus and A. arbustorum to the biocontrol product Nemaslug® (P. hermaphrodita) in the laboratory.

2. Materials and methods

2.1. Field collection of gastropods

The four species of gastropods were collected from two sites with mixed forest and open habitat in South-Eastern Norway: Site A in As Municipality, Akershus County (3500 m²), open habitat with approximately 30 cm tall grass, forest floor sparsely vegetated and relatively dry, 59°40'20''N, 10°46'04''E); Site B in Eidsberg Municipality, Østfold County (15000 m²), open habitats with cereal and strawberry crops, forest floor moist and richly vegetated, 59°33'15''N, 11°14'37''E). The gastropods were collected by searching the lower vegetation layer, and identified morphologically in the field using von Proschwitz (2009). Arion circumscriptus was separated from the similar A. silvaticus in the laboratory by examining the reproductive system.

2.2. Survey of naturally occurring parasites

Gastropods were collected monthly in both localities from June to October 2013. They were stored at 4 °C and weighed and dissected consecutively within four weeks after collection. Slug dissections followed the protocol provided in Appendix 1, and snail dissections the online protocol by White-McLean (2011). Each specimen was categorized as juvenile, sub-adult or adult based on the developmental stage of its reproductive organs. Parasites found inside the gastropods were counted, or number estimated by subsampling, and classified morphologically as either nematodes or trematodes (other groups were not included in this study).

2.3. Bioassays with Phasmarhabditis hermaphrodita

Two identical bioassays were performed, one in August-September and one in September-October 2013. Specimens of Arion vulgaris, A. fasciatus and Arianta arbustorum were collected in site A 14 days before each assay. In the pre-assay period the specimens were quarantined at 15 °C ± 1 and RH 80% in large plastic containers (H:W:L = 17:25:34 cm) lined with moist paper and fed Chinese cabbage and thin carrot slices. They were regularly inspected to remove those dead or with any signs of infection.

Phasmarhabditis hermaphrodita nematodes, Nemaslug®, were obtained from BASF Agricultural Specialities Ltd. Three treatments were carried out: 1) Control (10 ml tap water); 2) Low dose (64 nematodes per cm²); and 3) High dose (128 nematodes per cm²). Each of them was applied to 15 plastic boxes (H:W:L = 9:13:18 cm) with a 2 cm layer of moistened, peat soil (Profil GoJord from Degernes Torstrefabrikk A/S). Immediately after treatment application, one healthy looking and weighed gastropod was introduced into each box, there being five replicates of each gastropod species per treatment and bioassay (90 gastropods in total for the two assays). The top 3 cm of the inner walls of
the boxes were coated with Neudorff® Antischneck-Gel®, a non-toxic slippery substance gastropods avoid and are unable to cross. The boxes were then incubated for 20 days at LD 10:14 hrs, 15 °C ± 1 and RH 80%, and examined every second day to change food, remove and record dead specimens. To monitor food consumption and condition of the gastropods, they were fed cabbage leaf squares of 4×4 cm. The proportion eaten was noted when the square was replaced. To ensure exposure to nematodes, specimens found on the box walls were moved to the soil near the cabbage leaf square at every examination and the exposure to nematodes, specimens found on the box walls were moved to the soil near the cabbage leaf square at every examination and the frequency of this behaviour recorded. All dead specimens were dissected within a week to the soil near the cabbage leaf square at every examination and the frequency of this behaviour recorded. All dead specimens were dissected within the following day to record the presence of P. hermaphroditia and other nematodes or trematodes. At the end of each assay, all surviving gastropods were killed and then dissected within a week (following the same protocols as in the survey). The number of P. hermaphroditia found anywhere inside the gastropods were estimated, using the minimum estimated number in statistical analyses.

The P. hermaphroditia were morphologically identified based on Solveig Haukeland’s nematological expertise and experience from previous surveys in Norway (Ross et al., 2015). When in doubt, temporary mounts of the nematodes were examined under microscope and compared to reference material from the Nemaslug® product.

2.4. Statistics

We used binary logistic regression (BLR) with host species, site, developmental stage and site*host as explanatory variables to model parasite prevalences in the survey (SAS System, GLIMMIX Procedure). Differences in least square means with Tukey-Kramer adjustment were used for pairwise comparisons. To compare the parasite load of the four gastropod species, Mood’s median test (MMT) was used, excluding specimens without parasites.

In the bioassays, BLR was used to test the effect of gastropod species and assay number on infection by P. hermaphroditia, the effect of gastropod weight on mortality, and the effect of host species, treatment and assay number on occurrence of parasites other than P. hermaphroditia (MiniTab 18 Statistical Software). To compare P. hermaphroditia infection rates between host species, infection rates in the treatments for A. arbustorum, and mortality rates among host species, Fisher exact test (FET) was used. Fisher exact tests were also used to analyse the mortality data, as BLR returned quasi-complete separation errors. As with the survey data for nematodes and trematodes, MMT was used for host species comparison of P. hermaphroditia numbers in infected gastropods. To test the effect of host species, treatment, assay number, and host species*treatment on the amount of cabbage eaten per day (standardized to cm² per gram body weight at start of bioassay), a linear mixed model was used, with Tukey-Kramer adjustments for pairwise comparisons (SAS System, GLIMMIX Procedure). Finally, a one-way ANOVA with Tukey pairwise comparisons was used to analyse effect of treatment on the proportion of inspections in which A. arbustorum had moved away from the soil and were found attached to the box walls.

3. Results

3.1. Parasite prevalence survey

A total of 227 slugs and snails were collected from the two sites: 61 Arion vulgaris, 47 A. fasciatus, 54 A. circumscriptus, and 65 specimens of Arianta arbustorum. Nematode prevalence differed significantly among host species ($\chi^2_{3, 217} = 7.44, P = 0.0001$, BLR) and developmental stage ($\chi^2_{2, 217} = 5.61, P = 0.004$). Site and host*site were not significant factors ($\chi^2_{2, 217} = 1.23, P = 0.2681$ and $\chi^2_{2, 217} = 2.19, P = 0.090$, respectively). Similarly, trematode prevalence differed significantly among host species ($\chi^2_{3, 217} = 13.72, P < 0.0001$) and developmental stage ($\chi^2_{2, 217} = 9.17, P = 0.0002$), and also between sites ($\chi^2_{2, 217} = 6.13, P = 0.014$), but not host*site ($\chi^2_{3, 217} = 1.73, P = 0.163$). For both parasite groups, A. vulgaris had the highest prevalence, though the difference was not significantly higher than A. fasciatus regarding nematodes (Fig. 1.1). Adult hosts had significantly higher nematode prevalence than juveniles ($P = 0.003$, adjusted by Tukey-Kramer’s method) but not subadults ($P = 0.388$), and also higher trematode prevalence than both subadults and juveniles ($P = 0.028$ and 0.0002, respectively).

Among the infected gastropod specimens, there was a significant host species difference in the number of nematodes found ($P = 0.001$, MMT) (Fig. 1.2). Infected A. fasciatus generally hosted very few nematodes, and A. vulgaris had a significantly (corresponding confidence intervals do not overlap) higher nematode load than A. fasciatus (95% median CI, respectively: (4; 15.09), (1; 2)). For trematodes, there was no significant difference among host species ($P = 0.596$).

3.2. Bioassays

3.2.1. Infection and mortality

None of the gastropods in the control treatments were infected by P. hermaphroditia, but due to a high control mortality of A. fasciatus in the first assay, all data for this species in this assay were excluded from tests. No other control specimens died in any of the assays. There was
no effect of assay number on the proportion of specimens infected by *P. hermaphrodita* ($\chi^2_{1, 71} = 0.08$, $P = 0.77$, BLR) or other parasites ($\chi^2_{1, 69} = 0.33$, $P = 0.57$), nor on the mortality ($P = 0.112$, FET), hence the two assays were pooled in all pairwise tests (FET).

There was however a significant effect of host species ($\chi^2_{2, 71} = 13.44$, $P = 0.001$, BLR) on the proportion infected by *P. hermaphrodita* (Fig. 2.1). All individuals of the two slugs *A. vulgaris* and *A. fasciatus* treated with nematodes were infected, whereas in the snail, *A. arbustorum*, only 7 of the 20 exposed specimens were infected in total. Its infection rate was significantly lowest of the three species (*A. vulgaris*: $P \leq 0.0005$; *A. fasciatus*: $P = 0.001$) and similar for the two nematode dosages ($P = 1.0$). As expected from the survey, the natural parasite prevalence in the assayed gastropods (occurrence of other parasites than *P. hermaphrodita*) differed between species ($\chi^2_{2, 69} = 12.18$, $P = 0.002$, BLR). It did not differ among treatments ($\chi^2_{2, 69} = 0.69$, $P = 0.617$).

The mortality rate also varied between host species, with *A. fasciatus* having a significantly higher mortality than both *A. vulgaris* ($P = 0.045$) and *A. arbustorum* ($P < 0.0005$) (Fig. 2.1). The mortality of exposed *A. vulgaris* was not significantly higher than that of *A. arbustorum* ($P = 0.106$). Most of the *A. vulgaris* and *A. fasciatus* dying during the assays showed symptoms indicative of *P. hermaphrodita* infection, such as swelling of the mantle, developing holes at the rear end of the mantle, or staying in a curled position. The occurrence of other
parasites than \( P. \) hermaphrodita did not significantly correlate with mortality \( (P = 0.488) \), nor was there any significant correlation between body weight and mortality for nematode exposed \( A. \) arbustorum \( (\chi^2_{18} = 2.54, P = 0.111, \) BLR) or \( A. \) fasciatus \( (\chi^2_{8} = 2.66, P = 0.103) \).

The number of \( P. \) hermaphrodita significantly differed between the host species \( (P = 0.022, \) MMT) (Fig. 2.2). Infected \( A. \) arbustorum hosted very few specimens of \( P. \) hermaphrodita, their load of this parasite being significantly different from both \( A. \) vulgaris \( (95\% \) median CI: \( A. \) vulgaris \( (21.18; 101.47), A. \) fasciatus \( (15.66; 37.5), A. \) arbustorum \( (1; 3.8)) \).

3.2.2. Feeding inhibition

There was a significant host species*treatment interaction in the amount of cabbage, relative to start body weight, eaten by the bioassay gastropods \( (F_{4, 60} = 3.49, P = 0.012, \) linear mixed model). With a near complete feeding inhibition, the native species \( A. \) arbustorum was feeding significantly less than \( A. \) vulgaris when exposed to the high dose of \( P. \) hermaphrodita \( (P = 0.011, \) Tukey-Kramer). \( A. \) fasciatus was feeding significantly less when exposed to either dose of nematodes compared to controls \( (P = 0.002) \). The target species \( A. \) vulgaris did not show significant feeding inhibition in any treatment \( (P = 0.973) \).

We frequently found \( A. \) arbustorum snails situated on the box walls, often with dried mucus covering the shell opening. In contrast, the slugs stayed on the soil or the food and were hardly ever found on the walls, and never in a settled position. There was a significant effect of nematode treatment in the proportion of observations with \( A. \) arbustorum found on the walls \( (P < 0.0005, \) one-way ANOVA, each snail observed 14–17 times). The snails in the controls were on the average found on the walls in 17.1% of the observations, which was significantly less than in the low and high dose nematode treatments, with 60.8% and 58.7%, respectively \( (P < 0.0005, \) Tukey vs. Control). There was no significant difference in soil avoidance between high and low nematode dose \( (P = 0.973) \).

4. Discussion

\( A. \) vulgaris had a higher trematode prevalence than all the three other host species surveyed, but the number of trematodes in infected specimens was similar in the four species. Regarding nematode infection, two species, \( A. \) circumscriptus and \( A. \) arbustorum, had lower prevalence than \( A. \) vulgaris. The nematode parasite load in \( A. \) vulgaris was also high, and definitely higher than in \( A. \) fasciatus. Our findings support those by Ross et al. \( (2015) \), suggesting that \( A. \) vulgaris success is not due to parasite release. We could speculate that the high prevalence of parasites recorded in \( A. \) vulgaris may be explained by parasite spillover from the closely related native species \( A. \) ater.

Hence the invading \( A. \) vulgaris may have become a new suitable host for parasites already present in the habitat, and it seems to tolerate these parasites well. As demonstrated in the laboratory bioassays where we tested susceptibility to \( Phasmarhabditis \) hermaphrodita, \( A. \) vulgaris did not succumb to nematode infection to the same degree as \( A. \) fasciatus, although all individuals of both these slug species were infected and the number of \( P. \) hermaphrodita in infected specimens were not significantly different between them. \( A. \) vulgaris further demonstrated its robustness to infection by continuous feeding regardless of nematode dose, which contrasted the response of \( A. \) fasciatus. However, due to the cut in sample size of \( A. \) fasciatus, further tests are necessary.

For the snail \( A. \) arbustorum, no mortality and low levels of infection by \( P. \) hermaphrodita was observed in the bioassays, mirroring the low parasite prevalences found for this species in the survey. The low infection in the bioassay could be explained by its propensity to avoid nematode infested soil, a behaviour also found in the slug species \( D. \) reticulatum and \( A. \) ater \( (Wilson et al., 1999) \) and possibly the aquatic snail \( L. \) stagnalis \( (Morley and Morritt, 2006) \). Interestingly, Rae \( (2017) \) found that \( A. \) arbustorum can encapsulate \( P. \) hermaphrodita in the shell, a general immune response to infection. We did not find encapsulated nematodes in our dissections, but in our bioassay specimens were exposed for half the time of those of Rae’s. Furthermore, our \( A. \) arbustorum had a near complete feeding inhibition with the highest dose of nematodes, whilst in Rae’s study there was no significant feeding inhibition in either dose. Our high dose was however 42% higher than Rae’s, which could explain the difference.

In addition to probably being the first report on effects of \( P. \) hermaphrodita on \( A. \) fasciatus, our study is one of the first to report the presence and prevalence of trematodes in \( A. \) vulgaris, \( A. \) fasciatus, \( A. \) circumscriptus and \( A. \) arbustorum. We sent some samples of the trematodes \( (metacercariae) \) that were found in high numbers in \( A. \) vulgaris and \( A. \) arbustorum to Raul Iglesias at the University of Vigo, Spain. He confirmed they belong to the Brachylaimidae, supporting earlier observations by Cragg et al. \( (1957) \) and Foster et al. \( (1958a,b) \), and also an unpublished study \( (led \ by \ Solveig \ Haukeland) \) on the prevalence of trematodes in \( A. \) vulgaris from a coastal area in SE Norway, in which a
Deletion 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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://hdl.handle.net/11250/2646812.

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H. Antzée-Hyllseth, et al.

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