IJP: Parasites and Wildlife 8 (2019) 156–163

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Wild horse populations in south-east Australia have a high prevalence of *Strongylus vulgaris* and may act as a reservoir of infection for domestic horses

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ARTICLE INFO

Keywords:
Wild horse
Equine
Strongylus vulgaris
Faecal parasitology
Faecal egg counts

ABSTRACT

Australia has over 400,000 wild horses, the largest wild equid population in the world, scattered across a range of different habitats. We hypothesised that wild horse populations unexposed to anthelmintics would have a high prevalence of *Strongylus vulgaris* infections. Verminous endarteritis and colic due to migrating *S. vulgaris* larvae is now absent or unreported in domestic horses in Australia, yet wild horses may pose a risk for its re-emergence. A total of 289 faecal egg counts (FECs) were performed across six remote wild horse populations in south-east Australia, of varying densities, herd sizes and habitats. Total strongyle egg counts ranged from 50 to 3740 eggs per gram (EPG, mean 1443) and 89% (257/289) of faecal samples had > 500 EPG, classifying them as 'high level shedders'. There were significant differences in mean total strongyle FECs between different locations, habitats and population densities. Occurrence of *S. vulgaris* was not predictable based on FECs of total strongyle eggs or small (< 90 μm) strongyle eggs. A high prevalence of *S. vulgaris* DNA in faecal samples was demonstrated across all six populations, with an overall predicted prevalence of 96.7%. This finding is important, because of the ample opportunity for transmission to domestic horses. The high prevalence of *S. vulgaris* suggests vigilance is required when adopting wild horses, or when domestic horses graze in environments inhabited by wild horses. Appropriate veterinary advise is required to minimize disease risk due to *S. vulgaris*. Monitoring horses for *S. vulgaris* using larval culture or qPCR remains prudent. Gastrointestinal parasites in wild horse populations may also serve as parasite *refugia,* thus contributing to integrated parasite management when facing emerging anthelmintic resistance.

1. Introduction

Australia is home to the largest population of free-roaming wild horses in the world, estimated to be between 400,000–1 million (Dobbie et al., 1993; Dawson et al., 2006). Whilst population ecology of Australian wild horses has been the focus of some research (Dyring, 1990, Berman, 1991; Dawson 2005, 2009; Dawson and Hone, 2012; Cairns and Robertson, 2015; Zabek, 2015), gastrointestinal parasites have not previously been investigated. There are very few studies of gastrointestinal parasites in wild horses worldwide (Rubenstein and Hohmann, 1989; Slivinska et al. 2006, 2009; Debaffe et al., 2016; Cain et al., 2018).

Gastrointestinal parasites of horses may cause significant intestinal pathology, adversely affecting gastrointestinal function and potentially impacting on horses' body condition, health, reproduction and longevity (McCraw and Slocombe, 1976; Love et al., 1999; Anderson et al., 2014; Debaffe et al., 2016; Pihl et al. 2017, 2018). Year-round management of gastrointestinal parasites of owned horses, through routine anthelmintic administration, has significantly reduced the impact of parasites worldwide (Herd, 1990; Gomez and Georgi, 1991; Geary, 2005; Gokbulut and McKellar, 2018; Nielsen and Reinemeyer, 2018). The introduction of macrocyclic lactones in the 1980s has dramatically reduced the prevalence of verminous endarteritis due to migrating *Strongylus vulgaris* larvae (Herd, 1990; Geary, 2005; Nielsen et al., 2014a,b; Pihl et al., 2018). While clinical disease due to *S. vulgaris* has largely disappeared, the ubiquitous cyathostomines (small strongyles) remain a problem in most if not all domestic horse populations, particularly due to their increasing resistance to anthelmintics (Herd, 1990; Prichard, 1990; Love et al., 1999; Kaplan, 2002; Kaplan and Nielsen, 2010; Nielsen et al., 2014a,b; Scott et al., 2015; Becher et al.,

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https://doi.org/10.1016/j.ijppaw.2019.01.008
Received 13 September 2018; Received in revised form 30 January 2019; Accepted 30 January 2019

2018; Bellaw et al., 2018; Salas et al., 2018; Scare et al., 2018). Absence of clinical disease caused by *S. vulgaris* may either mean that (i) the parasite is no longer prevalent and potentially extinct or that (ii) the parasite remains prevalent, but is controlled by current strategies including the use of macrocyclic lactones (Nielsen et al., 2012; Kaspar et al., 2017). Sustained prevalence may indicate the presence of a reservoir such as untreated but owned horses or wild/free roaming horses that have never received anthelmintics (Cain et al., 2018).

Higher strongyle faecal egg counts (FECs) were found in wild horses on Shackleford Banks (North Carolina, USA), Sable Island (Nova Scotia, Canada) and Fort Polk (Louisiana, USA) compared to domestic horses (Rubenstein and Hohmann, 1989; Debaffe et al., 2016; Cain et al., 2018). Shackleford Banks horses were shown to shed *S. vulgaris* based on larval culture (Rubenstein and Hohmann, 1989) and over two thirds of the Fort Polk horses' fecal samples were positive for *S. vulgaris* DNA (Cain et al., 2018). Furthermore, *S. vulgaris* has been identified in a very small wild horse population in Poland (Slivinska et al., 2009) and in free-ranging Przewalski horses in Ukraine (Slivinska et al., 2006).

The aim of this study was to investigate the prevalence of *S. vulgaris* in free-roaming wild horses in south-east Australia. To do so, we performed FECs across six remote wild horse populations of varying densities, herd sizes and habitats. Presence of *S. vulgaris* was demonstrated using larval culture and diagnostic *S. vulgaris* specific real-time PCR (qPCR). We hypothesised that (i) wild horse populations never exposed to anthelmintics would have a high prevalence of *S. vulgaris* infection, and that (ii) FECs would vary significantly both within and between the different populations.

2. Materials and methods

2.1. Study sites

The populations sampled were part of a larger study concerning the population ecology and welfare of wild horses (University of Technology Sydney Animal Ethics Research Authority 2015000490, Parks Victoria scientific license 10008189, National Parks and Wildlife scientific license 101626, WaterNSW access license D2015/128332). Study sites comprised five sites across the Australian Alps in both New South Wales and Victoria, and one site in Blue Mountains National Park (Fig. 1). These wild horse populations have never been in active anthelmintic management programs.

Sites comprised of differing habitats, horse population densities and herd sizes (Table 1). Predominant habitat types were taken from previous vegetation surveys (McRae, 1989) and verified by on-ground surveys during the study period. Horse densities for the Kosciusko National Park (KNP) sites and Bogong High Plains are from estimations from previous aerial surveys (Cairns and Robertson, 2015), and population size estimates and mean herd sizes for Cooleman Plain and Cowombat Flat are from a recent population survey of on-ground observations and camera trapping (Butler 2016–17, personal communications, Watts, 2017). Population and herd size estimates for Kedumba Valley, Tin Mines and Lower Snowy River were performed as part of a wider ecological study, based on mark-recapture surveys from direct observations and camera trap images (Harvey 2015–17, unpublished data).

2.2. Sample collection and storage

Faecal samples were collected from Kedumba Valley at seven different time points between February 2016 and April 2017, and from the other study sites at two different time points between December and March of 2016 or 2017.

As horses in these populations are wild, and not habituated to human presence, they could not be approached closely, with recorded flight initiation distances varying from 40 to 217 m (Watts, 2017). Observations of horses were, therefore, most commonly performed from 200 to 500 m away using binoculars and a spotting scope. Consequently, it was rarely possible to identify individuals that the samples were collected from. Most of the samples were collected immediately after observing a known herd grazing, and thus, such samples were known to have been passed within one hour prior to collection. Some samples deemed to have been recently passed, based on warmth and moistness, were collected opportunistically.

Samples collected were from a minimum of three different herds in Kedumba Valley, 15 herds in Cooleman Plain, 12 herds in Cowombat Flat, one herd in Bogong High Plains, four herds in Tin Mines and five herds in the Lower Snowy River region.

Within half an hour of collection samples were placed in a 4 °C portable refrigerator for storage until FECs were performed. Faecal sample aliquots (200–400 mg) were placed in 1.5 mL Eppendorf tubes and frozen at −20 °C for later DNA extraction.

2.3. Faecal egg counts

Faecal egg counts (FECs) were performed within two weeks of sample collection. Three grams of faeces were dissolved in 60 mL of saturated salt solution (NaCl, specific gravity 1.20). A Whitlock universal 4 chamber worm egg counting slide (J.A.Whitlock & Co, Eastwood, Australia) was used, counting eggs in four 0.5 mL compartments (detection limit 10 EPG) according to described methodology (Gordan and Whitlock, 1939). No larvated eggs were observed. All strongyle eggs were included in the total strongyle egg count. Strongyle eggs were further subdivided into strongyle eggs < 90 μm length and ≥90 μm length, to investigate the historically assumed association of smaller eggs with presence of large strongyles such as *S. vulgaris* (Thienpont et al., 1979). Strongyle eggs > 120 μm length were recorded as *Triodontophorus*-like eggs. *Parascaris* spp. and *Anoplocephala* spp. eggs were also counted and recorded (Fig. 2).

2.4. Larval culture

Faecal samples that had stronglye eggs < 90 μm with counts above 200 EPG were selected for larval culture (n = 20). Samples were cultured within 10 days of sample collection. Larval culture was performed using described methodology (Dunn, 1978). Following 10 days of incubation, larvae were retrieved and 100 larvae from each sample were identified (limit of detection 1%), using an identification key (Thienpont et al., 1979), to determine the proportions of *S. vulgaris, S. equinus, S. edentatus, Triodontophorus* and *Trichonema* larvae.

2.5. Strongylus vulgaris diagnostic qPCR

DNA was isolated from 134 faecal samples across the six populations using the MagAttract PowerMicrobiome DNA/RNA Kit (Cat No./ID: 27600-4-KF, Qiagen, Australia) optimised for KingFisher® Duo. Each sample was homogenized for 40 s at 6.0 m/s on a FastPrep-24 benchtop homogeniser (MP Biomedicals, Australia). Samples were extracted in batches of 12 including an extraction blank per batch. DNA was eluted into 100 μL stored at −20 °C.

The diagnostic *S. vulgaris* qPCR was performed using described methodology (Nielsen et al., 2008). The qPCR reaction included 2 μL of DNA template in a total volume of 20 μL using the SensiFAST™ Probe No-ROX Kit (Cat No.: BIO-86020, Bioline, Australia). The oligonucleotides used in a final concentration of 400 nM for primers and 100 nM for the probe per reaction were: Forward primer 5′-GTA TAC ATT AAA TAG TGT CCC CCA TTC TAG-3'; Reverse primer 5′-GCA AAT ATC ATT AGA TTT GAT TCT TCC G-3'; Probe 5′-FAM-TGG ATT TAT TCT CAC TAC TTA ATT GTT TCG CGA C-BHQ3-3'. Primers and probe were synthesized by Macrogen (Seoul, Korea). The qPCR conditions included 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The qPCR assay was run on CFX96 TouchTM Real-Time PCR Detection System (BioRad, Australia), with samples run in duplicate. Real-time PCR results were analysed using BioRad CFX Manager 3.1 (BioRad, Australia). Positive results were determined if one or more repeats yielded C_t values < 40.00 and negative results were

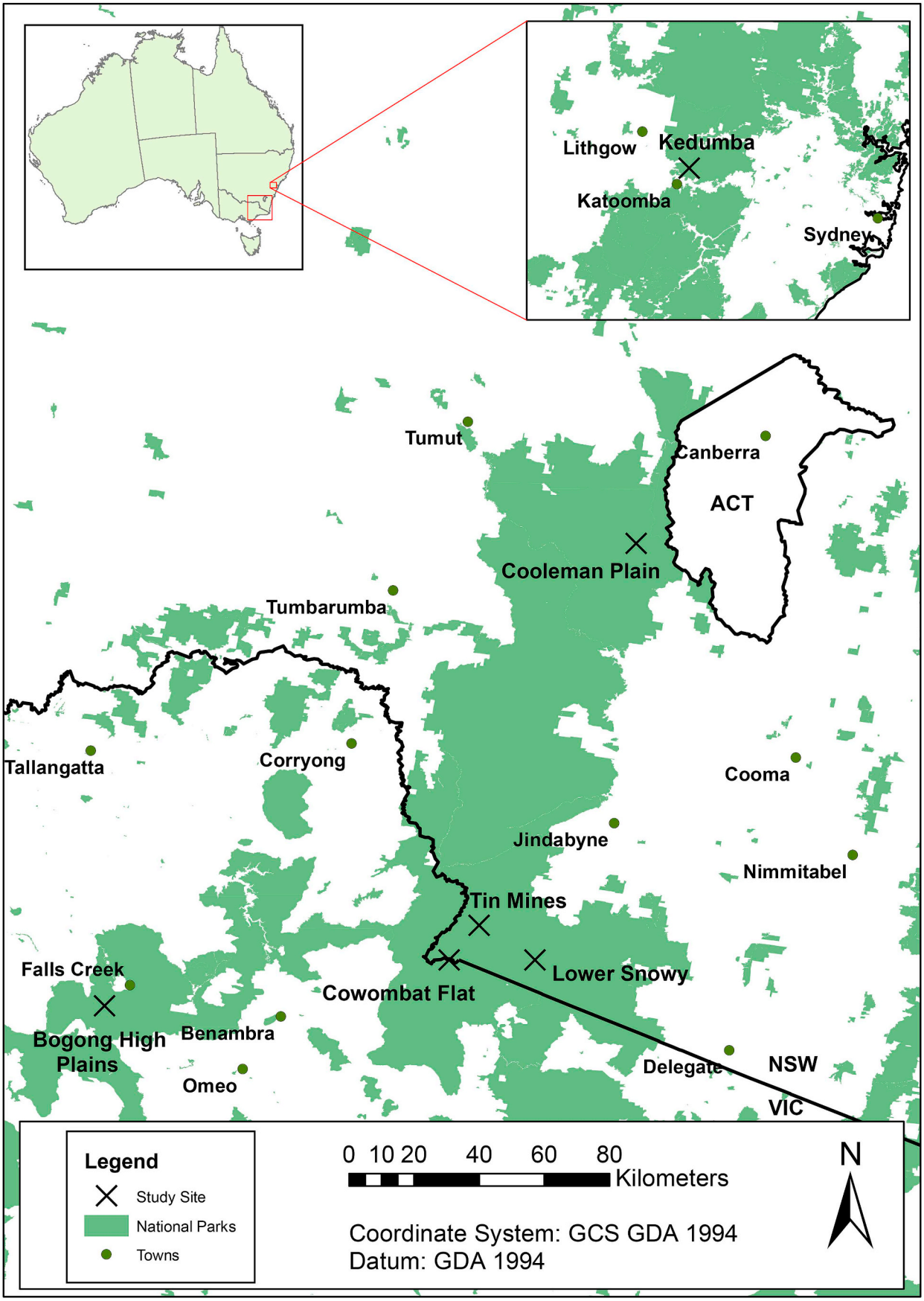

Fig. 1. Map illustrating location of sampling sites.

Table 1
Details of study sites, number of samples collected from each site at each different time points, and predicted means and standard errors for Strongyle and Parascaris spp. faecal egg counts in each population.

Site	Horse density	Estimated total no. of horses	Mean herd size (range)	Habitat type	Date collected	No. samples collected	Mean total strongyle EPG (SE)	Mean EPG eggs < 90 μm (SE)	Mean EPG eggs ≥90 μm (SE)	Mean *Triodontophorus*-like EPG (SE)	Mean *Parascaris* spp. EPG (SE)
Kedumba valley	Low	28	4 (2–7)	Eucalyptus woodland	02/2016 03/2016 04/2016 10/2016 01/2017 02/2017	9 14 5 8 6 6	466 (27)	80 (7)	368 (21)	N/A	N/A
Lower Snowy River	Low	~80	3 (2–5)	Eucalyptus woodland	12/2017 02/2018	11 10	1352 (67)	148 (14)	1185 (56)	16 (3)	46 (41)
Bogong High Plains	Low	~50	4 (3–6)	Alpine heathlands	01/2017 03/2017	2 4	1992 (151)	202 (31)	1780 (128)	14 (7)	10 (13)
Cowombat Flat	Medium	85	6 (3–10)	Open grassland	01/2017 03/2017	60 35	1746 (36)	281 (9)	1434 (29)	23 (2)	66 (13)
Tin Mines	Medium	~100	5 (2–6)	Alpine heathlands	12/2017 02/2017	9 7	2016 (93)	220 (20)	1745 (78)	28 (6)	107 (97)
Cooleman Plain	High	390	10 (4–25)	Open grassland	12/2016 02/2017	70 37	1287 (29)	179 (7)	1085 (24)	18 (2)	30 (9)

SE Standard Error.
EPG Eggs per gram.
Low density = estimated < 1 horse/km2, medium density = estimated 1–2 horses/km2, high density = estimated > 2 horses/km2 (Cairns and Robertson, 2015; Watts, 2017).

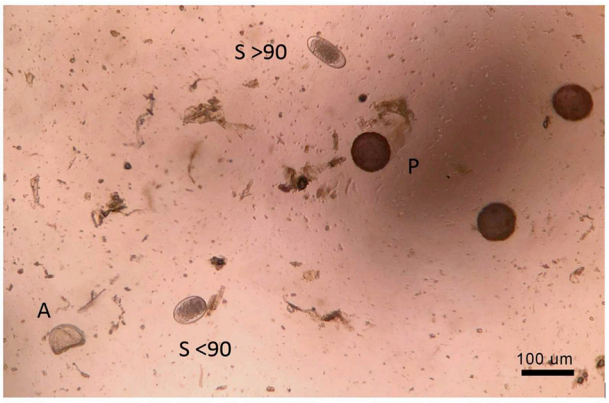

Fig. 2. Microscopic view of the different eggs. **A** = *Anoplocephala* spp. eggs, **S < 90** = strongyle eggs < 90 μm length, **S > 90** = strongyle eggs ≥90 μm length, **P** = *Parascaris* spp. eggs.

determined if both repeats did not cross the threshold ($C_t \geq 40$). Each qPCR run included positive control from DNA extracted from known adult *S. vulgaris* (courtesy of Ian Beveridge, University of Melbourne), negative control (water), plus the DNA extraction blank control.

2.6. Statistical analysis

All statistical analyses were conducted using GenStat v. 17 (VSNi). A Restricted maximum likelihood model (REML) was used to analyse continuous variables. An Anderson-Darling Test for normality was conducted for each variable, with those failing either square root or natural log transformed to assume a normal distribution. Outcome variables were the counts of total strongyle eggs (eggs per gram; EPG), strongyle eggs < 90 μm (EPG), strongyle eggs ≥ 90 μm (EPG), *Triodontophorus*-like eggs (EPG), *Parascaris* spp. (EPG) and *S. vulgaris* qPCR C_t values. Mean FECs were calculated based on FEC of all samples. Fixed effects of Location, Density and Habitat along with the random effect of Sample were included in the model. As there were limited cases where *Anoplocephala* spp. eggs were recorded, this variable was converted to a binary outcome, where *Anoplocephala* spp. were present or absent. *Anoplocephala* spp. and *S. vulgaris* PCR results were analysed separately using a General Linear Mixed Model with a binomial distribution. Fixed effects were Location, Density and Habitat, with the random effect of Sample.

3. Results

3.1. Strongyle faecal egg counts

A total of 293 faecal samples were collected (Fig. 1, Table 1). The most intensively sampled localities were Cooleman Plain (n = 107) and Cowombat Flat (n = 95), sampled over two time points, and Kedumba Valley (n = 48), which was sampled over six time points (Table 1).

Strongyle eggs were detected in 100% of faecal samples (n = 289). Four samples from Kedumba Valley were omitted because the consistency suggested that these were not fresh faecal samples. Total strongyle FEC ranged from 50-3740 EPG, with an overall mean of 1443 EPG (Table 1, Fig. 3). There were significant differences between the populations, classed by location (Table 1, Fig. 3), density and habitat type ($P < 0.001$), with the highest FECs in medium density populations and alpine healthland habitats.

If the arbitrary strongyle FEC values used in domestic horse populations for classifying 'low' (< 200 EPG), 'moderate' (200–500 EPG) and 'high' (> 500 EPG) level shedders are applied, then for Kedumba Valley 5/44 (11%) samples were from low level shedders, 21/44 (48%) samples were from moderate level shedders, and 18/44 (41%) samples were from high level shedders, for Cooleman Plain 5/107 (5%) samples were from moderate level shedders and 102/107 (95%) samples were from high level shedders, from Cowombat Flat 1/95 (1%) were from moderate level shedders and 94/95 (99%) from high level shedders, and for Bogong High Plains, Tin Mines and the Lower Snowy River 100% of samples (6/6, 16/16, 21/21 respectively) were from high level shedders.

When strongyle eggs were divided into those < 90 μm and ≥90 μm, the FEC for eggs < 90 μm ranged from 0 to 1310 EPG (mean 229) and for eggs ≥ 90 μm ranged from 30-2900 EPG (mean 1205) (Table 1). There were significant differences between the different populations, classed by location (Table 1), density and habitat type ($P < 0.001$). The highest FECs for eggs < 90 μm being in medium density populations and open grassland habitat, and the highest FECs for ≥90 μm being in medium density populations and alpine heathland habitat

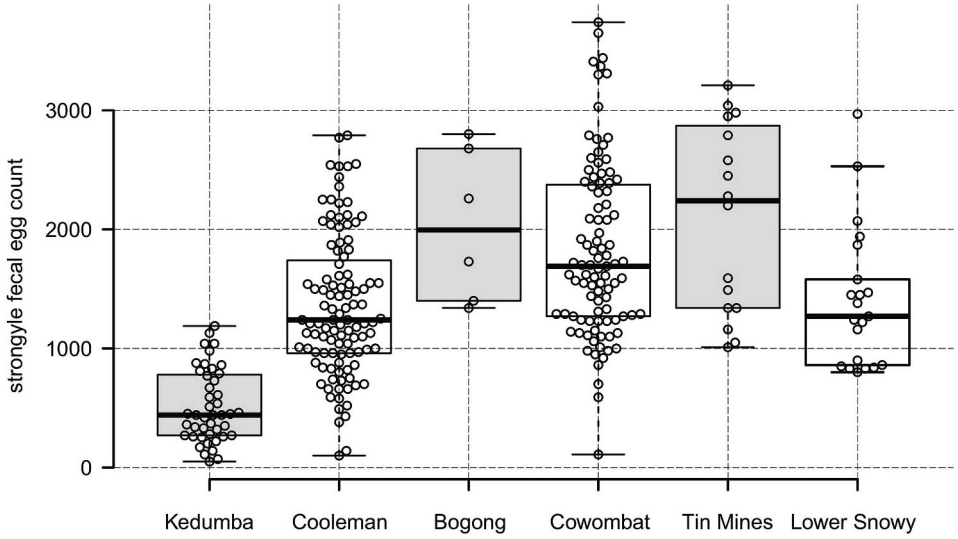

Fig. 3. A box and whisker plot (with individual data points) of the total strongyle egg counts across the different populations, showing the highest FECs were from samples from Bogong High Plains and Tin Mines, both alpine heathland habitats. Overall 89% of samples had FECs > 500 EPG, classed as 'high level shedders'.

Table 2
Significant differences in Australian wild horse parasite faecal egg counts between the locations, population densities and habitat type.

Variable	Total strongyle EPG	EPG eggs < 90 µm	EPG eggs ≥ 90 µm	EPG *Triodontophorus*-like	EPG *Parascaris* spp.	EPG *Anoplocephala* spp. (binary)
Location	< 0.001*	< 0.001*	< 0.001*	0.145	0.07	0.001*
Population density	< 0.001*	< 0.001*	< 0.001*	0.045*	0.011*	0.237
Habitat	< 0.001*	< 0.001*	< 0.001*	0.225	0.209	0.032*

EPG – eggs per gram; **significant differences, P < 0.05*.

(Table 2). *Triodontophorus*-like FEC ranged 0–190 EPG (mean 11) (Table 1) with the only significant difference (P = 0.045) being between population densities, the highest FEC occurring in medium density populations (Table 2).

3.2. Parascaris spp. and Anoplocephala spp. faecal egg counts

Parascaris spp. eggs were present in 25% (74/289) of samples, with FEC ranging from 0 to 1750 EPG (mean 31). There was a significant difference in *Parascaris* spp. FECs between the different populations, when classed by population density (P = 0.01), with the highest FECs being in medium density populations. There were no significant differences when classed by location and habitat type (Table 1). The prevalence of *Anoplocephala* spp. eggs across all populations was low with only 26 of the 289 samples testing positive for *Anoplocephala* spp. eggs (range 10–30 EPG). Statistical modelling indicated a 2% prevalence of *Anoplocephala* spp. eggs in all populations.

3.3. Strongylus vulgaris qPCR

S. vulgaris DNA was detected in 123/134 (92%) samples (C_t values ranged from 21.35 to 39.12, mean 26.58). The blank controls remained negative. A high prevalence of *S. vulgaris* DNA in faecal samples was demonstrated across all six populations, with an overall predicted prevalence of 96.7% (Table 3). No significant differences between populations were detected when classed by location, density or habitat type. There was no significant correlation between *S. vulgaris* C_t values and either total strongyle FEC or FEC of eggs < 90 µm, illustrating that presence of *S. vulgaris* cannot be predicted based on FECs of strongyle eggs < 90 µm.

3.4. Larval culture

Presence of *S. vulgaris* was confirmed by larval culture, with *S. vulgaris* larvae identified in 13/20 samples. Out of 100 larvae from each sample, 1–4 (mean 2.8) were identified as *S. vulgaris* (Supplementary Table S1). All 20 samples were positive for *S. vulgaris* DNA. Other larvae identified included *S. equinus* in 5/20 samples, *S. edentatus* in all 20 samples, *Triodontophorus*-like spp. in 19/20 samples, as well as cyathostomins ('*Trichonema*' spp. or small strongyles) in all 20 samples.

Table 3
Summary of qPCR results for *Strongylus vulgaris* DNA in Australian wild horses.

Location	qPCR *S. vulgaris DNA* positive[a]	Predicted prevalence	Predicted mean C_t value (SE)
Kedumba valley	96% (23/24)	95.8%	26.80 (0.68)
Lower Snowy River	90% (10/11)	90.9%	26.12 (1.03)
Bogong High Plains	100% (6/6)	99.9%	29.95 (1.3)
Cowombat Flat	91% (32/35)	91.4%	26.89 (0.57)
Tin Mines	89% (8/9)	88.9%	27.24 (1.23)
Cooleman Plain	90% (44/49)	89.8%	25.76 (0.49)

[a] Number of samples testing positive/tested samples; SE - Standard error.

4. Discussion

It is widely accepted that *S. vulgaris* is the most pathogenic gastrointestinal parasite in horses, with an infective L_3 stage, and a migratory arterial L_4 stage, which may cause arteritis, hypertrophy and fibrosis of mesenteric arteries, and thrombosis (McCraw and Slocombe, 1976; Ogbourne and Duncan, 1985; Nielsen et al., 2016; Pihl et al., 2018). *Strongylus vulgaris* infection has been rarely reported since the 1960's following the advent of effective anthelmintics (Herd, 1990; Geary, 2005; Kaplan and Nielsen, 2010). We demonstrate a high predicted prevalence (88.0–99.9%; overall 96.7%) of *S. vulgaris* DNA in six wild horse populations in south-east Australia. Such a high prevalence across large wild horse populations has not been reported previously.

As a result of the rising resistance of cyathostomins (small strongyles) to anthelmintics, recommended anthelmintic strategies have changed to focus on increased parasite surveillance through FECs, and reduced anthelmintic treatment intensity (Duncan and Love, 1991; Kaplan, 2002; Nielsen et al., 2014a,b; Becher et al., 2018). In Denmark, this has even led to anthelmintics becoming prescription only, with most veterinarians only prescribing anthelmintics if FECs are above a certain cut-off value (Nielsen et al., 2006). This approach, however, raises concerns about the potential re-emergence of *S. vulgaris* (Nielsen et al., 2008, 2012, 2014, 2016).

Historically, non-invasive diagnosis of *S. vulgaris* infection has been challenging. Eggs can not be definitively distinguished from other strongyle eggs, larval culture is time consuming, false negative results are common, expertise is required for species identification, and freshly collected faeces are required (Nielsen and Reinemeyer, 2018). The requirement for fresh faeces is challenging when collecting samples in remote locations. The qPCR assay used in this study enabled easier, more reliable, species-specific detection of *S. vulgaris* DNA in faeces (Nielsen et al. 2008, 2012; Kaspar et al., 2017). Applying this qPCR, 20/84 (24%) horses tested in Denmark were positive for *S. vulgaris*, validating concerns about its re-emergence with changes in anthelmintic strategies (Nielsen et al., 2008, 2012, 2014, 2016). Our study found 92% of samples from wild horses to be qPCR positive for *S. vulgaris* DNA. This suggests that if domestic horses graze on habitat occupied by infected wild horses, re-emergence in domestic horses is likely, if they are not routinely receiving anthelmintics.

Egg shedding patterns in domestic horses have been described to almost always follow the 20/80 rule, meaning that 20% of the horses' harbor 80% of the parasites in any given population (Kaplan and Nielsen, 2010; Nielsen and Reinemeyer, 2018). Even in the absence of anthelmintics, only a small proportion of 'high level shedders' (20–30%) produce the moderately large numbers of eggs responsible for environmental contamination (Nielsen et al., 2006; Becher et al., 2010; Kaplan and Nielsen, 2010). Whether similar egg shedding patterns occur in wild horse populations has not been previously described. In the populations of horses in our study, the 20/80 rule was not followed. A total of 257/289 (89%) wild horses from six different regions would be considered 'high level shedders' of strongyle eggs, if the cut-offs used in domestic horses are applied (Nielsen and Reinemeyer, 2018). The FECs are likely to be representative, as studies have shown that egg-shedding of individual horses stays consistent over time (Scheuerle et al., 2016). Our findings are consistent with those reported in Sable Island (Nova Scotia, Canada) wild horses, where a mean total strongyle FEC of 1543 EPG (SD 209.94) was identified across 447 horses (Debaffe et al., 2016), and in Fort Polk (Lousiana, USA) where wild horses had higher FECs than domestic horses, with 63.4% being high level shedders (Cain et al., 2018).

It is not well understood why some domestic horses shed higher numbers of eggs and others shed low numbers, but there are several factors known to influence shedding (Nielsen and Reinemeyer, 2018). Host immunity plays a complex role in transmission and progression of parasite infections, and is itself influenced by other factors such as age (Chapman et al., 2003; Nielsen et al., 2006; Becher et al., 2010; Nielsen and Lyons, 2017), genetics (Stear et al., 1984; Gasbarre et al., 1990; Kornas et al., 2015), and a range of stressors including nutritional, reproductive, thermal, exertional, social and health stressors (Segerstrom and Miller, 2004; Nielsen and Reinemeyer, 2018). Grazing behaviour and parasite factors also play a role (Herd and Willardson, 1985; Nielsen and Reinemeyer, 2018). We can, therefore, hypothesise that perhaps differences in the age distribution, genetics, stressors, and grazing behaviours of wild horses may account for the differences in egg shedding compared to domestic populations. A major limitation of this study, which makes interpretation of FECs challenging, is attributable to the nature of wild horses, meaning that information such as age, health and reproductive status, was not able to be obtained or linked to the individual from which each sample was collected.

In our study, mean total strongyle FEC was significantly higher in medium density populations and alpine heathland habitats. The aforementioned limitations of this study make it difficult to speculate about the reasons for these differences. The alpine heathland habitats are moister with higher rainfall, at higher elevations and lower temperatures. FECs are also affected by seasonality, influenced by temperature and rainfall, with higher FECs expected during spring and summer (Herd and Willardson, 1985; Wood et al., 2012; Misuno et al., 2018). In this study, all samples for most sites were collected during summer. Some samples for Kedumba were collected during autumn and spring, which could, in part, contribute to the lower mean FECs at this site.

The clinical significance of the 'high' FECs in these wild horses is difficult to determine, since FECs are not necessarily correlated with the size of worm burdens (Nielsen et al., 2010). The cut-offs of low/moderate/high level shedders are arbitrary values used in domestic horses simply to characterize the level of environmental contamination from that individual and thus guide targeted anthelmintic treatment, rather than estimating the impact of the parasite burden on an individual horse (Nielsen and Reinemeyer, 2018). Nevertheless, it is likely that the consistently high FECs in the horses in this study are associated with burdens of small strongyles that may be causing significant intestinal disease (Love et al., 1999; Debaffe et al., 2016).

Most horses develop very strong acquired immunity to *Parascaris* spp., and egg shedding eventually ceases (Donoghue et al., 2015; Fabiani et al., 2016). Consequently, infections are most commonly observed in foals, weanlings and yearlings, and only occasionally seen in horses over 18 months-old (Fabiani et al., 2016). It is challenging, however, to interpret the clinical significance of *Parascaris* spp. FECs as developing immunity can result in a negative egg count even in the presence of a potentially pathogenic adult burden (Nielsen et al., 2015).

FECs have a very low sensitivity for detecting *Anoplocephala* spp. infection, as, unlike nematodes, they do not regularly release eggs. The predicted prevalences based on FECs are, therefore, likely to be substantially underestimated (Nielsen and Reinemeyer, 2018).

Further work to assess parasite burdens and associated pathology in wild horses is needed to determine the clinical significance of the high prevalence of *S. vulgaris* DNA, the apparently high strongyle FECs, and the pathology and clinical significance associated with *Parascaris* spp. and *Anoplocephala* spp. infections, and other gastrointestinal parasites not detected with these diagnostic techniques.

On a positive note for domestic horses, small and large strongyles, as well as ascarids from wild horse populations serve as parasite *refugia* for strains that are not under anthelmintic selection pressure. Parasites that have never been exposed to anthelmintics, and thus have escaped selection for resistance, are known to delay the development of resistant parasites by outcompeting the resistant strains (Prichard, 1990; Van Wyk, 2001, Waghorn et al., 2008). Maintenance of diverse horse parasites under no anthelmintic pressure is an important component of integrated parasite management when facing the emerging anthelmintic resistance in horses (Nielsen et al., 2014a,b; Nielsen and Reinemeyer, 2018).

5. Conclusions

Despite 30 years of macrocyclic lactone anthelmintic use in domestic horses throughout Australia, *S. vulgaris* remains endemic in wild populations. There is ample opportunity for transmission to domestic horses, since wild horses from studied regions are regularly trapped, rehomed and domesticated.[1] Furthermore, domestic horses are ridden through some of these wild horse habitats and allowed to graze during rest periods.[2] It is important for horse owners who may adopt wild horses, or whose domestic horses may graze in wild horse habitats, to

[1] https://www.environment.nsw.gov.au/research-and-publications/publications-search/kosciuszko-national-park-horse-management-plan, http://parkweb.vic.gov.au/explore/parks/alpine-national-park/plans-and-projects/feral-horse-operational-plan http://parkweb.vic.gov.au/explore/parks/alpine-national-park/plans-and-projects/feral-horse-operational-plan.

[2] www.visitnsw.com/destinations/snowy-mountains/horseriding, http://parkweb.vic.gov.au/visit/popular-activities/horse-riding.

be educated about *S. vulgaris*, and advised appropriately to minimize the risk of their horses acquiring *S. vulgaris* infection. Monitoring domestic horse populations for re-emergence of *S. vulgaris* is prudent, particularly those in rural and remote locations that may not receive routine anthelmintic treatment. Since strongyles and ascarids from wild horse populations have not been exposed to anthelmintics, they may also become useful for tackling the emerging issues with anthelmintic resistance in domestic horses.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

AH's PhD is supported by an Australian Government Research Training Program Scholarship, and University of Technology Sydney Chancellors Research scholarship. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgements

Many thanks to Katrina Gilchrist for assistance in the lab, and particularly with assistance in performing larval cultures, to Catherine Butler for collaboration in field work, to field volunteers that assisted in collecting samples, to Richard Malik for assistance with the manuscript, to WaterNSW for permission to perform the research in Kedumba Valley and to National Parks and Wildlife Service and Parks Victoria for their advice regarding field sites, and their support throughout the field work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.01.008.

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