Short communication

Characterization of Internal Transcribed Spacer of Nuclear Ribosomal DNA of *Trichuris ovis* from Goat in Hunan Province, China

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Abstract

Trichuris ovis parasitizes the caeca and colon of hosts, causing trichuriasis. Despite this parasite being of animal health significance, causing considerable socio-economic losses globally, little is known of the molecular characteristics of *T. ovis*. In the present study, the entire first and second internal transcribed spacer (ITS-1 and ITS-2) regions of nuclear ribosomal DNA (rDNA) of *T. ovis* from goats in Hunan province, China were amplified by polymerase chain reaction (PCR), and the representative amplicons were subjected to sequencing in order to estimate sequence variation. The ITS rDNA sequences for the *T. ovis* samples were 1289-1303 bp in length. Sequence analysis revealed that the ITS-1 rDNA, 5.8S and ITS-2 rDNA of these whipworms were 725-734 bp, 163 bp and 401-406 bp in size, respectively. Sequence variation in ITS rDNA within and among *T. ovis* was also examined. Excluding nucleotide variations in the simple sequence repeats, the intra-species sequence variation in the ITS-1 was 0-2.7%, and ITS-2 was 0-4.1%. The inter-species sequence variation among the *T. ovis* and other whipworms were more than 45.7% for ITS-1 rDNA and 51.3% for ITS-2 rDNA. These results demonstrated that the ITS rDNA sequences provide additional genetic markers for the identification and differentiation of the *T. ovis*. These data should be useful for studying the epidemiology, population genetics of *T. ovis*, as well as for the diagnosis of trichuriasis in sheep and goats.

Keywords: China, internal transcribed spacer (ITS), sequence variation, *Trichuris ovis*

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Introduction

Nematodes of the genus *Trichuris* are soil-transmitted parasites, which are located in cecum of their hosts (Bethony et al. 2006). Its common name "whipworm" is derived from adult worm of whip handle-like posterior part. Because of its simple life cycle, whipworms have a broad range of host, including human (*T. trichuris*), pigs (*T. suis*), dogs (*T. vulpis*), rodents (*T. muis*), sheep, goats, cattles (*T. ovis*, *T. globulosa*, *T. skrjabini* and *T. discolor*) (Hansen et al. 2013). More importantly, trichuriasis caused a global burden of about 600,000 disability-adjusted life-years which is a serious public health problem in some countries (Pullan et al. 2014). Whipworms also infect sheep and goats (*T. ovis*), causing clinical signs such as anorexia and bloody diarrhoea (Wilmsen et al. 2014).

Sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) have been widely used as useful genetic markers for the identification and differentiation of many nematodes (Mejía-Madrid and Aguirre-Macedo, 2011; Lin et al. 2012; Liu et al. 2014). For whipworms, previous studies have indicated that sequence differences between *T. trichiura* and *T. suis* specimens were significantly higher in ITS rDNA, suggested that *T. trichiura* and *T. suis* is different nematode species (Cutillas et al. 2009; Nissen et al. 2012; Liu et al. 2014). These studies showed that ITS rDNA is useful genetic markers for the identification and differentiation of *Trichuris* species from different animals (Cutillas et al. 2009; Nissen et al. 2012; Liu et al. 2014). Recently, Liu and colleagues based on mitochondrial (mt) and ITS rDNA sequences support a new *Trichuris* species in the endangered françois' leaf-monkey (Liu et al. 2013). However, there is yet been characterized the ITS rDNA sequences of *T. ovis*.

Nine species, namely *T. ovis*, *T. discolor*, *T. skrjabini*, *T. concolor*, *T. gazellae*, *T. globulosa*, *T. indicus*, *T. wuweiensis* and *T. longispiculus*, have been identified in sheep and goats worldwide. In China, however, *T. ovis* is considered as predominant species in sheep and goats. Therefore, the objectives of the present study were to characterize *T. ovis* from Boer goats in China by their ITS rDNA sequences and to study sequence variations within *T. ovis*.

Materials and Methods

Adult specimens of *Trichuris* (n=16) were obtained from naturally infected goats that located in different regions of Hunan province, China (Table 1). Adult worms from each animal were washed separately in physiological saline, identified morphologically (Cutillas et al. 1995), then fixed in 70% (v/v) ethanol and stored at -20 °C before DNA extraction. Individual worms were treated with SDS/proteinase K (Wizard™ DNA Clean-Up, Promega). The total genomic DNA was extracted according to the manufacturer's recommendations. Their sample codes, geographical origins and hosts of ITS rDNA are listed in Table 1.

Table 1 Sample codes, geographical origins and hosts of *Trichuris ovis* samples used in the present study.

Sample codes	Geographical origin	Hosts
CZA	Chenzhou	Goat
CZB	Chenzhou	Goat
CZC	Chenzhou	Goat
CZD	Chenzhou	Goat
LYA	Liuyang	Goat
LYB	Liuyang	Goat
LYC	Liuyang	Goat
NYA	Ningyuan	Goat
NYB	Ningyuan	Goat
NYC	Ningyuan	Goat
XPA	Xupu	Goat
MYA	Mayang	Goat
MYB	Mayang	Goat
CSA	Changsha	Goat
CSB	Changsha	Goat
CSC	Changsha	Goat

The full ITS rDNA region was amplified by polymerase chain reaction (PCR) from individual DNA using primers NC5 (forward; 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2 (5′-TTAGTTTCTTTTCCTCCGCT-3′) described preciously (Zhu et al. 2000). PCR reactions (25 µL) were performed in 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 4 mM $MgCl_2$, 200 µM each of dNTP, 50 pmol of each primer and 2 U *Taq* polymerase (Takara) in a thermocycler (Biometra) under the following conditions: initial denaturation at 94 °C for 5 min, then 37 cycles of 94 °C for 30 s (denaturation); 54.8 °C for 30

s (annealing) 72 °C for 70 s (extension), followed by a final extension at 72 °C for 5 min. One microlitre (5~10 ng) of genomic DNA was added to each PCR reaction. Five microlitres of each amplicon was examined by 0.8% (w/v) agarose gel electrophoresis to validate amplification efficiency. Positive amplicons were sent to Sangon Company (Shanghai, China) for sequencing from both directions.

The sequences representing the 5′ end and 3′ end of the ITS-1 and ITS-2 rDNA of *T. ovis* were determined by comparison with those of *T. ovis* derived from roe deer in Czech Republic (GenBank accession number JX218218). Sequences of the ITS-1 and ITS-2 rDNA of different samples were aligned using the computer program Clustal X 1.81 (Thompson et al. 1997) and modified by eye. In addition, the DNAStar 5.0 software (Burland, 2000) was used to analyze sequence similarity of different *Trichuris* species.

Results and Discussion

One amplicon of 16 individual *Trichuris* samples appeared as single band with approximately 1300 bp in length (not shown). The ITS rDNA sequences for the *T. ovis* samples were 1289-1303 bp in length. Sequence analysis revealed that the ITS-1 rDNA, 5.8S rDNA and ITS-2 rDNA of these whipworms were 725-734 bp, 163 bp and 401-406 bp in size, respectively. Sequence variation in ITS rDNA within and among *T. ovis* was examined. Excluding nucleotide variations in the simple sequence repeats, the intra-species sequence variation in the ITS-1 rDNA was 0-2.7%, and ITS-2 rDNA was 0-4.1%. Sequence variations in ITS-1 were 0-2.5% for *T. ovis* from goats and 0-1.3% for samples from sheep. Sequence variations in ITS-2 were 0-4.1% for *T. ovis* goats and 0-3.9% for samples from sheep. The inter-species sequence differences among the *T. ovis* and other whipworms (*T. suis* and *T. trichiura*) were more than 45.7% for ITS-1 rDNA and 51.3% for ITS-2 rDNA, which were consistent with previous study (Nissen et al. 2012). Recently, Liu et al have determined the mt genomes of *T. trichiura* and *T. suis*, and found a significant difference between *trichiura* and *T. suis* (32.9%) (Liu et al. 2012a). In addition, Liu et al have also recently determined the mt genomes of *T. ovis* and *T. discolor*, and detected a substantial difference between them (21.68%) (Liu et al. 2012b). Many studies form ITS rDNA sequences (Cutillas et al. 2009; Liu et al. 2014) has demonstrated that *Trichuris* from different hosts were distinct whipworm species, so it is more likely that goats in China has been infected with more than one species of whipworms (Such as *T. ovis*, *T. globulosa*, *T. skrjabini* and *T. discolor*). Therefore, more *Trichuris* samples from much broader geographical localities of the world should be employed in further studies to better illuminate the taxonomy and population genetic structures of *Trichuris*.

Many nematodes are parasites of humans, animals and plants, and cause significant diseases and major socio-economic impact on a global scale (Cantacessi et al. 2012). Whipworm are responsible for neglected tropical diseases (NTDs) of humans in developing countries. In addition, whipworms also infect other animal hosts, including pigs, dogs and sheep (Hansen et al. 2013). For many years, whipworms (*Trichuris* spp.) have been described with a relatively narrow range of both morphological and biometrical features. Moreover, there has been insufficient discrimination between congeners (or closely related species) (Robles, 2011). Due to the limitations of morphological approaches, various molecular methods have been used widely for the identification and differentiation of *Trichuris* species. Although mt gene is reliable for identifying related species in many parasites (Lin et al. 2012; Li et al. 2013; Liang et al. 2013), ITS rDNA has previously been described as a useful marker for the identification and differentiation of *Trichuris* species (Cutillas et al. 2009; Liu et al. 2014). Therefore, the present study used ITS rDNA to study the genetic variation in *T. ovis*. At least nine *Trichuris* species, including *T. ovis*, *T. discolor*, *T. skrjabini*, *T. concolor*, *T. gazellae*, *T. globulosa*, *T. indicus*, *T. wuweiensis* and *T. longispiculu* could be identified by their ITS rDNA sequences. So, we believe it is still necessary to carry out more experimental research to provide additional evidence for the hypothesis. Future studies could (i) employ other more variable molecular markers and larger number of samples to confirm the phylogenetic relationships among *Trichuris* taxa, (ii) detailed morphological re-description of these *Trichuris* nematodes, (iii) characterize the ITS rDNA of these *Trichuris* from different hosts.

In conclusion, the present study characterized *T. ovis* from goats in Hunan province, China by their ITS rDNA sequences. Sequence comparison revealed that the inter-specific sequence differences among the two *Trichuris* species were significantly higher than intra-specific sequence variations within *T. ovis*.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No. 31372431).and Outstanding Young Scientific Research Project of the Education Department of Hunan Province (Grant No.14B092).

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บทคัดย่อ

การศึกษาคุณสมบัติของตำแหน่งภายในกลุ่มยีนของนิวเคลียร์ไรโบโซมอลดีเอ็นเอของ *Trichuris ovis* จากแพะในมณฑลหูหนานประเทศจีน

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Trichuris ovis อาศัยอยู่ภายในกระเปาะลำไส้ใหญ่และลำไส้ใหญ่ของโฮสต์ซึ่งเป็นสาเหตุของโรคพยาธิแส้ม้า แม้ว่าพยาธิชนิดนี้จะสำคัญต่อสุขภาพของสัตว์ ซึ่งส่งผลต่อความสูญเสียทางสังคมและเศรษฐกิจไปทั่วโลก แต่ยังเข้าใจคุณสมบัติทางโมเลกุลของพยาธิชนิดนี้เพียงเล็กน้อย ในการศึกษาครั้งนี้ได้ใช้ปฏิกิริยาลูกโซ่เพื่อเพิ่มจำนวนของ นิวเคลียร์ไรโบโซมอลดีเด็นเอ (*rDNA*) ที่ตำแหน่ง *ITS-1* และ *ITS-2* ของ *T. ovis* ที่ได้จากแพะในมณฑลหูหนานประเทศจีนด้วย และนำดีเอ็นเอสายใหม่มาวิเคราะห์หาลำดับเบสเพื่อประเมินความหลากหลายของลำดับเบสดีเอ็นเอ พบว่าดีเอ็นเอของ *ITS rDNA* จากตัวอย่าง *T. ovis* มีความยาว *1289-1303 bp* จากการวิเคราะห์หาลำดับเบสแสดงว่า *ITS-1 rDNA, 5.8S* และ *ITS-2 rDNA* ของพยาธิแส้ม้ามีความยาว *725-734 bp, 163 bp* และ *401-406 bp* ตามลำดับ รวมถึงการทดสอบความหลากหลายของลำดับเบสดีเอ็นเอใน *ITS rDNA* ทั้งภายในและระหว่างสายพันธุ์ของ *T. ovis* เมื่อตัดความหลากหลายของลำดับเบสดีเอ็นเอในตำแหน่งที่มีลำดับเบสซ้ำกันออกไป พบว่าความหลากหลายของลำดับเบสดีเอ็นเอภายในสายพันธุ์ที่ตำแหน่ง *ITS-1* คือ *0-2.7%* และ *ITS-2* คือ *0-4.1%* สำหรับหลากหลายของลำดับเบสดีเอ็นเอระหว่างสายพันธุ์ของ *T. ovis* และพยาธิแส้ม้าสายพันธุ์อื่นที่ตำแหน่ง *ITS-1 rDNA* มีมากกว่า *45.7%* และที่ตำแหน่ง *ITS-2 rDNA* มีมากกว่า *51.3%* ผลการศึกษาแสดงว่าลำดับเบสของ *ITS rDNA* เหมาะสมที่จะใช้เป็นตัวชี้วัดทางพันธุกรรมเพื่อระบุและจำแนกชนิดของ *T. ovis* ข้อมูลเหล่านี้น่าจะเป็นประโยชน์เพื่อการศึกษาทางระบาดวิทยา ประชากรพันธุกรรมของ *T. ovis* รวมถึงเพื่อการวินิจฉัยโรคพยาธิแส้ม้าในแกะและแพะ

คำสำคัญ: ประเทศจีน Internal transcribed spacer Sequence variation *Trichuris ovis*,

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