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Microsatellite analysis reveals marked genetic differentiation between *Haemonchus contortus* laboratory isolates and provides a rapid system of genetic fingerprinting

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Abstract

Many of the *Haemonchus contortus* isolates currently used for experimental work were originally derived from different regions of the world and are commonly exchanged between laboratories. In most cases, these are largely genetically uncharacterised other than the analyses conducted on specific genes of interest. We have used a panel of eight microsatellite markers to genetically characterise five different commonly used *H. contortus* isolates including MHco3 (ISE), the isolate chosen for full genome sequencing as part of the *H. contortus* genome project. There is an extremely high level of genetic differentiation between each of the isolates except the two which have a common origin, MHco1 (MOSI) and MHco3 (ISE). We have investigated the amplification of microsatellite markers from pooled DNA as a potential method for fingerprinting different isolates. Good estimates of the true allele frequencies can be made by amplification from either pooled adult DNA or bulk L3 DNA for seven out of the eight markers tested. Both single worm genotyping and bulk DNA fingerprinting revealed no genetic differentiation between adult worms in the host and larvae derived from faecal culture. Furthermore, none of the eight markers showed genetic changes when isolates were passaged through different individual hosts. Hence the microsatellite genotyping of bulk larval DNA samples provides a simple and rapid method to genetically define and monitor laboratory isolates, and to determine their relationship with particular field populations.

Keywords: Nematode; Microsatellites; *Haemonchus*; Genetic fingerprinting; Population genetics

1. Introduction

The parasitic nematode *Haemonchus contortus* is an important model for both applied and basic parasitology research, including studies on vaccine development, drug target identification and mechanisms of anthelmintic resistance, as well as being a major pathogen of small ruminants (Knox et al., 2003; Gilleard, 2006). Experimental work has been conducted on a variety of laboratory-maintained and field isolates that have been derived from many different locations throughout the world. There has been relatively little detailed genetic analysis of these populations, other than the analysis of specific genes of interest, and there are currently no simple methods to genetically define and monitor laboratory-maintained isolates or compare those with field populations.

In recent years it has become apparent that *H. contortus* is an extremely genetically diverse organism (Prichard, 2001; Troell et al., 2006). Studies have shown that mtDNA sequence diversity for *H. contortus* and other

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trichostrongyloid nematodes is very high, being up to 10 times that seen in vertebrates (Blouin et al., 1995; Anderson et al., 1998; Blouin, 1998). Nuclear loci show similarly high levels of genetic diversity; single nucleotide polymorphisms (SNPs) in coding and non-coding sequences, transposable element insertions and microsatellite loci all show very high levels of genetic diversity within populations (Beech et al., 1994; Hoekstra et al., 1997, 2000; Blackhall et al., 1998; Otsen et al., 2000a,b). It seems likely that this is a function of the extremely large effective population size of this parasite since individual hosts can contain thousands of adult worms (Urquhart et al., 1996). The extent of genetic diversity between populations, both in different hosts and in different geographical regions, is less well characterised. Until recently, in common with other trichostrongyloid nematode populations, this has been anticipated to be low (Blouin et al., 1995; Anderson et al., 1998). This view was originally proposed in a study of mtDNA sequence variation on trichostrongyloid nematode populations of cattle and sheep in North America in which *H. contortus* populations from several different geographical locations showed little genetic differentiation (Blouin et al., 1995). However, other studies aimed at developing genetic markers such as SNPs, microsatellites and transposon insertions, have suggested the existence of significant genetic differences between separate *H. contortus* populations, although this was based on genotyping only a very small number of worms (Otsen et al., 2000a,b). Although few phenotypic comparisons between *H. contortus* isolates have been conducted, differences in protease secretions based on substrate gel profiles and differences in vulval morphology have been reported (Le Jambre et al., 1995; Karanu et al., 1997). More recently, marked genetic differentiation has been shown between *H. contortus* populations derived from several continents using both mtDNA sequence and amplified fragment-length polymorphism (AFLP) analysis (Troell et al., 2006). Our understanding of the genetic structure of *H. contortus* populations is still very limited and there is a need for more detailed genetic analysis of parasite isolates being studied both in the field and in the laboratory.

Microsatellites are repetitive sequences consisting of 2–3 bp tandem repeats and are commonly used as population genetic markers in a wide range of organisms (Schlotterer, 1998). We have used microsatellite markers to undertake a detailed analysis of several *H. contortus* isolates that are commonly used in experimental studies. A profound degree of genetic differentiation is apparent between these isolates which has important implications for their experimental use. We have also exploited this genetic differentiation to develop a method of genetic fingerprinting based on amplifying microsatellite loci from bulk DNA preparations. This approach is both simple and rapid and should provide a useful routine approach for defining, monitoring and comparing both laboratory and field isolates.

2. Materials and methods

2.1. Nematode populations

We have used the following nomenclature scheme to identify the isolates used in this study. When an isolate was obtained from another laboratory and then passaged by experimental infection at the Moredun Institute, it was given a name with the prefix "MHco" followed by a unique number. We retained the name of the original isolate in brackets to allow its source to be apparent. This system allows each genetically defined isolate to be uniquely identified relative to other versions and related isolates in other laboratories. The genetically defined isolates have all been cryopreserved and archived. The five laboratory isolates of *H. contortus* characterised in this study were chosen because they are derived from isolates that are commonly used in a number of different laboratories. MHco1 (MOSI) is the laboratory isolate routinely used at the Moredun Institute and is susceptible to all the main classes of anthelmintics. It has been maintained at Moredun since the late 1950s and is thought to have been isolated in East Africa. This has been referred to as the SE isolate in the past (Hoekstra et al., 1997; Otsen et al., 2001). MHco3 (ISE) isolate is susceptible to all main classes of anthelmintic and was produced by experimental passage of the ISE isolate which was originally derived from the SE isolate by multiple generations of inbreeding from progeny of a single adult female worm (Otsen et al., 2001; Roos et al., 2004). The MHco3 (ISE) isolate has been adopted as the standard isolate for the *H. contortus* genome sequencing project (http://www.sanger.ac.uk/Projects/H_contortus/). MHco4 (WRS) was produced by experimental passage of an ivermectin-resistant isolate known as the White River Strain (WRS) that has been passaged for many years by experimental infection of sheep and was originally isolated from the field in South Africa (van Wyk and Malan, 1988). MHco10 (CAVR) was produced by experimental passage of the Chiswick Ivermectin Resistant Isolate (CAVR) that had been previously passaged for many years by experimental infection of sheep and was originally isolated from the field in Australia (Le Jambre et al., 1995). Finally, the HcSwe (VAST) was not passaged at Moredun but DNA samples were obtained from a laboratory isolate originally isolated from the field in Vastermorland, Sweden (Troell et al., 2003). Experimental infections were performed at the Moredun Institute using orally administered infections of 5000 L3s into 4- to 12-month-old Greyface cross Suffolk lambs that had been reared and maintained indoors under conditions designed to minimise the risk of infection with trichostrongyloid nematodes.

2.2. Preparation of DNA templates

All adult male *H. contortus* were identified in accordance to the Manual of Veterinary Parasitology Laboratory Techniques using male tail and spicule morphology