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Invited review

Mutation scanning-coupled tools for the analysis of genetic variation in *Taenia* and diagnosis – Status and prospects

Robin B. Gasser *, Bronwyn E. Campbell, Aaron R. Jex

Department of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia

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ABSTRACT

Cestodes of the genus *Taenia* occur as adult tapeworms in the small intestine of carnivorous definitive hosts and are transmitted to particular mammalian intermediate hosts, in which they develop as fluid-filled larvae in tissues, causing the disease cysticercosis or coenuriasis. A number of species are of medical importance and/or cause losses to the meat and livestock industry mainly due to the condemnation of infected muscle and offal. The control of taeniid cestodes relies upon epidemiological data, including the precise identification and characterization of the causative agents. Traditional, phenetic techniques have limitations for specific diagnosis. Although there has been progress in the establishment of molecular tools, there has been relatively limited application of mutation scanning approaches to species of *Taenia*. In the present article, we briefly review key genetic markers used for the specific identification of taeniids and tools for the analysis of genetic variation within and among populations and the diagnosis of taeniasis and cysticercosis/coenuriasis. We also discuss the advantages and disadvantages of selected techniques and emphasize the benefits of utilizing mutation scanning-based approaches in achieving detailed insights into the population genetics and epidemiology of *Taenia* species.

Contents

1. Introduction

Adult tapeworms of the genus *Taenia* (family Taeniidae = taeniids) infect the small intestines of carnivorous definitive hosts and are transmitted (via eggs) to mammalian intermediate hosts in which they become established as larval stages (= cysts) in specific tissues, causing the disease cysticercosis or coenuriasis (Bowman, 2008). The larval (including cysticerus and coenurus) stages of a number of *Taenia* species cause losses to the meat industry due to the condemnation of infected meat or offal, and/or are zoonotic (Jones and Pybus, 2001; Ito et al., 2003a; Carabin et al., 2005; Flisser et al., 2006; Schantz, 2006).

The specific diagnosis of *Taenia* infections in both definitive and intermediate hosts is central to the epidemiology and control of cysticercosis and coenuriasis. Traditionally, the specific identification of *Taenia* species has been based predominantly on ecological, biological and/or morphological criteria, including the features of the adult stage (such as the number, size and shape of the rostellar hooks, the presence or absence of a vaginal sphincter, the location of the genital pore along the segment margin and the number of

* Corresponding author. Tel.: +61 3 9731 2000; fax: +61 3 9731 2366.
E-mail address: robinbg@unimelb.edu.au (R.B. Gasser).

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principal lateral branches of the gravid uterus, the distribution of the testes, and the shape of the cirrus-sac and its extent relative to the longitudinal osmoregulatory canals), the morphology and type of asexual reproduction of the larval stage, and the level of host specificity in different geographical regions (Verster, 1969; Beveridge and Gregory, 1976; Edwards and Herbert, 1981; Rausch, 1994; Loos-Frank, 2000; Chervy, 2002; Rausch, 2003). However, based on these criteria, unequivocal identification is often difficult.

Biochemical and traditional molecular approaches, such as multilocus enzyme electrophoresis (MEE) and Southern blot-coupled restriction fragment length polymorphism (RFLP) analysis, have assisted in the genetic characterization and identification of *Taenia* spp. from different hosts (reviewed by McManus, 1990a,b; McManus and Bowles, 1996). Techniques based on the use of the polymerase chain reaction (PCR; Saiki et al., 1988) have found broader applicability to epidemiological and/or population genetic studies of some taeniid cestodes, particularly *Echinococcus*, mainly because their sensitivity permits the analysis of particular genes from tiny amounts of genomic DNA from fresh, frozen or even ethanol fixed parasite material (see McManus, 2006; Yamasaki et al., 2006a). Nonetheless, there has been limited molecular study of the broad range of species of *Taenia* recorded to date (Verster, 1969; Loos-Frank, 2000). In the present article, we provide an account of genetic markers employed for the identification of *Taenia* species, and tools for the analysis of genetic variation within and among species and the diagnosis of cysticercosis/coenuriasis and/or taeniasis. We also discuss the advantages and disadvantages of selected techniques and emphasize the benefits of utilizing "analytical" and "diagnostic" mutation scanning to achieve better insights into the systematics, epidemiology and population genetics of members of the genus *Taenia*.

2. Key genetic markers and methods used, and their attributes

PCR-coupled techniques, employing specific primer pairs/sets for the selective amplification of different genetic loci, followed by enzymatic cleavage, or sequencing, have been used often to identify, characterize or classify *Taenia* species or "genotypes" (Campbell et al., 2006; McManus, 2006; Varcasia et al., 2006; Yamasaki et al., 2006a). The key loci used are within mitochondrial (mt) genes or nuclear ribosomal DNA.

Mitochondrial DNA (mtDNA) has been used most commonly for the identification and delineation of closely related species because of its relatively rapid rate of evolution. This DNA is haploid, appears to be maternally inherited and does not recombine (Avise, 1994), thus simplifying sequencing and analysis. Complete or almost complete mtDNA sequences have been determined for a number of taeniid cestodes (Le et al., 2000), and they provide a rich source of genetic markers for systematic and genetic studies (Le et al., 2002). The mitochondrial genomes of the *Taenia* species sequenced to date (i.e. *T. crassiceps*, *T. solium*, *T. saginata* and the operational taxonomic unit (OTU) designated "Asian *Taenia*" or "*T. asiatica*") are similar in structure to those of species of *Echinococcus* or other metazoans (see Le et al., 2000; McManus, 2006). They contain 12 protein-coding genes, including those within the nicotinamide dehydrogenase (*nad*1–*nad*6 and *nad*4L subunits) and cytochrome *c* oxidase (*cox*1–*cox*3 subunits) complexes, and cytochrome *b* (*cob*) and adenosine triphosphatase subunit 6 (*atp*6). These genes are transcribed in the same direction, lack introns and usually abut one another or are separated by a small number of nucleotides; some genes, such as *nad*4 and *nad*4L, can overlap. In accordance with a range of other helminths (reviewed by Hu and Gasser, 2006), no *atp*8 gene is present, which contrasts the situation for other metazoan groups (Hu et al., 2004; Hu and Gasser, 2006). Two ribosomal RNA genes, the large subunit (*rrn*L) and small subunit (*rrn*S) are present as well as 22 tRNA genes. In taeniids, there are two relatively long non-coding regions (NRs) which are likely to be associated with replication and/or transcription. To date, the mitochondrial markers most commonly applied to systematic and population genetic studies of taeniid cestodes are within the *cox*1, *nad*1, *cob* and rRNA genes (reviewed by McManus, 2006; Yamasaki et al., 2006a). Some of these mitochondrial and/or selected nuclear markers have been utilized also in diagnostic systems based on specific enzymatic amplification (e.g., González et al., 2000, 2002a,b, 2004, 2006; Nunes et al., 2003; Stefanic et al., 2004; Yamasaki et al., 2004, 2005, 2006b; Harrison et al., 2005; Abuseir et al., 2006; Mathis and Deplazes, 2006; Sato et al., 2006; Geysen et al., 2007; Trachsel et al., 2007; Hernández et al., 2008; Mayta et al., 2008; Jeon et al., 2009). Recently, Nkouawa et al. (2009) evaluated a loop-mediated isothermal amplification (LAMP) assay for differential diagnosis of infections with *Taenia* species using markers in the *cox*1 or a cathepsin L-like cysteine peptidase (*clp*) gene. LAMP using *cox*1 was shown to achieve the delineation among *Taenia solium*, *Taenia saginata* and "Asian *Taenia*", whereas this was not possible using *clp*. Based on the findings, the LAMP shows considerable promise, but needs to be assessed for a broader range of *Taenia* species.

Nuclear ribosomal DNA (rDNA) also provides specific and/or genotypic ('strain') markers. The rDNA of eukaryotes, including taeniids, represents a large multigene family, consisting of tandemly arrayed sequence repeats (often tens or hundreds), usually found in clusters in specific chromosomes (see Elder and Turner, 1995). Through various molecular processes, nuclear rDNA sequences exhibit patterns of "concerted evolution", which usually lead to sequence homogenisation that is greater within a species than between/among species (e.g., Arnheim, 1983; Dover, 1989; Hancock et al., 1989; Hancock and Dover, 1990; Schlötterer and Tautz, 1994; Elder and Turner, 1995; Gasser et al., 1998a). As a consequence of "concerted evolution", rDNA can provide useful specific markers for parasites. For example, internal and external transcribed spacers (ITS and ETS, respectively) as well as the 28S gene have been shown to provide useful genetic markers for taeniids and other helminths (Zarlenga, 1991; Zarlenga et al., 1991; reviewed by Gasser, 1999, 2006; McManus, 2006), applicable to PCR-based systems. Intra-specific variation in ITS rDNA can be low; however, some parasite groups, including taeniids, tend to exhibit significant sequence and/or length heterogeneity in this region, reflecting intra-isolate, intra-individual or population variation (e.g., Bowles and McManus, 1993a; Gasser and Chilton, 1995; van Herwerden et al., 2000).

In addition, repetitive microsatellite or anonymous markers (defined by random amplification of polymorphic DNA [RAPD] analysis; Welsh and McClelland, 1990; Williams et al., 1990), have been used for the purpose of detecting genetic variation within and among populations of a particular species. Satellite DNA (Tautz, 1989, 1993) has been used increasingly for population genetic studies of *Echinococcus multilocularis* (see Bart et al., 2006; Casulli et al., 2008; Knapp et al., 2008) – which had been considered previously as notorious for its genetic homogeneity (e.g., Haag et al., 1997) – but has not yet found broad applicability to taeniids. Anonymous markers displayed electrophoretically following RAPD have been employed to infer 'genetic' variation within species (e.g., *T. solium*; see Maravilla et al., 2003, 2008; Vega et al., 2003; Campbell et al., 2006). Although RAPD can be a useful approach, because of its ability to amplify from small amounts of genomic DNA and its capacity to rapidly screen the entire genome without requiring prior DNA sequence information, there are significant problems with the reproducibility and specificity of RAPD (see Ellsworth et al., 1993; MacPherson et al., 1993; Gasser, 1999). Importantly, the display of bands on agarose or polyacrylamide gels based on size alone provides phenetic (rather than genetic) characters; these characters are anonymous until defined by