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

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25 A B S T R A C T

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

46

47 *Keywords:*48 *Haemonchus placei*

49 Nematode

50 Ecology

51 Cattle

52 Geographical distribution

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 *H. 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 *H. 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 (Achi et al., 2003; Amarante et al., 1997; Jacquet et al., 1998).

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

78 predominantly winter rainfall. In high rainfall and coastal zones within this region, *H.*
 79 *contortus* regularly parasitizes sheep, with an occasional ‘spillover’ into cattle in situations of
 80 communal grazing.

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

90 2. Materials and methods

92 2.1. Study area and farms

93 The study area was in the south-west of WA (Fig. 1). In contrast to much of WA, the coastal
 94 rim maintains some green pasture throughout most of the year, including Kikuyu grass,
 95 annual ryegrass and clovers. The coastal city of Albany in the Great Southern Region of WA
 96 (latitude 35.03 °S, longitude 117.88 °E, elevation 3 m) has a temperate climate (temperature:
 97 winter (July) 8-15 °C; summer (January) 15.5-23 °C) and receives an annual rainfall of 600-
 98 800 mm (Australian Bureau of Meteorology; www.bom.gov.au). Beef cattle farms in the
 99 region represent mostly self-replacing herds, with an average of approximately 200 breeding
 100 cows, with some larger herds of up to 2,000 cattle, mostly of British breeds. The farms ($n =$
 101 7) involved in the present study were within 40 km of the coast, in the vicinity of Albany
 102 (Table 1).

103

104 2.2. *Coprological methods used for preliminary investigations*

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

112

113 2.3. *Experimental infection of sheep with Haemonchus larvae from cattle*

114 Based on size differentiation of cultured L3s derived from cattle faeces (section 2.2.), *H.*
 115 *contortus* and *H. placei* were suspected. To confirm the presence of *H. placei*, a sheep was
 116 infected with L3s to produce adult worms. Specifically, an adult, helminth-free Merino sheep
 117 was treated with PYRIMIDE 3-Way Combination Drench for Sheep® (abamectin 0.8 g/L,
 118 albendazole 20.0 g/L, levamisole 25.5 g/L, Novartis Animal Health, Australia). After three
 119 weeks, the sheep was orally infected with 5000 L3s, containing 27% *Haemonchus* larvae in a
 120 mixture with other strongylid nematode larvae, and housed for 40 days before euthanasia, to
 121 collect adult worms. Abomasal contents were passed through a 1 mm sieve and resuspended
 122 in a tray, from which adult *Haemonchus* were isolated. Spicule length and vulval flap
 123 morphology of these individual adults were recorded according to previously published
 124 articles (Roberts et al., 1954; Bremner, 1956). The worms were stored in a mixture of alcohol
 125 (70%) and glycerol (5%) until molecular characterisation (September 2012).

126

127 2.4. *Collection of eggs to confirm the presence of Haemonchus 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 to 9- months old, from each of seven farms) were collected. Faecal egg counts were performed to establish the presence of strongyles, 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 μ l 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.

2.5.2. PCR amplification, single-strand conformation polymorphism (SSCP) and sequencing

The second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (including flanking sequence) was amplified by PCR from the genomic DNA (10-20 ng template) from individual worms using primers NC1 (5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (5'-TTAGTTTCTTTTCTCCGCT-3'). PCRs were conducted in 50 μ l volumes containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl (Promega), 3.5 mM MgCl₂, 200 μ M of each deoxynucleotide triphosphate (dNTP), 50 pmol of each primer and 1 U of GoTaq polymerase (Promega) using the following cycling conditions: 94°C for 5 min, then 35 cycles of 94°C for

30 s, 55°C for 30 s and 72°C for 30 s, followed by 72°C for 10 min. Negative (no-DNA) and known positive (*H. contortus*) controls were included in each set of PCRs. Amplicons were subjected to agarose (1.5%) gel electrophoresis, and photographed upon transillumination using a GelDoc system (BioRad, Hercules, USA). *Haemonchus* DNA was detected in genomic DNA from strongylid eggs from faeces using a real-time PCR method (Bott et al., 2009).

ITS-2 amplicons from 92 samples of *Haemonchus* spp. were subjected to SSCP analysis using protocol B of Gasser et al. (2006) to screen for sequence variation within and among individual worms. One to five amplicons representing each unique SSCP profile were treated with shrimp alkaline phosphatase and exonuclease I (Fermentas Inc., USA), and sequenced (BigDye® Terminator v.3.1 chemistry, Applied Biosystems, USA) using primers NC1 and NC2 in separate reactions. The quality of individual sequences was assessed visually using the program Geneious Pro 5.6.5 (Biomatters Ltd., New Zealand).

2.5.3. Sequence comparisons and phylogenetic analyses

Prior to phylogenetic analyses, ITS-2 sequences were subjected to BLASTn analysis (<http://blast.ncbi.nlm.nih.gov>) to identify the best matches to all nucleotide sequences available in current databases. Sequence differences were calculated by pairwise comparison. Subsequently, all distinct ITS-2 sequences determined in the present study were aligned with a selected subset of closely related reference sequences using the program Clustal X (Larkin et al., 2007), and alignments were adjusted manually. Phylogenetic analyses of the sequence data (ITS-2) was conducted by Bayesian inference (BI), employing the Monte Carlo Markov Chain (MCMC) method in MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and distance-based Neighbour Joining (NJ) methods. For BI, the likelihood parameters were set based on the Akaike Information Criteria (AIC) test in Modeltest v.3.7 (Posada and Crandall, 1998). The general time-reversible model of evolution,

with gamma-distribution and a proportion of invariable sites (GTR + Γ + I), was utilised for the analysis of the sequence data. Sequence data were also analyzed using the Neighbour-Joining (NJ) method employing PAUP (PAUP 4.0b10) where molecular distances were estimated by the general time-reversible model of evolution and the nodes were tested for robustness by 100,000 bootstrap replicates. Phylogenetic trees constructed using the BI and NJ methods were examined for concordance in topology.

3. Results

3.1. Morphological identification of L3 and adult stages of *Haemonchus*

Anthelmintic resistance testing of cattle in 2010 and 2011 on 19 farms throughout Western Australia had identified gastrointestinal nematodes of the genera *Cooperia onchophora*, *Haemonchus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi* and/or *Trichostrongylus* spp. (J. Cotter, unpublished). *Haemonchus* was found on 17 of the 19 farms, and results from larval culture and differentiation had indicated the existence of *H. placei* in cattle on 17 of these farms. FECs for *Haemonchus* in cattle ($n = 1425$) from these farms ranged from a mean of 0 to 100 eggs per gram.

In faecal samples from cattle on 7 farms in 2013, lectin binding assays on eggs isolated from the samples after worm egg counts indicated that *Haemonchus* was present on all farms, with mean FEC from 2 to 90 eggs per gram (this technique does not allow differentiation to the species level).

On the basis of morphological measurements (spicules, and vulval morphology), both *H. placei* (80%) and *H. contortus* (20%) were identified from adult worms recovered from the abomasa from the sheep experimentally infected with L3s originally derived from pooled faeces from cattle from one farm.

3.2. Molecular identification

In order to verify the existence of *H. placei* in the study area, adults of *Haemonchus* from the experimentally infected sheep and strongylid nematode eggs from cattle faeces were subjected to molecular investigation.

ITS-2 amplicons of the expected size (~310 bp) were produced from the genomic DNAs from individual adults of *Haemonchus* ($n = 92$) collected from the experimentally infected sheep. Ten distinct ITS-2 profiles (designated A–J) were detected by SSCP analysis (Table 2). Selected ITS-2 amplicons ($n = 1-5$) representing each of these SSCP profiles were sequenced. The lengths of individual ITS-2 sequence types, mean nucleotide frequencies, polymorphic positions (if any), G+C content and respective accession numbers are listed in Table 2. Sequencing revealed ten different sequence types (231 bp; GenBank accession nos. KF364623-KF364632).

These ten sequence types were aligned over 243 positions with ITS-2 reference sequences for *H. contortus* ($n = 13$) and *H. placei* ($n = 5$) (Stevenson et al., 1995; Cerutti et al., 2010; Brasil et al., 2012; Gharamah et al., 2012) and for *Bunostomum phlebotomum*, *Nematodirus rupicaprae*, *Oesophagostomum columbianum* and *Trichostrongylus axei* ('outgroups') ($n = 4$) (Hoste et al., 1995; Newton et al., 1998; Gasser et al., 1999; Jex et al., 2009) and subjected to phylogenetic analyses. The analyses unequivocally identified *H. placei* and *H. contortus* with strong nodal support (posterior probability values 0.8-1.00; bootstrap values 80-98%) (Fig. 2). The topologies of the trees constructed using the two different algorithms were the same with only minor variation in nodal/bootstrap support values (see Fig. 2). This analysis revealed 78 and 14 adults of *H. placei* and *H. contortus*, respectively. Five distinct genotypes were characterized within each species, which differed in sequence by 0.5-3.5% (accession

nos. KF364623-KF364627) and 0.5-0.9% (accession nos. KF364628-KF364632), respectively, upon pairwise comparison.

SSCP analysis and sequencing of ITS-2 amplicons from the strongylid eggs from cattle faeces, recovered in 2013 (section 2.4), revealed *H. placei* (accession no. KF364623) on farms 1-6 and *H. contortus* (accession no. KF364628) on farm 7 (Table 1) based on a perfect match to the sequence of *H. placei* (genotype A; accession no. KF364623) or *H. contortus* (genotype F; accession no. KF364628).

237

238

4. Discussion

Morphological examination of L3s cultured from faeces and of adult worms from sheep with experimental infections with such larvae revealed the presence of *H. placei* in young cattle in the temperate southern region of WA, which was confirmed by molecular study. This was a new and unexpected finding. While *H. placei* is endemic in tropical and subtropical zones in Australia, including in the Kimberley region in the far north of WA, it had not been found previously in more temperate, southern regions. *H. placei* might have been occasionally introduced to southern regions via cattle transported from the northern, endemic zone, but environmental constraints that limit the distribution of *H. contortus* were expected to apply to an even greater degree to the less cold-tolerant *H. placei*. In southern WA, studies with *H. contortus* in the relatively temperate south-coastal environment of the Albany region (Besier and Dunsmore, 1993a,b) indicated that the development of the free-living stages on local pastures was limited to short periods in autumn and spring, and ceased for several months in the hot, dry summer periods and during the relatively cold winters. As the environmental extremes of both summer and winter are greater in inland and more northern areas, the ecological requirements explain the geographical distribution of *H.*

contortus, which is found largely in the milder coastal and high-rainfall areas of the state. Rarely, *H. contortus* is found in laboratory submissions from sheep in arid inland parts of southern WA, but FECs are invariably negligible (B. Besier, unpublished findings).

In the present study, we also confirmed that that *H. placei* was still cycling on the farms even after one year where it was detected first time. The incapability of *H. placei* to tolerate lower temperature was expected to severely restrict its establishment within the agricultural region of south-western WA (Fig. 1). For example, minimum temperatures of $\leq 13^{\circ}\text{C}$ have been shown to prevent the hatching of *H. placei* eggs under laboratory conditions, while *H. contortus* eggs are reported to hatch at $10\text{--}11^{\circ}\text{C}$ (Le Jambre 1981, Besier, 1992). Within the southern agricultural region, mean monthly minimum temperatures of more than 13°C are recorded only for the months of December to March (south of Perth), and November to April (north of Perth) (Australian Bureau of Meteorology; www.bom.gov.au). Under these conditions, in the months sufficiently warm enough to allow *H. placei* egg development, conditions in most of southern WA are too dry (generally less than 25 mm rainfall per month) and hot (mean monthly temperatures of more than 25°C) for the successful development of *H. contortus* eggs, particularly on pastures composed of annual plant species (Besier and Dunsmore, 1993a; O'Connor et al., 2006). The only location where summer temperatures (for at most a 2-month period) are sufficiently mild enough to allow *H. contortus* development is along the south coast (mainly between Albany and Esperance), although conditions are also dry at this time. This ecological model indicates little opportunity for the development of L3s of *H. placei* on pasture, and supports the lack of previous reports from southern WA.

Detection of *H. placei* in areas previously considered unfavourable raises questions about the applicability of environmental data from studies with *H. contortus* in sheep for these predictions. However, the taxonomic similarity of *H. contortus* and *H. placei* (see Gibbons, 1979) suggests that the ecological behavior of both species will also be similar, and studies of

Ostertagia (now *Teladorsagia*) *circumcincta* from sheep and *O. ostertagi* from cattle (Young, 1983) showed little difference in over-summer L3 survival rates in relation to the nature of host faecal deposits. A more likely explanation of the survival of *H. placei* in southern WA is that micro-environmental conditions allow L3 development in situations not indicated by general ecological models. Considerably better survival of L3s of *H. contortus* occurred for eggs from sheep faeces when deposited on to pastures of perennial (summer-green) grass species, compared with those deposited on to dry pastures (Besier and Dunsmore, 1993b). Importantly, perennial pastures (particularly Kikuyu grass, *Pennisetum clandestinum*) were present on all farms studied here and on which *H. placei* was found. In addition, all farms on which *H. placei* was located in the relatively temperate south coast region, where summer temperatures are mostly mild and summer rainfall is common. Although *Haemonchus* was found in cattle outside of this area, the species in these samples has not yet been investigated. Further study should assist in determining whether *H. placei* is restricted to more temperate parts of the southern agricultural region, or whether the ecological determinants for this species should be revised to include survival and development in more extreme environments of WA. A potential role for hypobiosis (seasonally arrested development) cannot be excluded, but is not considered likely. Although well-recognized in *H. contortus* in extremely dry or cold climates (Gibbs 1986), hypobiosis has not yet been reported in either *Haemonchus* species in southern parts of Australia.

The presence of both *Haemonchus* species in cattle on one farm, and of *H. contortus* only on another, is not unexpected. *Haemonchus* spp. are occasionally detected in faecal samples submitted to local laboratories from young cattle in this region, though never in association with overt parasitism (B. Besier, unpublished), and, until recently, were considered to be *H. contortus*, as cross-infection with this species among ruminant hosts is well-recognized (Riggs, 2001; Akkari et al., 2013). Local patterns of occurrence are likely to be determined

by the opportunity for infection with each species. For example, *H. contortus* is common on sheep farms in the more temperate parts of the southern agricultural region of WA, and young cattle on these properties are likely to acquire short-term infection with *H. contortus*, although bovine haemonchosis has not been detected in this region. In the present study, it could not be established whether *H. placei* was recently introduced to southern WA by the introduction of cattle from the Kimberly region and/or other parts of the country, or whether this species of nematode was always present in the region and was found incidentally. Most of the farms studied here had closed herds and did not introduce cattle from other regions of WA and or elsewhere. However, some farms (nos. 2, 4 and 5, Table 1) introduced cattle from the Kimberley region about 18 years ago, and it is possible that *H. placei* was brought to southern WA with these cattle. Based on the low *Haemonchus* egg counts found in cattle, clinical disease would not be expected, and it is possible that inapparent *H. placei* infection had been present for some time.

It has been suggested that climate change will alter the risk of infectious disease outbreaks, including for ruminant nematodes, by extending the seasonal window for parasite growth and by increasing the rate of transmission (Kenyon et al., 2008, Morgan and van Dijk, 2012). Rainfall in the South West agricultural region has declined significantly over the past 30 years compared with early 20th century (Carmody, 2010), with a greater variability in seasonal patterns and a greater proportion of rainfall in summer. In addition, it has been proposed that by 2030 the average annual temperatures are likely to rise 0-2 °C in southern WA (Carmody, 2010). Therefore, it is possible that changes in environmental conditions in recent decades might explain the presence of *H. placei* in southern WA, and increased summer rainfall may increase its importance in the region. However, further investigations of the ecology of *H. placei* are necessary to provide environmental data for modelling predictions of the likely response to changes in climate.

Based on the PCR–coupled mutation scanning analysis of the ITS-2 sequences determined herein, we defined five genotypes each for *H. contortus* and *H. placei*. Two genotypes of *H. placei* were identical to previously reported ITS-2 sequences (GenBank accession nos. JN128896 and JQ342249) from Brazil (Brasil et al., 2012), whereas the other three matched with those (JQ342248, X78812, JN128895) reported from Australia and Brazil (Stevenson et al., 1995; Brasil et al., 2012) (see Fig. 2). Similarly, *H. contortus* sequences determined herein matched previously published sequences of the ITS-2 from Australia, Brazil, Italy, Malaysia and Yemen (see Fig. 2) (Stevenson et al., 1995; Cerutti et al., 2010; Brasil et al., 2012; Gharamah et al., 2012).

In conclusion, this report demonstrates the advantage of using a combined morphological/molecular approach for the differential diagnosis of nematode infections, particularly where species identification is essential for the interpretation of new epidemiological information. The approach is applicable irrespective of the developmental stage of the parasite involved, thus providing a reliable and powerful tool for understanding the ecology of the free living stages of parasites. Although there is an extensive literature available on the development and behavior of the free-living stages of *H. contortus* in sheep, only a few studies have been undertaken to understand the epidemiology of *H. placei* in cattle. Further studies are required to elucidate the ecology of free living stages of *H. placei* in this region, and to indicate the prevalence and potential impact of *H. placei* on young cattle in the southern parts of Australia.

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469

470 **Figure legends**

471

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

476

477 **Fig. 2.** Phylogenetic analysis of the ITS-2 sequence data representing *Haemonchus* spp. from
478 the southern Western Australia (present study: bold-type) and sequence data for previously
479 published sequences. *Bunostomum phlebotomum*, *Nematodirus rupicaprae*,
480 *Oesophagostomum columbianum* and *Trichostrongylus axei* represent outgroups. Bayesian
481 inference (BI) and neighbour-joining (NJ) methods were used to infer phylogenetic
482 relationships. Nodal support is given as a posterior probability (pp) for BI (top) and bootstrap
483 value for NJ (bottom). The scale bar indicates distance.

484

485

486

487 **Fig. 1.**

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490

491 Fig. 2.

Table 1

Characteristics of beef cattle farms near Albany and Denmark sires, Western Australia

Farm No. Local location	Breed of cattle (age in months)	Introduction of cattle in the last five years and/or earlier	Sheep on and/ or adjoining farms	Communal grazing ^a
1 Marbellup	Red Angus/Sussex/ South Devon weaners (~8 -10 months)	Original cows from within agricultural (Ag) region. Bulls sourced from within Ag region.	Sheep were run concurrently until 10 years ago	None
2 Narrikup	Red Angus weaners (~ 8-10 months)	Kimberley cattle brought to Ag region (1994), transferred to current property 12 months later. Other cattle sourced from within Ag region. Semen imported	Sheep were run concurrently until 1995	None - occasional ovine or bovine stray
3 Denbarker	Murray Grey weaners (~8-10 months)	Original cows from within Ag region. Bulls sourced within Ag region.	Sheep were run concurrently until 5 years ago	None
4 Kentdale	Angus and Murray Grey weaners (~8-10 months)	Kimberley cattle brought on in 1993. Bulls sourced from within the Ag region.	No sheep and no neighbors with sheep	None
5 Narrikup	Angus weaners (~8-10 months)	Original cows from within Ag region, 1995.	Small sheep flock run concurrently and goats run on adjoining farm	Yes, periodically with goats on adjoining farm
6 Young's Siding	Murray grey/Angus /Simmental weaners (~8-10 months)	Original cows from within Ag region. Bulls sourced from within the Ag region.	Sheep were run concurrently until 5 years ago	none
7 Kalgan	Hereford weaners (~8-10 months)	Original cows from within Ag region. Bulls sources from within the Ag region.	Sheep are run concurrently	None

^a Grazing with other animals, including sheep, goats, alpacas and horses; .

Table 2

The classification of adult *Haemonchus* spp. specimens based on single-strand conformation polymorphism (SSCP) profiles for the ITS-2 used in the present study. The sequence linked to each unique SSCP profile is represented by its GenBank accession number, its length, polymorphism and G+C content. Mean nucleotide frequencies for the main sequence types are also provided.

Genotype		ITS-1							
	SSCP profile (no. of samples with this profile)	Accession no.	Length (bp)	Polymorphism ^a (alignment position)	GC content (%)	Mean nucleotide frequencies			
						A	C	G	T
A	Hp-1 (28)	KF364623	231	---	32.90	0.30303	0.16017	0.17749	0.35931
B	Hp-2 (47)	KF364624	-do-	C/G (21)	32.90	---	---	---	---
C	Hp-3 (1)	KF364625	-do-	R (24), T/A (65), Y (103), R (219)	32.90	---	---	---	---
D	Hp-4 (1)	KF364626	-do-	T/A (65)	32.90	---	---	---	---
E	Hp-5 (1)	KF364627	-do-	C/G (21), T/A (65)	33.33	---	---	---	---
F	Hc-1 (7)	KF364628	231	---	33.77	0.30736	0.15152	0.17749	0.36364
G	Hc-2 (1)	KF364629	-do-	W (196)	33.77	---	---	---	---
H	Hc-3 (1)	KF364630	-do-	G/C (21)	32.47	---	---	---	---
I	Hc-4 (2)	KF364631	-do-	T/A (196)	33.77	---	---	---	---
J	Hc-5 (3)	KF364632	-do-	T/C (22)	33.77	---	---	---	---
Total (nematodes)		92							

^aPolymorphism for each sequence type was assessed by aligning these sequences with the reference sequences (Stevenson et al., 1995). R = A/G; K = G/T; S = C/G; Y = C/T

505 **Highlights**

- 506 • *Haemonchus placei* is an abomasal parasite of cattle
- 507 • This parasite primarily occurs in summer rainfall areas of the world
- 508 • Here we show that *H. placei* also occurs in a winter rainfall area of Australia

509

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