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Human tapeworms in north Vietnam

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Summary Sixty-five *Taenia* samples were collected from patients in a referral hospital in Hanoi, north Vietnam, for species identification by morphological and molecular techniques. PCR-RFLP of a mitochondrial 12S rDNA fragment, developed for this study, allowed direct differentiation between all *Taenia* spp., overcoming the disadvantages of classical morphological examination, which failed on disintegrated samples. *Taenia saginata asiatica* was the most common species (55.4%) followed by *T. saginata* (38.5%) and *T. solium* (6.2%). This report demonstrates the complexity of the epidemiology of *Taenia* spp. in Vietnam and the need for further work to reveal transmission patterns of these species.

1. Introduction

Three adult *Taenia* (sub)species are known to occur in humans, *Taenia solium*, *T. saginata* and *T. saginata asiatica*. The natural intermediate host of *T. solium* and *T. s. asiatica* is pigs, whilst metacestodes of *T. saginata* develop in cattle. *Taenia solium* is of greater medical importance than the other species as humans may also act as intermediate hosts where the tapeworm may cause neurocysticercosis (NCC) when cysticerci establish in the central nervous system (Garcia et al., 2003). Different species often co-exist in a region, making identification of *Taenia* spp. necessary to understand transmission patterns and to focus intervention programmes. Identification of the adult tapeworm in the final host is difficult because the eggs of all species are identical and because morphological characteristics of the proglottids overlap. In addition, identification based on morphological characteristics allows differentiation between *T. solium* and *T. saginata* but not between *T. saginata* and its Asian subspecies *T. s. asiatica*. Recently, molecular techniques were developed that allow conclusive identification to subspecies level (Eom et al., 2002; Yamasaki et al., 2004).

In Vietnam, a taeniasis prevalence of 0.1–5.3% has been reported based on microscopic examination of stool samples. Based on morphological characterisation, 78–80% were identified as *T. saginata* and 20–22% as *T. solium* (cited by Willingham et al., 2003). In 2001, the use of molecular techniques allowed demonstration of the first, and so far only,

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case of *T. s. asiatica* in Vietnam (De et al., 2001). Asian *Taenia* is common in Taiwan, Korea and China and has been reported in Indonesia, Malaysia, the Philippines and Thailand (González et al., 2004). Whereas on a genetic basis it is considered a subspecies of *T. saginata*, it has distinct morphological and biological characteristics.

In the present study, *Taenia* spp. collected from individuals living in north Vietnam were identified by morphological and molecular techniques. The study aimed at completing the knowledge on human cestode infections in the country.

2. Materials and methods

Tapeworm proglottids were collected from 65 patients originating from 14 provinces of north Vietnam from June 2002 to October 2003 at a referral hospital in Hanoi. Patients presented themselves to the hospital after self-diagnosis of proglottids. Samples were obtained following treatment with praziquantel (15 mg/kg body weight) and $MgSO_4$ (30 g). The proglottids were preserved in 70% ethanol until analysis. Morphological identification of proglottids was done using the Semichon's acetocarmine staining method. Differentiation between *T. saginata* and *T. solium* was based on the number of uterine branches in gravid segments and/or the presence/absence of two ovary lobes in mature segments (Morgan and Hawkins, 1949). To confirm (sub)species identification, the proglottids were subjected to PCR-RFLP based on the mitochondrial 12S rDNA gene, slightly modified from the protocol described by Rodríguez-Hidalgo et al. (2002). DNA extraction was performed according to Boom et al. (1990). A highly specific cestode primer pair (TAEnF/ITMTnR) developed for use on faeces samples was used (R. Rodríguez-Hidalgo et al., unpublished results). The specificity of the primers was checked through alignment of the 12S rDNA sequences from *T. solium*, *T. saginata* and *T. s. asiatica* with the primers. GenBank BLAST of the primers did not reveal any homology with human and non-*Taenia* DNA sequences.

To differentiate between *T. solium*, *T. saginata* and *T. s. asiatica* by RFLP, the amplified products were digested by the restriction enzymes *Dde*I and *Hinf*I (10 U/μl; Boehringer Mannheim GmbH, Indianapolis, IN, USA) in the same assay and visualised on polyacrylamide gels by SYBR® Green I (Cambrex, Rockland, ME, USA) (Rodríguez-Hidalgo et al., 2002). The use of both restriction enzymes in the same assay has been previously validated on documented samples.

Patients were informed about the risks and consequences of the cestodicidal treatment. Permission to conduct the study was obtained from the National Institute of Malariology, Parasitology and Entomology and the Vietnamese Ministry of Health.

3. Results

Morphological examination showed that 35 of the 65 samples were *T. saginata*, whilst 4 samples could be identified as *T. solium*. Species identification was not possible on 26 samples owing to advanced disintegration of the expelled proglottids.

DNA from *Taenia* could be detected in all 65 samples. Amplification of the mitochondrial 12S rDNA gene gave a product of 890 bp (data not shown). Non-specific bands were not present in the PCRs.

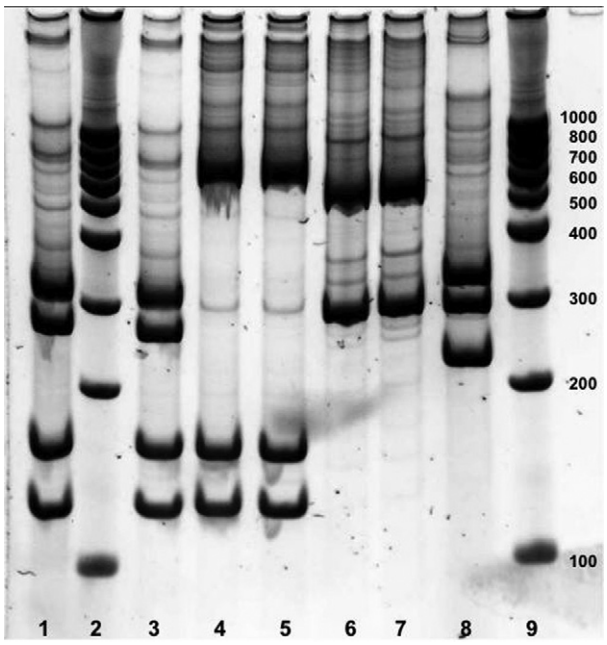

Figure 1 RFLP profiles following digestion of 12S rDNA amplicons with *Dde*I and *Hinf*I on a polyacrylamide gel. Lanes 2, 9: DNA size marker; lanes 1, 3: profile of *Taenia saginata asiatica* (VN1, VN4); lanes 4, 5: profile of *T. saginata* (VN13, VN14); lanes 6, 7: profile of *T. solium* (VNO1, VNO2); lane 8: *T. crassiceps* profile as positive control.

RFLP showed that 36 samples (55.4%) were *T. s. asiatica*, 25 samples (38.5%) were *T. saginata* and 4 samples (6.2%) were *T. solium*. The RFLP patterns are shown in Figure 1. The four *T. solium* samples agreed with the morphological identification.

4. Discussion

The present study demonstrated that a PCR-RFLP assay using primers derived from the 12S rDNA fragment is a reliable tool for (sub)species identification of *Taenia*. In contrast to morphological examination, PCR-RFLP allowed differentiation between *T. saginata* and *T. s. asiatica* and could also be performed on disintegrated samples. The PCR-RFLP in this study performed as well as a multiplex PCR based on the mitochondrial COX-1 recently established for differential diagnosis of *Taenia* spp. (Yamasaki et al., 2004).

The proportion of *T. solium* (6.2%) among the collected tapeworms was lower than expected. NCC is recognised as a common health problem in north Vietnam. Approximately 150 new clinical cases of NCC are reported each year in referral hospitals in Hanoi (Dorny et al., 2004; Willingham et al., 2003). Seroprevalence of human cysticercosis established in cross-sectional studies ranged from 0% to 5.7% (Erhart et al., 2002; Somers et al., 2006). Although the sampling in this study covered a wide geographical area in north Vietnam, we are aware that the given figure may underestimate the true proportion of *T. solium*. Indeed, the parasitic material was not collected from random surveys but from

a hospital following self-diagnosis by the tapeworm carriers. *Taenia saginata* proglottids generally leave the host through active migration, whilst expulsion of *T. solium* is mostly during defecation. Therefore, self-diagnosis is likely to overestimate the occurrence of *T. saginata* compared with *T. solium*. Community-based sampling may provide a better estimate of *Taenia* spp. distribution in north Vietnam. On the other hand, these results support the findings of Rodríguez-Hidalgo et al. (2003) that tapeworms collected in NCC-endemic areas should not automatically be considered as *T. solium*.

The high proportion of *T. s. asiatica* among the tapeworms was unexpected because so far only one case of this subspecies has been reported in Vietnam (De et al., 2001) and the larvae of *T. s. asiatica* have never been reported in Vietnamese pigs. Transmission of *T. s. asiatica* occurs through consumption of undercooked liver and visceral organs (Eom and Rim, 2001), which is uncommon in Vietnam in contrast to the tradition of eating dishes containing raw or undercooked meat (Somers et al., 2006; Willingham et al., 2003).

The results of this study raise some questions about the epidemiology of the taeniasis/cysticercosis complex in Vietnam. Realising the limitations of this preliminary study, we recommend randomised prevalence and identification surveys on taeniasis and cysticercosis in several regions in Vietnam based on recently developed molecular tools.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

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