Infection, Genetics and Evolution 13 (2013) 236–241

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

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

Population genetics of *Parascaris equorum* based on DNA fingerprinting

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ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 27 September 2012
Accepted 28 September 2012
Available online 17 November 2012

Keywords:
Parascaris equorum
Population genetics
AFLP

ABSTRACT

The large roundworm of horses, *Parascaris equorum* is considered ubiquitous in breeding operations, and is regarded as a most important helminth pathogen of foals. Over the past decade, this parasite has been reported increasingly resistant to anthelmintic drugs worldwide. This paper reports analysis of the population genetic structure of *P. equorum*. Adult parasites (n = 194) collected from Sweden, Norway, Iceland, Germany, Brazil and the USA were investigated by amplified restriction fragment length polymorphism (AFLP) analysis. The genetic variation was low (Hj = 0.12–0.4), for the global population of worms. This was accompanied by a weak degree of population structure (Fst = 0.2), low gene flow (Nm = 1.0) and low mutation rate (4 Nμ = 0.07). Thus, the low genetic diversity is probably a result of a low mutation rate in DNA, although the gene flow (due to global movement of horses) is large enough to allow the spread of novel mutations. Surprisingly, isolates from Icelandic horses were not found to be different from other isolates, in spite of the fact that these have been isolated for thousands of years. The study indicates that the global *P. equorum* population is essentially homogenous, and continents do not appear to be strong barriers for the population structure of this species. Consequently, the potential spread of rare anthelmintic resistance genes may be rapid in a homogenous population.

1. Introduction

The large roundworm of horses, *Parascaris equorum,* is the most pathogenic parasite of foals and yearlings worldwide. Infection may cause nasal discharges, coughing and impaired growth, and large burdens may even lead to death caused by obstruction or penetration of the small intestine (Boyle and Houston, 2006; Cribb et al., 2006).

Anthelmintic resistance (AR) has become a major problem in veterinary medicine and constitutes a threat to animal welfare and productivity (Kaplan, 2004). Failure of macrocyclic lactone (ML) treatment to reduce the shedding of *P. equorum* eggs has been reported in several countries, such as the Netherlands (Boersema et al., 2002), Canada (Hearn and Peregrine, 2003), the United Kingdom (Stoneham and Coles, 2006), the United States (Craig et al., 2007), Denmark (Schougaard and Nielsen, 2007), Germany (von Samson-Himmelstjerna et al., 2007), Brazil (Molento et al., 2008), Sweden (Lindgren et al., 2008), Italy (Veronesi et al., 2009) and Finland (Näreaho et al., 2011). In ML-resistant populations of *P. equorum*, treatment with pyrantel pamoate, fenbendazole and oxibendazole has been successful (Slocombe et al., 2007; Lyons et al., 2008; Schougaard and Nielsen, 2007; Hearn and Peregrine, 2003). One study has reported apparent lack of pyrantel efficacy against *P. equorum* (see Lyons et al., 2008), while there are no published studies reporting benzimidazole (BZ) resistance in this parasite. However, BZ resistance might be developing, as the use of this drug class in foals is likely to increase due to the reduced efficacy of MLs. Little is still known about how anthelmintic resistance arises, and how genes conferring resistance are spread among parasite populations.

Molecular techniques have provided valuable tools to conduct detailed population genetic investigations of almost all organisms. For parasitic nematodes of veterinary interests it has been shown that gene flow and diversity are determined by the life history of both the parasite and its host (Anderson et al., 1998). For example, in the closely related large roundworm of pigs, *Ascaris suum,* host dispersal effectively determines and controls the patterns of gene flow (Nadler, 1986). Thus, the potential spread of AR may be rapid due to extensive transport of infected hosts across large geographical distances. While this is still only a preliminary conceptual idea for the spread of AR, it has been shown that large nationwide transport of domestic pigs has led to a homogenisation of the gene pool of *A. suum* in Denmark (Nejsum et al., 2005).

Accordingly, an investigation of the population genetic structure and diversity of *P. equorum* can be used to predict the pattern of spread of this parasite, and also to explore whether there is

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http://dx.doi.org/10.1016/j.meegid.2012.09.022

evidence for distinct isolates of *P. equorum* representing local subpopulations, or whether the members can rather be regarded as a single panmictic population. This knowledge is essential for understanding whether there is an imminent risk for the selection and spread of drug-resistance genes in response to an excessive and indiscriminate use of anthelmintics as practiced in many equine establishments. Clearly, the rate of spread of any genes conferring anthelmintic resistance will be directly correlated with the degree of gene flow among hosts and farms (Anderson et al., 1998; Hawdon et al., 2001; Nejsum et al., 2005).

In Sweden, transportation of horses between farms is readily apparent. Many horse farms routinely accommodate mares and foals, especially during the breeding and grazing seasons. Although *P. equorum* lives for less than a year in its host, foals become infected on pasture by parasite eggs deposited from the previous generation of foals on the same farm (Lindgren et al., 2008). Moreover, these eggs are reported to be very resistant to both climatic changes and disinfectants and, apparently, they can remain viable in the soil for up to 10 years (Urquhart et al., 1996). Thus, *P. equorum* possesses numerous potential pathways for gene flow between different host generations.

The aim of this study was to investigate the population structure of *P. equorum* among young horses in Sweden and to compare it with other isolates both from Europe and other continents using the DNA fingerprinting technique amplified fragment length polymorphism (AFLP). We have addressed the question as to what extent geographical locations have influenced the population structure of *P. equorum*. AFLP is commonly used in population genetic studies (Bensch and Åkesson, 2005; Bonin et al., 2007; Meudt and Clarke, 2007) and one of the major advantages of the AFLP technique are the detection of a large number of polymorphism and the generation of a unique genetic pattern for each individual (Vos et al., 1995). Another advantage is that it is efficient to use on worms with no previous knowledge of the DNA sequence (Höglund et al., 2004; Nejsum et al., 2005). Identification of nematodes analysed in the AFLP was also performed with universal primers targeting the conserved genes flanking the spacers of the internal transcribed spacers 2 (ITS-2) of the ribosomal rDNA gene array.

2. Materials and methods

2.1. Parasite material

In Table 1 sample code of parasite isolates, geographical origin, collection method and breed of horses are given. The *Parascaris* isolates from Sweden and Norway were sampled from faeces that were collected 1–2 days after treatment with fenbendazole. Expelled worms (a minimum of 10 adult worms from each farm) were collected in a plastic bag by the horse manager and immediately submitted to the laboratory at the Section for Parasitology at the Swedish University of Agricultural Sciences (SLU) in Uppsala. The isolates from Brazil and Iceland were collected from the intestine at slaughter. One isolate from the US (U1) was obtained from a well-characterised, closed research horse herd, maintained at the University of Kentucky without anthelmintic intervention since 1979 (Lyons et al., 1997), and parasites maintained here are therefore ML-naïve. The second US isolate (U2) was collected during routine necropsy performed at the Veterinary Diagnostic Laboratory at University of Kentucky. All worms collected outside Sweden and Norway were preserved in ethanol (70–90%) and sent by airmail to the laboratory at the Section for Parasitology at the Swedish University of Agricultural Sciences (SLU) in Uppsala. Upon arrival all worms were stored in −70 °C until DNA extraction was performed.

2.2. DNA extraction

DNA was extracted from individual worms using nucleospin tissue (Macherey–Nagel, Düren, Germany) according to the manufacturer's recommendation. RNase A (400 μg/sample) was used to obtain RNA-free samples. The purified DNA was stored at 4 °C until further analysis.

2.3. AFLP

A total of 194 worms were used for AFLP analysis (Table 1). As an internal control replicate analyses (5) were performed for one of the isolates from farm S3 (Heby). The AFLP procedure was performed as described by Applied Biosystems (ABI, Carlsbad, California, USA) in their Plant Mapping Protocol and as described by Höglund et al. (2004). All reagents were supplied in the AFLP™ Plant Mapping Kit except the restriction enzymes and the T4 DNA ligase, which were from New England Biolabs (Ipswich, Massachusetts, USA).

For all worms, the maximum allowed volume (5.5 μL), of extracted DNA was used. The DNA from each worm was digested with *Mse*I and *Eco*RI (ABI, Carlsbad, California, USA), and adaptors for *Mse*I and *Eco*RI restriction products were ligated in the same

Table 1
Parascaris isolate code, geographical origin, collection method and breed of horses.

Code	Continent	Geographical origin	Collection method	Number of worms	Breed of horses
S1	Europe	Sweden, Boden	Expelled by fenbendazole, faecal sample	10	Standardbred
S2	Europe	Sweden, Borlänge	Expelled by fenbendazole, faecal sample	10	Standardbred
S3	Europe	Sweden, Heby	Expelled by fenbendazole, faecal sample	10	Standardbred
S4	Europe	Sweden, BrO	Expelled by fenbendazole, faecal sample	10	Standardbred
S5	Europe	Sweden, Ekerö	Expelled by fenbendazole, faecal sample	10	Standardbred
S6	Europe	Sweden, Tystberga	Expelled by fenbendazole, faecal sample	10	Standardbred
S7	Europe	Sweden, Västervik	Expelled by fenbendazole, faecal sample	10	Standardbred
S8	Europe	Sweden, Västervik	Expelled by fenbendazole, faecal sample	10	Standardbred
S9	Europe	Sweden, Landskrona	Expelled by fenbendazole, faecal sample	10	Standardbred
S10	Europe	Sweden, Svenljunga	Expelled by fenbendazole, faecal sample	10	Standardbred
S11	Europe	Sweden, Sjöbo	Expelled by fenbendazole, faecal sample	10	Standardbred
G	Europe	Germany	Collected at an abattoir	10	Standardbred
I1	Europe	Iceland, Hvolsvöllur	Collected at an abattoir	9	Icelandic horse
I2	Europe	Iceland, Hella	Collected at an abattoir	9	Icelandic horse
I3	Europe	Iceland, Hvolsvöllur	Collected at an abattoir	9	Icelandic horse
N1	Europe	Norway, Vestfold	Expelled by fenbendazole, faecal sample	11	Standardbred
N2	Europe	Norway	Expelled by fenbendazole, faecal sample	10	Standardbred
B	S. America	Brazil	Collected at an abattoir	6	Not known
U1	N. America	US, Kentucky	Collected during necropsy	10	Mixed light breed
U2	N. America	US, Kentucky	Collected during necropsy	10	Thoroughbred

reaction as the digestion. After digestion, the mixture was diluted to 200 μL with $TE_{0.1}$ (20 mM Tris–HCl, 0.1 mM EDTA, pH 8.0).

For the preselective amplification, 4 μL of the diluted restriction-ligation reaction was mixed with ABÍs preselective primer pairs and ABÍs Core Mix. Amplified products were run on 1.5% agarose gel. Most samples were diluted to 200 μL, but samples that appeared weak on the gel were diluted to 105 μL.

For the selective amplification, one primer combination was used: *Mse*I-CTA and *Eco*RI-ACT. The *Eco*RI selective primer was labelled with the fluorescent dye FAM. For the electrophoresis, 1 μL of the selective amplification was mixed with 9.6 μL deionized formamide and 0.4 μL GeneScan-500 size standard (ABI, Carlsbad, California, USA). The samples were loaded onto the Genetic Analyser (ABI PRISM® 3100, Carlsbad, California, USA), and data were collected using Gene Mapper Analysis software 4.0 (ABI, Carlsbad, California, USA). The tabular data for all peaks greater than 50 U were summarised. The values were converted to binary characters (0, absence of peaks; 1, presence of peaks) for further analysis.

2.4. Data analysis

Most of the AFLP data analyses (genetic diversity Hj, Ht, Hs; population structure Fst, population mutation rate 4 Nμ; gene flow NM; proportion of neutral loci; re-allocation success; genetic distance between individuals and populations) followed Höglund et al. (2012) and Bonin et al. (2007), with the calculations performed using the latest version of each program. The genetic rarity index was calculated using the AFLPdat package (Ehrich, 2006), with significant deviations from expectation tested using 10,000 randomizations. The population mutation rate (Nm) was adjusted to incorporate the estimated mutation rate (4 Nμ) (i.e. NM = ((1-Fst)/(4Fst)–(4 Nμ/4)). Following Morrison (2010), the between-individual genetic distances were displayed using the neighbour-net network, rather than a phylogenetic tree, computed using the SplitsTree (version 4.11.3) program of Huson and Bryant (2006). These between-population distances were also compared to the geographical distances between the farms in Sweden via Mantel tests, with 10,000 randomisations, using the ZT program of Bonnet and van der Peer (2002). For the test of genetic differences between countries, the Mantel tests, with 10,000 randomisations, used the Mantel–Struct program of Miller (1999).

There are no established criteria that we could use for choosing a cut-off value for converting the AFLP peak heights into data for presence–absence data of fragments representing different loci. So, we heuristically chose a cut-off value based on (i) minimising the observed differences among the five replicate AFLP analyses (from a single worm) and (ii) maximising the success of re-allocating the worms to their isolates of origin.

2.5. Sequencing of ITS-2

A fragment of the internal transcribed spacer (ITS-2) within the rDNA gene array was amplified by PCR. Two combinations of primers were used. One combination with reverse-, NC2R, 5′-TTAGT TTCTTTTCCTCCGCT-3′ (Zhu et al., 2000) and forward primers NC13F 5′-GCTGCGTTCTTCATCGAT-3′ (Zhu et al., 2000) and one combination with forward primer OP642F 5′-AATGCCATATATGAAATAT ATACG-3′ designed downstream of the NC13 F based on the *P. eqourum* ITS-2 sequence (Y08274.1) together with NC2R. The amplification was performed in 25 μl reaction volumes containing 10 mM Tris–HCl pH 8.3, 3.2 μg Bovine Serum Albumin (BSA) 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 μM of each primer, 0.2 μM dNTP and 1 U AmpliTaq GOLD DNA polymerase. All reagents were from Applied Biosystems (ABI, Carlsbad, California, USA) apart from BSA that was supplied from New England Biolabs (Ipswich, Massachusetts, USA). The cycling parameters for the amplification consisted of an initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 30 s, and extension at 72 °C for 1 min with a prolongation of the final extension of 2 min (ABI, Thermal Cycler 2720, Carlsbad, California, USA). Aliquots of PCR products were analysed by electrophoresis on an agarose gel. The remaining products were purified using 10 U exonuclease I (Fermentas, St. Leon-Rot, Germany), according to the manufacturer's description prior to sequencing.

The amplicons were sequenced with Big Dye chemistry (Applied Biosystems) according to the manufacturer. The sequences were determined with a Genetic Analyser (ABI PRISM® 3100, Carlsbad, California, USA). The sequences were edited and analysed with CLC Main Workbench 5.6.1 (Aarhus, Denmark), and the resulting contigs were compared with the *P. eqourum* ITS-2 accession Y08274.1 in GenBank.

3. Results

3.1. Population genetic analysis

With a cut-off value of 50 U, there were 11 AFLP markers that varied among the five replicate analyses, decreasing to one difference at a cut-off of 80, 90 and 100 U (Table 2). The success of re-allocating the worms to their isolates of origin (based on the AFLP markers) varied little, from 38.4% to 42.4%. At 80 U the cut-off value was 41.4% deemed to be the most appropriate for the subsequent data analyses, as it minimised the detected differences among the replicates while maximising the re-allocation success.

With the given reaction conditions and a cut-off value of 80 U, a total of 198 informative AFLP markers were generated, of which 182 were variable among the worms. One isolate from Norway (code N2) had less than 10 informative peaks. Consequently, all of these worms were excluded from the geographical test and the phylogenetic network, because the genetic distances cannot be calculated. In the other population genetic analyses, similar patterns were observed with and without this Norwegian isolate (N2); thus it was included in those analyses.

Estimates of genetic diversity (or within-population heterozygosity) were small but consistent across the global isolates (Hj ranged from 0.12 to 0.04), with an Hj average of 0.08 (Table 3). Even among the 11 isolates from Sweden, the genetic diversity was low (Hj ranged from 0.12 to 0.6; Table 3).

The number of rare markers matched expectations (based on the number and distribution of variable markers in a randomly mating population) in nine of the populations (Table 3). However, two Swedish isolates, one Norwegian isolate and one North American isolate had a larger number of rare markers (1.4–2.5; Table 3). All isolates from Iceland, three isolates from Sweden and the German isolate had a smaller number of rare markers than expected (0.6–0.3; Table 3).

Table 2
AFLP markers at different peak cut-off levels. Cut-off value 80 U was the most appropriate for subsequent data analyses.

Cut-off	Total peaks	Variable peaks	Duplicate differences*	Re-allocation
50	245	225	11	42.2%
60	224	205	7	41.4%
70	210	190	3	39.4%
80	198	182	1	41.4%
90	195	174	1	39.9%
100	184	157	1	41.4%
110	176	153	1	38.4%
120	171	149	1	39.4%

* Replicate analyses (5) were performed on the isolate from S3.

Table 3
Values of the measures of genetic diversity (Hj) and rarity of isolates from 20 different farms.

Code	Continent	Genetic diversity (Hj)	Rarity	–*
S1	Europe	0.08	0.5	Small
S2	Europe	0.11	2.0	Large
S3	Europe	0.06	0.7	ns
S4	Europe	0.08	0.6	ns
S5	Europe	0.09	0.9	ns
S6	Europe	0.11	0.6	Small
S7	Europe	0.06	0.3	Small
S8	Europe	0.09	1.2	ns
S9	Europe	0.08	0.7	ns
S10	Europe	0.12	2.5	Large
S11	Europe	0.07	0.8	ns
G	Europe	0.05	0.3	Small
I1	Europe	0.09	0.3	Small
I2	Europe	0.08	0.4	Small
I3	Europe	0.10	0.5	Small
N1	Europe	0.09	1.2	ns
N2	Europe	0.04	1.4	Large
B	S.America	0.07	0.6	ns
U1	N.America	0.1	1.7	Large
U2	N.America	0.1	1.1	ns
Mean		0.08		

* ns, not statistically different from expected; large, larger than expected; small, smaller than expected.

The measures of total genetic diversity (Ht) and average diversity (Hs) within the global isolates were low (Table 4), as it also was among the Swedish isolates (Table 4). The resulting population structure (Fst) was low, but was considerably higher in the analysis with all data sets compared with the analysis exclusively with the Swedish isolates (Table 4). The Fst value among the Swedish isolates was very small and indicates that there is almost as much variation among worms within a farm as there is among farms. The population mutation rate for the nematodes ($4\,N\mu$) was low and consistent in both the global samples as well as in the Swedish samples. The gene flow among isolates (Nm) was moderate, being seven times higher among the Swedish isolates compared with the full data set (Table 4).

Estimates of the similarity of each nematode to the other members of the same isolate (re-allocation) were low both at a global and at a national scale (Table 4). Nevertheless, the test for neutral evolution (neutrality) for the 182 variable AFLP markers was rejected in almost half of the cases (Table 4), suggesting that mating is not completely random.

The weak structuring of genetic variation between and within populations is most appropriately displayed as a phylogenetic network (Fig. 1). This network illustrates clearly the weak global structure among the isolates as well as considerable within-and inter-population variation among all isolates.

The geographical test of genetic similarity among the global isolates (Table 4) did not show any distinct pattern between continental areas. On a national level, there was no geographical correlation among the 11 isolates in Sweden (Table 4).

3.2. ITS-2

The ITS amplicons produced by primers NC2 and NC13 were c. 400 bp in size, and the amplicons produced by primer NC2 and OP642 were c. 290 bp in size. Limited sequence but no consistent sequence variation was detected among the individual nematodes (data not shown). The sequence identity with the database ITS-2 sequence was 97–100% for all amplicons representing 20 representative geographical samples.

4. Discussion

The population genetics of the intestinal nematode *P. equorum* of foals was studied using AFLP. The most important result is that no genetic differentiation occurs among continental areas and that there was no correlation between genetic and geographic distance, indicating that continents are not strong barriers for the population structure of *P. equorum*. Notably, the Icelandic isolates were not detected as different from other isolates, in spite of the long-term restriction (more than 700 years ago) on horse importation and, thus, potential gene flow for the worms. Similarly, it was also surprising that the specimens obtained from the ML-naïve herd in Kentucky were no different from other isolates. The worms maintained in this herd are naïve to MLs, and it was, therefore, our expectation to reveal genetic differences in AFLP. From an historical point of view, one could speculate that the population structure of *P. equorum* was already genetically homogenous due to widespread transportation of horses within Europe (thus allowing the mixing of the parasite gene pool) before the introduction of horses to Iceland in the 9th century and the re-introduction of horses to America at the end of 15th century. Subsequently, genetic drift has dominated, as indicated by the small population mutation rate ($4\,N\mu = 0.07$) within the global *P. equorum* population, and the weak population structure has therefore remained. Thus, no locally adapted isolates have emerged during the past 600 years.

This study did not show any difference in AFLP markers of genetic adaptation to the intensive anthelmintic treatment regimens adopted on many commercial farms. However, this is in line with AFLP analysis in *Haemonchus contortus*, which showed no reduction during benzimidazole selection (Otsen et al., 2001). The genetic constitution of the *H. contortus* population changed, but did not lead to a decrease in the genetic diversity (Otsen et al., 2001). Apparently, the changes in the genome due to benzimidazole selection in *H. contortus* or the different selection pressure of anthelmintic in the *P. equorum* isolate included in this study, appear to be too small to be found by the AFLP analysis.

One isolate from Norway (code N2) had less than 10 informative peaks. The reason for this is unknown, since this isolate passed all quality checkpoints in the AFLP-procedure, such as gel electrophoresis for verifying intact DNA and gel electrophoresis to ensure that amplification has occurred in the preselective amplification. Moreover, the isolate was rerun twice from step one in the AFLP procedure and resulted in less than 10 informative peaks both times. One potential explanation could be the fact that two different *Parascaris* species are described infecting the horse. Apart from *P. equorum*, which is the species usually referred to as the equine roundworm, there is also another species named *P. univalens*. These species are morphologically similar and, thus far, the only established methods for discriminating them are karyotyping for the enumeration of chromosomes (Goday and Pimpinelli, 1986) or gel electrophoresis for evaluation of isoenzyme patterns (Bullini

Table 4
Comparison of average values of genetic diversity, population structure, population mutation rate, gene flow between field isolates, re-allocation and neutrality in *Parascaris equorum* population between Sweden and the other countries.

	Genetic diversity (Ht/Hs)	Population structure (Fst)	Population mutation rate ($4\,N\mu$)	Gene flow (Nm)	Re-allocation (%)	Neutrality (%)	Geography test
Global	0.10/0.08	0.2	0.07	1.0	41	66	$p = 0.014$
Swe	0.09/0.08	0.03	0.07	7.1	34	58	$p = 0.120$

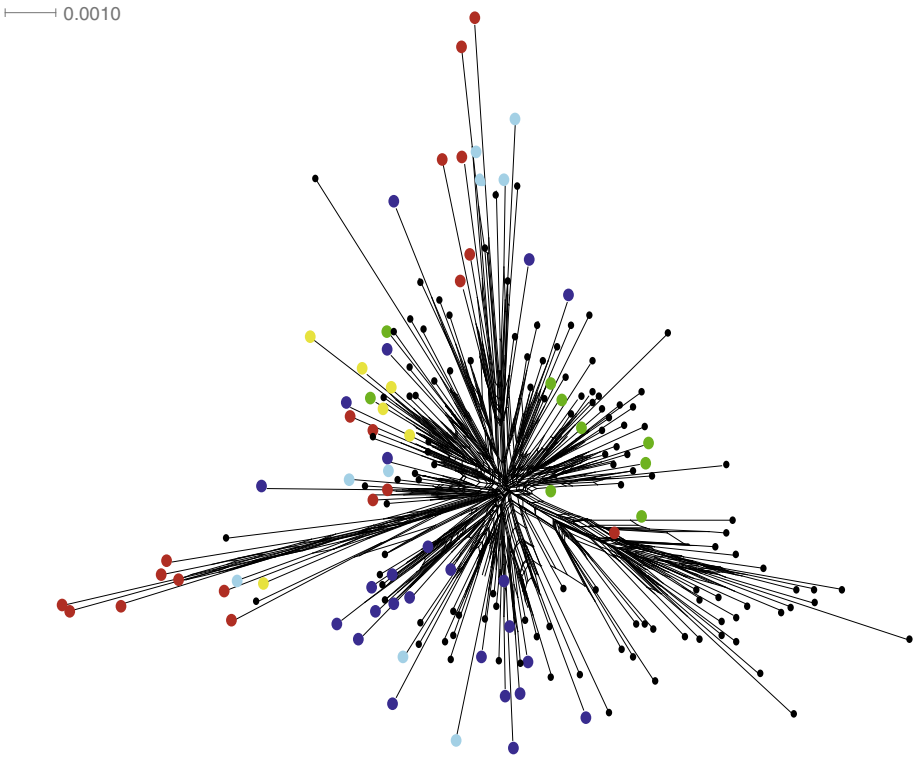

Fig. 1. Neighbour-net graph showing the genetic relationships among the worms based on the AFLP data. Each dot represents a single worm. Some of the worms are coloured: red, USA; blue, Iceland; green, Germany; light blue, Norway; yellow, Brazil. The worms from Sweden are labelled with a small black dot. There is very little common structure to the network, with each worm having a long terminal branch indicating only that it is different from the other worms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 1978). However, since the mid 1980s PCR-based sequencing approaches targeting the nuclear ribosomal DNA have proven to be useful for the species identification of nematode parasites of veterinary interest (Gasser, 1999). Using universal primers targeting ITS-2 no fixed differences among the worms in this study was identified, and all amplicons were 97–100% identical to the sequence of *P. eqourum* deposited in GenBank. If this Norwegian isolate (N2) was represented by a different *Parascaris* species, it still requires further investigation. Excluding the Norwegian isolate (N2), the AFLP generated 182 fragments, of which 92% were polymorphic in *P. equorum*. This is similar to the results from other AFLP studies in ascaroids, such as *A. suum* and *Ascaridia galli,* where 71% and 81%, respectively, of the scored fragments were polymorphic (Nejsum et al., 2005; Höglund et al., 2012). This further demonstrates the utility of AFLP for the characterisation of intraspecific polymorphism within and among nematode populations.

The AFLP results showed that the heterozygosity (genetic diversity) was low among the global isolates (Hj varied between 0.04 and 0.12), and also among the Swedish isolates (average Hj = 0.086). This finding is almost identical to the value reported from an isolate of *P. equorum* from Southern Louisiana in the USA (Hj = 0.085) using biochemical markers (Nadler, 1986). The low heterozygosity within isolates of *P. equorum* indicates the relative lack of genetic variation among the worms both at a global and at a national scale. Furthermore, the value for total genetic diversity (Ht) across all global isolates and among the Swedish isolates was also relatively low, respectively Ht = 0.10 and Ht = 0.09, as it also was for *A. suum* (Ht = 0.20) (Nejsum et al., 2005) and *A. galli* (Ht = 0.15) (Höglund et al., 2012). This information suggests that low genetic diversity is a general feature of ascaroid nematodes.

The herein observed genetic diversity among the isolates from different countries was associated with a weak population structure, indicating very weak genetic differentiation among populations. Furthermore, there were no consistent genetic patterns in the *P. equorum* population between the isolates from different continents (as shown by the network analysis), and, thus, no evidence for locally genetically adapted isolates. In contrast, high population structure and a correlation between genetic and geographic distance have been reported for the cattle lungworm, *Dictyocaulus viviparus,* (Höglund et al., 2004). In the latter case, it was suggested that host movements does not play a major role in the spread of the parasite among farms due to a high level of biosecurity and limited exchange of cattle that had been exposed to this parasite (e.g., most cattle in Sweden are moved between farms before they have been infected on pasture). Interestingly, AFLP analyses of *H. contortus* populations isolated from different continents revealed high levels of genetic differentiation between continents, implying that, at a global scale, there are strong barriers to gene flow (Troell et al., 2006). Also this pattern was explained by the poor dispersal ability of the parasite across the strong geographic barriers that differentiate the continents, along with restricted opportunities for host movement between these geographical locations.

At a national scale, the low population structure (Fst), relatively high gene flow (Nm) and low mutation rate (4 Nμ) of Swedish *P. equorum* indicate that the low genetic diversity might be a result of either a low mutation rate in the DNA, movement of worms among farms with their hosts, or a combination of both. Low population structure and high gene flow have also been reported for other nematode parasites using the AFLP approach, e.g., *A. suum* (Nejsum et al., 2005), and *N. americanus* (Gruitjer et al., 2006). These authors also found no correlation between genetic and geographic distance in any of these worms at a national scale. For example, data for *A. suum* suggest extensive gene flow between different populations due to translocation of parasites along with the movement of infected hosts.

The prevalence of *P. equorum* in foals is known to be high on many farms (e.g., Austin et al., 1990; Osterman and Christensson, 2009), and most foals, at least in Sweden, are often moved among farms. This mainly occurs from August to December, which is a critical time for the spread of infection, since it is the time of the year when foals become infected (Lindgren et al., 2008). This information, implies that there is a high risk for transferring this parasite between farms. This suggests that host movements do play a major role in the moderate gene flow, as seen in the population structure of *P. equorum* in Sweden.

5. Conclusion

In summary, it is possible to understand how a parasite population will respond to an imposed selection pressure, such as the frequent use of anthelmintics, only when the population genetic structure and patterns of gene flow of the population are known. The results from this study show that the global *P. equorum* populations appear to be genetically homogenous.

This probably reflects the movement of infected horses within Europe before the introduction of horses to Iceland and America, creating a homogenous worm population, which is in line with what has been observed for trichostrongylid parasites of ruminants in the USA (Blouin et al., 1995).

Acknowledgements

This work could not have been completed without the valuable contribution of worms from Inger Sofie Hamnes (Norwegian School of Veterinary Science, Norway), Georg von Samson Himmelstjerna (Freie Universität Berlin, Germany) and Solange Maria Gennari (University of São Paulo, Brazil). The work was financially supported by the Foundation for Equine Research, Sweden.

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