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Unexpected occurrence of *Haemonchus placei* in cattle in southern Western Australia

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ABSTRACT

Haemonchus placei is an abomasal parasite of cattle, primarily in tropical and subtropical areas of the world. In Australia, this nematode can be extremely pathogenic in summer rainfall areas, particularly in the hot, sub-tropical Kimberley region, in the far north of the state of Western Australia (WA). Although cattle are occasionally transferred to southern parts of WA, it was believed that *H. placei* did not occur in southern regions of WA, as it is less cold-adapted than *Haemonchus contortus*, and the free-living stages would not develop during the cold winter and dry summer periods. Here, we show that, although *H. contortus* is found in cattle in the temperate southern region of WA, it appears that *H. placei* also occurs in southern WA. While investigating the prevalence of anthelmintic resistance in nematodes of cattle in WA, the existence of *H. placei* was suspected on a range of participating farms, following the morphological examination of third-stage larvae cultured from faeces, and of adult worms recovered from sheep experimentally infected with these larvae. Genomic DNAs from individual worms as well as eggs from pooled faecal samples from seven farms in southern WA were subjected to PCR-based mutation scanning and sequence analyses of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA. The results showed that both *H. contortus* and *H. placei* were harboured by cattle. This first record of *H. placei* in cattle in southern WA raises questions as to the prevalence and distribution of this parasite in other temperate and cool climatic regions of Australia. Although clinical disease due to *H. placei* has not yet been seen in southern WA, global, climatic trends might suggest an increased importance of this parasite in the longer term.

1. Introduction

Members of the genus *Haemonchus* Cobb, 1898 (Nematoda: Trichostrongyloidea) are important abomasal nematodes of domestic ruminants (Anderson, 2000) and are responsible for significant economic losses in sheep and goats in tropical and subtropical regions of the world (O'Connor et al., 2006). These nematodes are transmitted orally from contaminated pasture to the host through a complex life cycle (cf. Veglia, 1916; Anderson, 2000): eggs are excreted in host faeces; the first-stage larva (L1) develops inside the egg to then hatch and moult to the second- (L2) and third-stage (L3) larvae. The host becomes infected when infective L3s are ingested, which then exsheath and, after a histotrophic phase, develop via fourth-stage larvae (L4s) to dioecious adults. *Haemonchus* spp. feed on blood from capillaries in the abomasal mucosa, and cause haemorrhagic gastritis, anaemia, oedema and associated complications, often leading to death in severely affected animals, particularly sheep and goats (Anderson, 2000).

Important species are *Haemonchus contortus* (Rudolphi 1803) Cobb, 1898, which principally infects sheep and goats, but can also be found in cattle and some species of deer (Eve and Kellogg, 1977; Anderson, 2000), and *Haemonchus placei* (Place 1893) Ransom 1911, which is primarily a parasite of cattle (Anderson, 2000). However, it is also known that both species can simultaneously infect cattle and small ruminants, particularly on communal pastures (Amarante et al., 1997; Jacquiet et al., 1998; Achi et al., 2003).

In Australia, *H. placei* in cattle and *H. contortus* in sheep can be extremely pathogenic in summer rainfall areas (O'Connor et al., 2006). In Western Australia (WA), *H. placei* has been recognised as a common nematode of young beef cattle in the Kimberley district (Fig. 1), an area in the north of WA (summer rainfall zone), although disease outbreaks are rare (B. Besier unpublished findings) (Fig. 1). By contrast, the agricultural region in southern WA is in a Mediterranean climatic zone (Fig. 1), characterised by hot dry summers and receiving predominantly winter rainfall. In high

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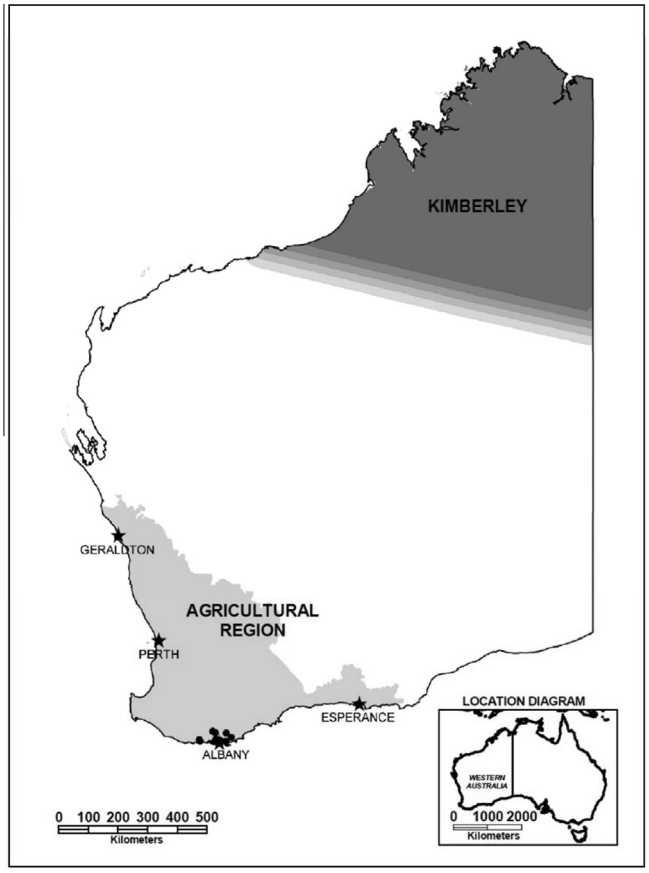

Fig. 1. Map showing geographical location of the study area in the agricultural region in Western Australia. Black dots in this area near Albany show the location of farms included in the study. An endemic area, i.e., Kimberly region, in the state endemic for *Haemonchus placei* is also shown. Inset shows an Australian map.

rainfall and coastal zones within this region, *H. contortus* regularly parasitizes sheep, with an occasional 'spillover' into cattle in situations of communal grazing.

While investigating the prevalence of anthelmintic resistance in young cattle in southern WA (J. Cotter, unpublished), the existence of *H. placei* was suspected on a range of participating farms, following the morphological examination of L3s cultured from cattle faeces, and of adult worms recovered from sheep following experimental infection with these larvae. The present study was conducted to confirm that, although *H. contortus* is occasionally found in cattle in southern WA, *H. placei* also occurs in this region. We used morphological and molecular methods to characterise *H. placei* and *H. contortus* present here in young cattle.

2. Materials and methods

2.1. Study area and farms

The study area was in the south-west of WA (Fig. 1). In contrast to much of WA, the coastal rim maintains some green pasture throughout most of the year, including Kikuyu grass, annual ryegrass and clovers. The coastal city of Albany in the Great Southern Region of WA (latitude 35.03°S, longitude 117.88°E, elevation 3 m) has a temperate climate (temperature: winter (July) 8–15 °C; summer (January) 15.5–23 °C) and receives an annual rainfall of 600–800 mm (Australian Bureau of Meteorology; www.bom.gov.au). Beef cattle farms in the region represent mostly self-replacing herds, with an average of approximately 200 breeding cows, with some larger herds of up to 2000 cattle, mostly of British breeds. The farms ($n = 7$) involved in the present study were within 40 km of the coast, in the vicinity of Albany (Table 1).

2.2. Coprological methods used for preliminary investigations

In 2010 and 2011, faecal samples were collected from young cattle (8–10 months of age) during the course of a series of anthelmintic resistance trials (manuscript in preparation). For each treatment and control group, individual faecal egg counts (FEC) were performed on 4 g of faeces from individual cattle ($n = 1425$) on participating farms ($n = 19$) using the modified McMaster technique (Whitlock, 1948), and the remaining pooled faeces from each farm were subjected to larval culture for seven days at 25 °C. Cultured L3s were identified as previously described (Dikmans and Andrews, 1933; Keith, 1953; MAFF, 1986).

2.3. Experimental infection of sheep with Haemonchus larvae from cattle

Based on size differentiation of cultured L3s derived from cattle faeces (Section 2.2.), *H. contortus* and *H. placei* were suspected. To confirm the presence of *H. placei*, a sheep was infected with L3s to produce adult worms. Specifically, an adult, helminth-free Merino sheep was treated with PYRIMIDE 3-Way Combination Drench for Sheep® (abamectin 0.8 g/L, albendazole 20.0 g/L, levamisole 25.5 g/L, Novartis Animal Health, Australia). After 3 weeks, the sheep was orally infected with 5000 L3s, containing 27% *Haemonchus* larvae in a mixture with other strongylid nematode larvae, and housed for 40 days before euthanasia, to collect adult worms. Abomasal contents were passed through a sieve (1 mm mesh size) and resuspended in a tray, from which adult *Haemonchus* were isolated. Spicule length and vulval flap morphology of these individual adults were recorded according to previously published articles (Roberts et al., 1954; Bremner, 1956). The worms were stored in a mixture of alcohol (70%) and glycerol (5%) until molecular characterisation (September 2012).

2.4. Collection of eggs to confirm the presence of H. placei in cattle

In February 2013, in order to verify that *H. placei* was still cycling on the farms where it was detected one year before, pooled faecal samples (from 20 weaner cattle, 6–9- months old, from each of seven farms) were collected. Faecal egg counts were performed to establish the presence of trichostrongyloids, and a lectin binding assay (Palmer and McCombe, 1996; Colditz et al., 2002) was used to confirm the presence of *Haemonchus*. Strongylid eggs were isolated from faeces as described previously (Bott et al., 2009).

2.5. Molecular methods

2.5.1. Isolation of genomic DNA

Prior to DNA isolation, ethanol was removed from individual worms by rehydration. Then, individual adults of *Haemonchus* ($n = 9$) were incubated in ~200 µl of 20 mM Tris–HCl (pH 8.0), 100 mM EDTA, 1% sodium dodecyl-sulphate containing 10 mg/ml proteinase K (Amresco Inc., USA) at 37 °C for 18 h. Genomic DNA was isolated from the homogenised suspension using mini-columns (Wizard® DNA Clean-Up Kit, Promega, USA). Genomic DNA from strongylid eggs from faeces was isolated using PowerSoil® DNA Isolation Kit (MO BIO Labs, Inc., USA) according to the manufacturer's protocol.