Pattern of Prevalence of *Angiostrongylus vasorum* in Urban, Suburban, and Rural Slugs revealed by Real Time PCR

Nor Azlina A Aziz[1], Dena Azam[1], Simon Allen[1,2], Ben Rowson[3], Carolyn Greig[2], Dan Forman[2] and Eric R Morgan[1]

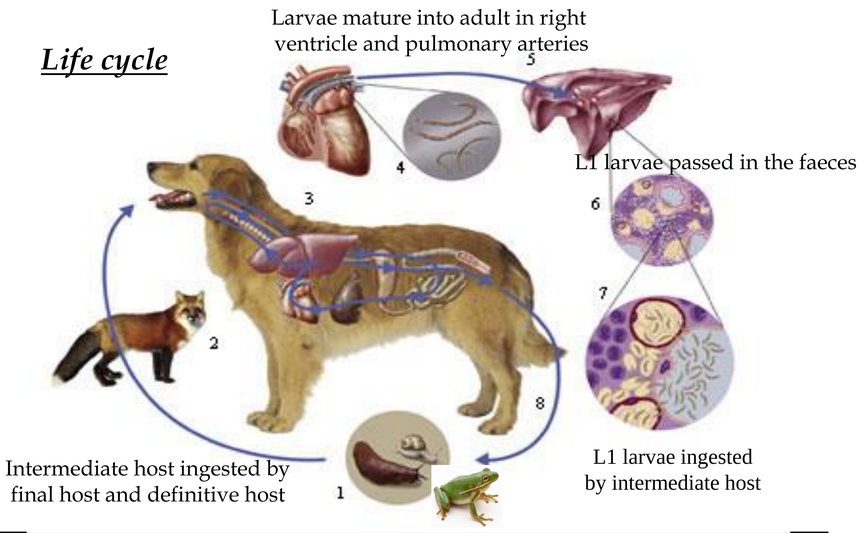
[1] *Veterinary Parasitology and Ecology, School of Biological Sciences, University of Bristol, England*
[2] *Swansea Ecology Team, Dept. Of Biosciences, Swansea University, Wales*
[3] *Dept. Natural Sciences, National Museum of Wales, Cardiff*

Introduction

- *Angiostrongylus vasorum* (Nematoda; Metastrongyloidea) is a parasite of the heart and pulmonary circulation of domestic and wild canids, including dogs (*Canis lupus familiaris*) and red foxes (*Vulpes vulpes*). Historically, in the United Kingdom, a few populations of *A. vasorum* have been reported in Cornwall (Simpson and Neal, 1982), southern Wales (Patteson et al. 1987) and the Southeast of England (Chapman et al. 2004). Recently, geographic expansion has been related to both sudden appearance in areas previously free of infection, and local expansion of known endemic foci (Morgan et al. 2008; Helm et al. 2009; Yamakawa et al. 2009).

- Infection is acquired by intentional and accidental ingestion of gastropods containing *A. vasorum* larvae (Moeremans et al. 2011). Information on which gastropod species can act as intermediate hosts, and the distribution of infected individuals is lacking. Such information is needed to evaluate the role played by slug populations as intermediate hosts of infection and the probability of transmission of this and many other endoparasites to domestic dogs and other canid species.

Life cycle

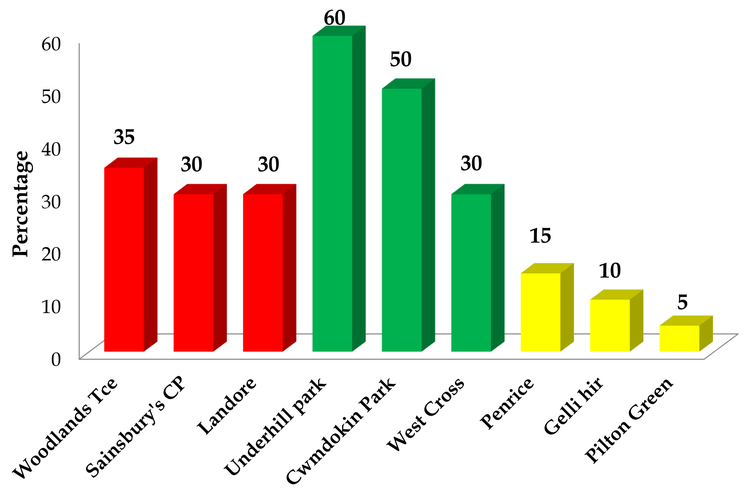


Aim and Hypothesis

Aim - To investigate the distribution of *A. vasorum* between infected slugs species in different areas (urban, suburban, rural) of Swansea, United Kingdom.

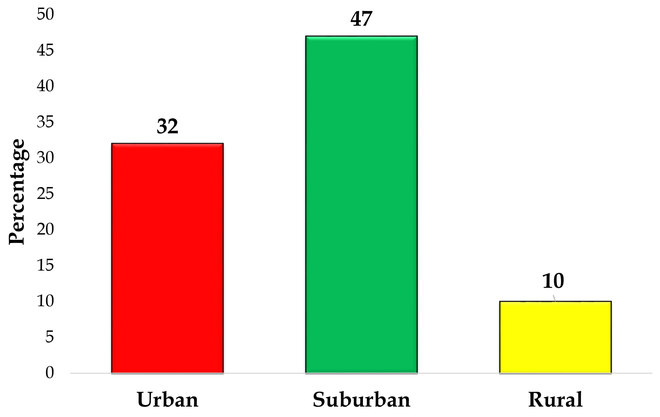
Hypothesis - The prevalence of *A. vasorum* in infected slug species should differ along an urban-rural gradient, reflecting variation in patterns of intermediate and final host parasite interaction.

Prevalence of *A. vasorum* in slugs



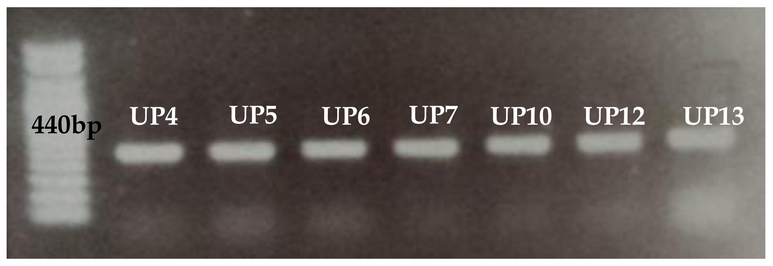
For the preliminary study, 20 slugs per area were tested for the presence of natural *A. vasorum* infection. Out of 180 examined slugs, 53 (29.4%) were infected with *A. vasorum*. The minimum prevalence was 5% in rural sites.

Prevalence of *A. vasorum* according to localities



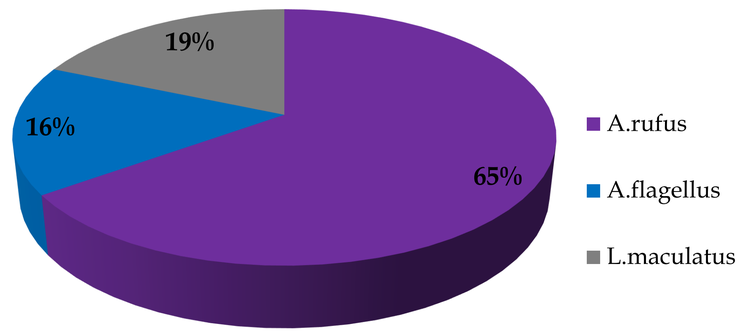
The preliminary results suggest that more than half of all infected slugs were found in suburban environments. The prevalence of infected slugs was significantly different between environments (Urban, suburban and rural; $\chi^2 = 14.78$, 2 d.f., $p < 0.001$). there was a statistically higher prevalence of *A. vasorum* in suburban and urban areas compared to rural areas ($\chi^2 = 12.00$, 1 d.f., $p < 0.001$). Infection was more common in suburban than in rural slugs ($\chi^2 = 14.16$, 1 d.f., $p < 0.001$).

Slug Species Identification



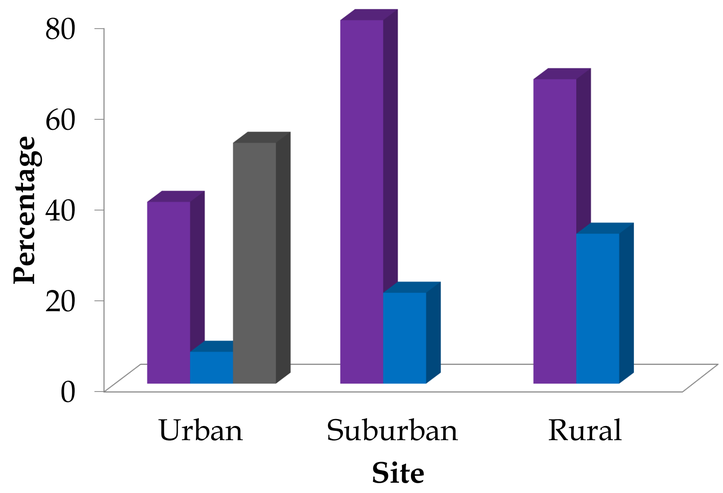
- 16S mitochondrial ribosomal DNA was amplified using conventional PCR for slugs sampled from the Underhill Park site.
- Sequence results were *Arion rufus* and *Arion flagellus*.

***A.vasorum* Infected Slugs**



- Three larger slug species from two families (Arionidae and Limacidae) were represented among 43 slugs that tested positive for *A. vasorum*.

A.vasorum Infected Slugs According to Site



- *A. rufus* is a more common host than *A. flagellus* in all three environments, and an additional host, *L. maculatus*, was utilised in urban environments.

Methods

Study Areas and Slug collection

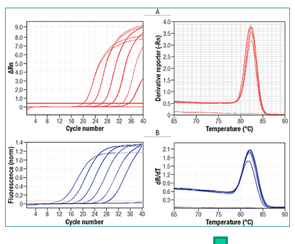
Terrestrial slugs were collected from woodlands and parks in area of Swansea, UK, during October to November 2012. Slugs were kept in the freezer until used for DNA extraction.

Site	Date Collected	Number of Slugs	Description
Underhill park	13/10/2012	100	Suburban
Cwmdokin Park	14/10/2012	141	Suburban
West Cross	18/10/2012	118	Suburban
Woodlands Tce	21/10/2012	96	Urban residential
Sainsbury's CP	23/10/2012	89	Urban municipal
Landore	25/10/2012	120	Urban brown field
Penrice	29/10/2012	45	Rural wooded
Gelli hir	30/10/2012	84	Rural wooded
Pilton Green	1/11/2012	84	Rural coastal farmland

Prevalence of *A. vasorum* in slugs (Jefferies et al. 2009)

10mg slug tissue cut and weighed

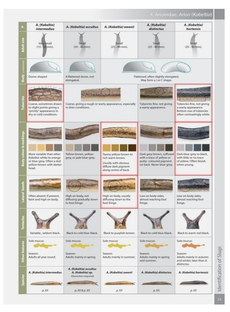
DNA extraction using DNeasy Blood and Tissue Kit (Qiagen, Germany)



Real Time PCR Assay

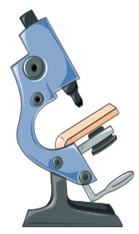
Slug Species Identification (Rowson et al. 2014)

1 Morphological Identification



Based on external characteristic

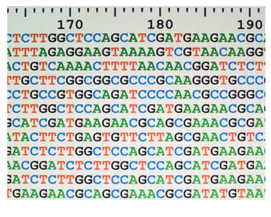
Based on internal characteristic



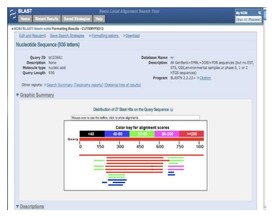
2 Molecular Identification -PCR was conducted on some slugs whose identification by morphological criteria was not possible

DNA extraction

Conventional PCR using genus specific primer – 16S



DNA sequencing



Analyze using NCBI BLAST

Conclusion

- The preliminary findings suggest that there is high prevalence of *A. vasorum* in suburban areas compared to rural and urban areas.
- Three larger slug species from two families were found to be infected with *A. vasorum*. *Arion rufus* was more commonly infected than *Arion flagellus* in all three environments. An additional host, *Limacus maculatus*, was utilised in urban environments.

References

1) Moeremans, I., et al. (2011). Vlaams Diergeneeskundig Tijdschrift 80: 319-326.
2) Jefferies, R., et al. (2009). Veterinary Parasitology 166:112–118.
3) Rowson, B., et al. (2014). PLoS ONE 9(4):e91907.
4) Simpson, V.R. and Neal, C. (1982). Veterinary Record 111: 303–304.
5) Patteson, M.W., et al. (1987). Veterinary Record 120: 349.
6) Chapman, P.S., et al. (2004). Journal of Small Animal Practice 45: 435–440.
7) Morgan, E.R., et al. (2008). Veterinary Parasitology 154: 48–57.
8) Helm, J., et al. (2009). Journal of Small Animal Practice 50: 255–259.
9) Yamakawa, Y., et al. (2009). Veterinary Record 162: 149–152.

Acknowledgements

This work was funded by the Malaysia Ministry of Higher Education (MOHE) and Universiti Sultan Zainal Abidin Malaysia (UniSZA).