



# Supplementary Materials: DNA Footprints: Using Parasites to Detect Elusive Animals, Proof of Principle in Hedgehogs

Simon Allen <sup>1,2,\*</sup>, Carolyn Greig <sup>3</sup>, Ben Rowson <sup>4</sup>, Robin B. Gasser <sup>5</sup>, Abdul Jabbar <sup>5</sup>, Simone Morelli <sup>6</sup>, Eric R. Morgan <sup>2,7</sup>, Martyn Wood <sup>1</sup> and Dan Forman <sup>3</sup>

<sup>1</sup> Gower Bird Hospital, Sandy Lane, Parkmill, Gower, Swansea SA3 2EW, UK; martyn@gowerbirdhospital.org.uk

<sup>2</sup> School of Biological Sciences, University of Bristol, Bristol Life Sciences Building, 24, Tyndall Avenue, Bristol BS8 1TQ, UK; eric.morgan@qub.ac.uk

<sup>3</sup> College of Science, Swansea University, Singleton Park, Swansea, Wales SA2 8PP, UK; c.greig@swansea.ac.uk (C.G.); d.w.forman@swansea.ac.uk (D.F.)

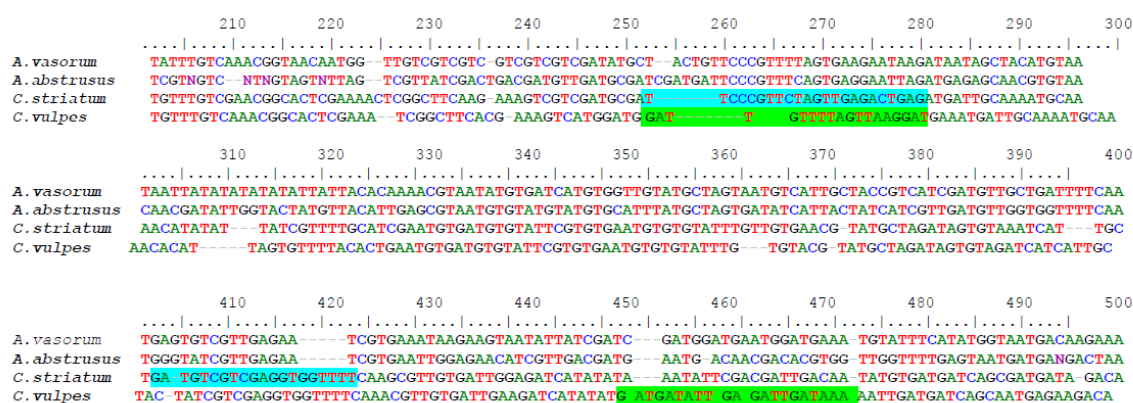
<sup>4</sup> Department of Natural Sciences, National Museum of Wales, Cardiff, Wales CF10 3NP, UK; ben.rowson@museumwales.ac.uk

<sup>5</sup> Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia; robinbg@unimelb.edu.au (R.B.G.); jabbara@unimelb.edu.au (A.J.)

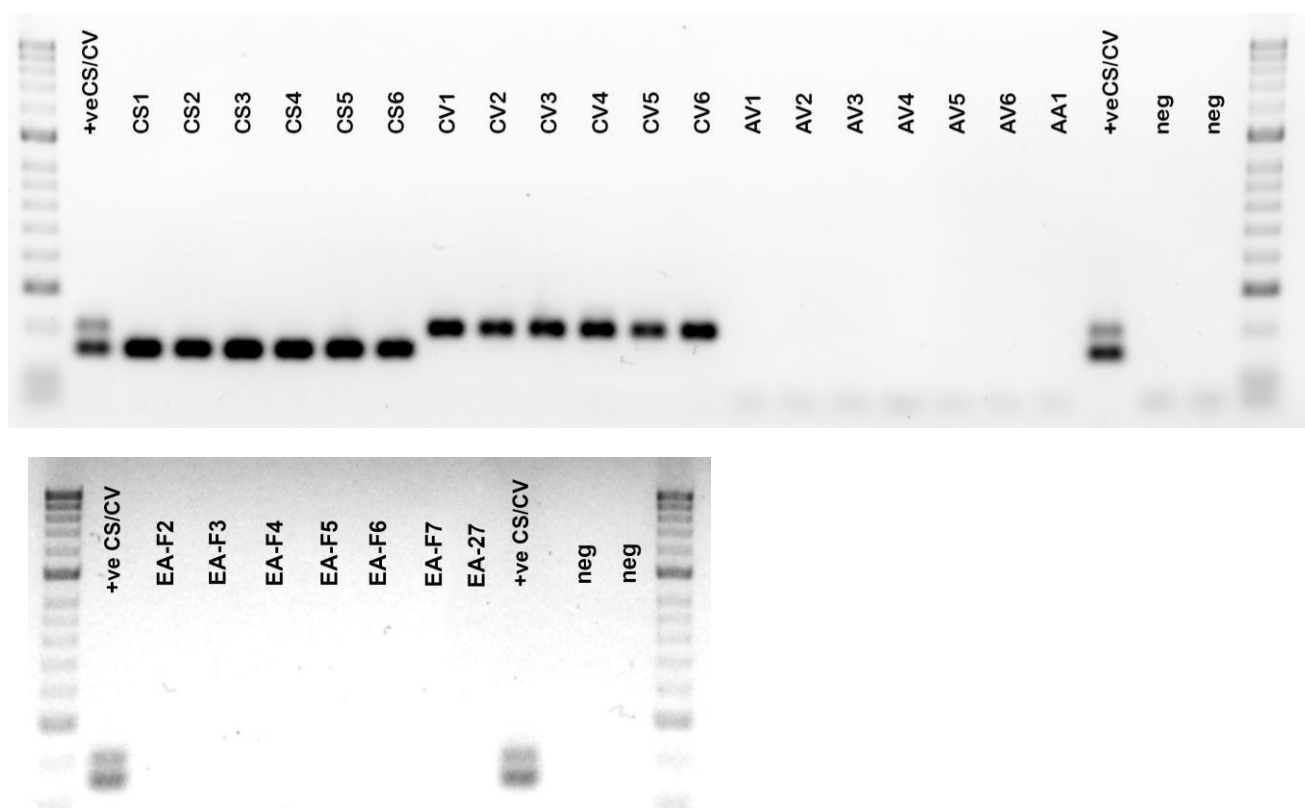
<sup>6</sup> Faculty of Veterinary Medicine, Teaching Veterinary Hospital, University of Teramo, 64100 Teramo, TE, Italy; smorelli@unite.it

<sup>7</sup> School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, Northern Ireland BT9 5DL, UK

\* Correspondence: simon@gowerbirdhospital.org.uk ; Tel.: +44-1792371630



**Figure S1.** Sequence alignment of ITS-2 sequences showing positions of discriminatory primers resulting in specific PCR products differing in length by 50bp.



**Figure S2.** PCR of extracted nematode DNA with CS/CV multiplexed primer set illustrating specific amplification.

PCRs produced expected bands: 157 bp for *C. striatum* (CS), 207 bp for *C. vulpis* (CV) and no product amplified for other nematode species tested. **Gel 1** shows PCR of DNA extracts from *Crenosoma striatum* (CS) (hedgehog), *Crenosoma vulpis* (CV) (fox), *Angiostrongylus vasorum* (AV) (fox), and *Aelurostrongylus abstrusus* (AA) (cat) (kindly donated by S. Morelli). **Gel 2** shows PCR of DNA extracts from *Eucoleus aerophilus* (*Capillaria aerophila*) (EA) (fox). PCRs included a positive control mixture of DNA from *Crenosoma striatum* and *Crenosoma vulpis* and negative H<sub>2</sub>O controls. All DNA was isolated from morphologically identified adult lungworms and validated for PCR with universal primers before testing.