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▣ BRIEF COMMUNICATION

Prevalence and Phylogenetic Analyses of *Trichuris suis* in Pigs in Hunan Province, Subtropical China

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Abstract: *Trichuris suis* infection in pigs is ubiquitous in intensive and extensive farms, which causes potential threat to human health. The objective of this research was to investigate the prevalence of *T. suis* in pigs in Hunan province. Total 2,267 fresh fecal samples distributed in 28 pig farms from 7 different administrative regions (Hunan province) were evaluated for the existence of *T. suis* eggs using saturated NaCl floating method. The average infection rate of *T. suis* in pigs was 8.91% in Hunan province. To determine genetic variation of the gained *T. suis* isolates in the present study, the internal transcribed spacer (ITS) regions from nuclear ribosomal DNA (rDNA) of 7 *T. suis* isolates were cloned and analyzed. Nucleotide diversities were 1.0-3.5% and 0-3.8% for ITS-1 and ITS-2, respectively. Phylogenetic analyses indicated that all isolates collected in the present study and *T. suis* available in Genbank generated a monophyletic clade. The present investigation revealed high infection rates of *T. suis* in pigs in Hunan province, which shed light on making effective measures to prevent and control *T. suis* infection in pigs in Hunan province.

Key words: *Trichuris suis*, prevalence, pig, internal transcribed spacer (ITS), Hunan province

Trichuris suis, belonging to genera *Trichuris*, which exists in various mammals, including domestic pigs, wild pigs, humans as well as other primates, is an intestinal parasite which can lead to Trichuriosis [1]. Pigs obtain *T. suis* after ingestion of raw water, fodder, and vegetables contaminated by cysts accidentally. The most common clinical symptoms include magersucht, anemia, diarrhea, mucosal hemorrhage and hydropsy [2]. *T. suis* is identified as one of the most widespread pathogenic parasites, leading to decreased feed efficiency of pigs, which brings serious economic losses to pig industries globally [3,4]. Pig-breeding has been a traditional and preponderant industry in Hunan province, the productive value of which has been exceeding grain plant since 1997. The same data also shows that pig-breeding industry already takes a crucial part in livestock husbandry of Hunan province that directly closes to the agricultural economy and lives of people. However, few reports are available associated with the prevalence and molecular characteristics of *T. suis* in pigs in Hunan province. The present research was conducted to identify the prevalence and epidemic characteristics of *T. suis* infection from intensive and extensive farms in Hunan province of subtropical China. Moreover, the phylogenetic tree based on ITS-2 sequences was reconstructed to ascertain evolutionary relationships between *T. suis* and other whipworm species.

The present research was conducted from March 2016 to November 2016 (Table 1). A total of 2,267 pigs aged from 2 weeks to 3 years were selected randomly from representative intensive farms (the number of reared pigs > 300) (n = 7) and extensive farms (the number of reared pigs ≤ 300) (n = 21) from 7 different administrative regions in Hunan province (Fig. 1). 20.0% and 10.0% of pigs were randomly selected from intensive and extensive farms according to the different growth stages, respectively, and fecal samples were collected individually, labeled, frozen with ice packs, and transported to the Department of Parasitology, College of Veterinary Medicine, Hunan Agricultural University for further processing. Each fecal sample (approximately 10-20 g) was examined for *T. suis* eggs using saturated NaCl floating method. The number

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Table 1. Prevalence of *T. suis* infection in pigs from different seasons and regions in Hunan province

Factor	Category	No. tested	No. positive	Prevalence (%)	Average intensity	χ^2 test[d]	*P*-value
Season	Spring[a]	852	53	6.22	1,400	54.2023	<0.0001
	Summer[b]	583	46	7.89	1,600		
	Autumn[c]	832	103	12.38	2,400		
Distinct	Changsha	324	38	11.37	1,900	20.8214	0.002
	Loudi	362	32	8.84	1,800		
	Xiangtan	347	47	13.54	2,300		
	Changde	305	21	6.89	1,400		
	Huaihua	294	25	8.50	2,000		
	Hengyang	315	16	5.08	1,100		
	Zhangjiajie	320	23	7.19	1,500		
Total		2,267	202	8.91	1,700		

[a]From March to May.
[b]From June to August.
[c]From September to November.
[d]Qualitative data between different groups.

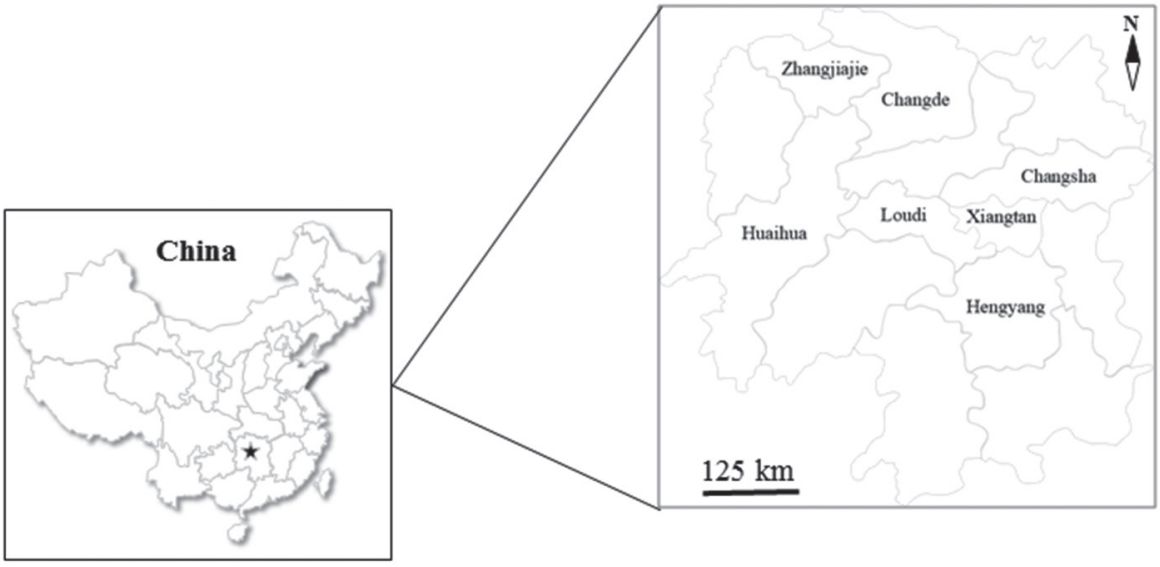

Fig. 1. Seven different administrative regions where a total of 2,267 fecal samples were collected to examine for *T. suis* infection in pigs were labeled in the map of Hunan province (Pentagram), China.

of eggs was counted from per gram fecal sample according to the McMaster method, and each detected egg was distinguished with its morphological features using the light microscope. The caeca of 7 *T. suis* infected pigs from different regions in Hunan province were collected and evaluated for the presence of adult *T. suis*. Each adult sample was detached and washed thoroughly in physiological saline, labeled and stored at -20˚C in 70% ethanol till it would be used.

The data of differences in the infection rates of *T. suis* in pigs of different seasons, geographical locations, growth periods, and raising systems in Hunan province were statistically analysed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA), with the value of $P<0.05$ being considered to be statistically significant. In addition, χ^2 test was conducted to compare the qualitative data between different groups.

Total genomic DNA of 7 adult *T. suis* isolated from the caeca of the tested pig was extracted using TIANamp Genomic DNA Kit (Beijing, China) following the instructions. The full ITS gene was amplified by PCR according to the report of Liu et al. [5]. Negative sample (no DNA) was used as the control, and then positive PCR products were purified and sequenced in duplicate.

The 3′ end and 5′ end of the sequence representing ITS-1 and ITS-2 regions were determined by comparison with these of *T. suis* isolates from pigs [5,6]. Sequences of ITS-1 and ITS-2 representing different isolates and reference sequences avail-

Table 2. Information of internal transcribed spacers (ITS) gene sequences for the *Trichuris* species employed in the present research

Species	Location	Sample codes	Genbank™ accession No.
Trichuris suis	Changde	HN-CD	MG656438
	Changsha	HN-CS	MG656439
	Huaihua	HN-HH	MG656440
	Hengyang	HN-HY	MG656441
	Loudi	HN-LD	MG656442
	Xiangtan	HN-XT	MG656443
	Zhangjiajie	HN-ZJJ	MG656444
	Hunan (Changsha)	-	AM992999
	Hunan (Miluo)	-	AM993003
	Hunan (Yiyang)	-	AM993005
	Guangdong (Zhanjiang)	-	AM993016
Trichuris trichiura	China	-	AM992990
	China	-	AM992996
Trichuris muris	Spain	-	FN543201
	Spain	-	AJ299407
Trichuris discolor	Spain	-	HE608848
Trichuris ovis	Spain (Andalucia)	-	AJ238220
Trichuris leporis	Spain (Andalucia)	-	AJ251321
Trichuris skrjabini	Unknown	-	AJ489248
Trichuris vulpis	Unknown	-	AM234616

able in the Genbank (Table 2) were aligned using Clustal W in MEGA5 [7]. Nucleotide variations between different samples were determined using the Megalign procedure in DNAStar 5.0 software [8]. Evolutionary relationship was evaluated based on ITS-2 rDNA sequences available in this study using maximum likelihood (ML) method in MEGA5 [7], and the stability of tree was calculated based on 1,000 bootstrap replicates. Meanwhile, other whipworm species were employed as in-groups, with *Ascaris suum* (Genbank accession number AB571302) as out-group.

A total of 2,267 fecal samples were collected in Hunan province to examine the existence of *T. suis* eggs in pigs. The average infection rate (8.91%) of *T. suis* was detected in Hunan province, which is higher than Guangdong province in China (5.07%) [4], Cambodia (6.6%) [9] and India (6.6%) [10], but lower than these reported in Chongqing province (10.13%) [11] and Tibet Autonomous Region in China (15.2%) [12]. These data revealed that *T. suis* infection is widely spread in Aisa, mainly in developing countries or regions. Furthermore, different infection rate of *T. suis* in pigs are mainly on account of distinctions in geographical locations, animal welfare, and climate characteristic. The prevalence of *T. suis* in pigs ranged from 5.08% to 13.54% from different geographical regions in Hunan province (Table 1), and the differences were statistically significant ($P<0.05$), while the highest prevalence was in Xiangtan city and the lowest prevalence was in Hengyang city.

Seasonal prevalence of *T. suis* infection in pigs ranged from 6.22% to 12.38% ($P<0.0001$). The highest prevalence was in autumn (12.38%), followed by summer (7.98%), and the lowest prevalence was in spring (6.22%) (Table 1). It was speculated that the wet and warm environment from April to July in Hunan province is one of the significant factors for the survival and multiplication of *T. suis*, increasing infection rate in pigs. As the pre-patent period of *T. suis* in the host is nearly 50 days (almost 2 month), which results in higher detection rates of *T. suis* in summer and autumn, similar with the other intestinal parasite (*Balantidium coli*) in pigs [13,14].

Infection rates of *T. suis* from pigs of different growth stages varied from 3.78% to 15.40% ($P<0.0001$), higher in the fatteners group (aged from 2 to 5 months) (15.40%) and breeding boars group (aged from 5 to 36 months) (9.75%), followed by growers group (aged from 1 to 2 months) (8.86%) and breeding sows group (less than 2 weeks) (8.62%) , lowest in weaners group (aged from 2 weeks to 1 month) (3.87%). Furthermore, 7.54% (85/1,127) and 10.26% (117/1,140) fecal samples were confirmed for the existence of *T. suis* eggs from intensive and extensive pig farms ($P=0.0278$), respectively. Obviously, prevalence of *T. suis* from extensive pig farms was more serious than those from intensive pig farms, which might attribute to its poor facilities and unscientific feeding system of extensive pig farms compared with those of intensive pig farms. 2.89% (17/242) of weaners group, 6.15% (22/358) of growers group, 14.58% (35/240) of fatteners group, 6.45% (6/93) of breeding boars group and 7.73% (15/194) of breeding sows group were demonstrated to be infected with *T. suis* from intensive pig farms. Altogether, of 5 groups from extensive pig farms, the infection rate was 4.46% (14/314), 12.01% (37/308), 16.24% (38/234), 20.0% (6/30) and 8.66% (22/254) ($P<0.0001$), respectively (Table 3). The prevalence of *T. suis* increased with the growth stage of pigs, showing that age is an important factor in *T. suis* prevalence in pigs.

Seven ITS rDNA sequences of 7 isolated adults *T. suis* were successfully amplified with a fragment length of 1,444-1,450 bp. The complete sequences of the ITS-1 rDNA (687-692 bp), 5.8S rDNA (154 bp) and ITS-2 rDNA (599-606 bp) were analyzed. The deletion/ insertion of nucleotides generated the differences of the length of ITS-1 and ITS-2 rDNA sequence. The GC content of ITS rDNA sequences were 61.07-61.30%. Nucleotide variations of ITS-1 and ITS-2 rDNA sequences were 0.6-2.1% and 0-3.2%. The result showed a higher genetic variabil-

Table 3. Prevalence of *T.suis* infection in pigs from different growth period groups and different raising systems in Hunan province, China

Raising system	Pig category	No. tested	No. positive	Prevalence (%)	Average intensity	χ^2 test[c]	*P*-value
I[a]	Weaners	242	7	2.89	800	25.7362	<0.0001
	Growers	358	22	6.15	1,000		
	Fatteners	240	35	14.58	2,100		
	Breeding boars	93	6	6.45	1,000		
	Breeding sows	194	15	7.73	1,300		
II[b]	Weaners	314	14	4.46	800	25.3812	<0.0001
	Growers	308	37	12.01	1,400		
	Fatteners	234	38	16.24	2,200		
	Breeding boars	30	6	20.0	2,700		
	Breeding sows	254	22	8.66	900		
I	Subtotal	1,127	85	7.54	1,300	4.8399	0.0278
II	Subtotal	1,140	117	10.26	1,700		
	Total	2,267	202	8.91	1,700		

[a]Intensive pig farms (the number of reared pigs > 300).
[b]Extensive pig farms (the number of reared pigs ≤ 300).
[c]Qualitative data between different groups.

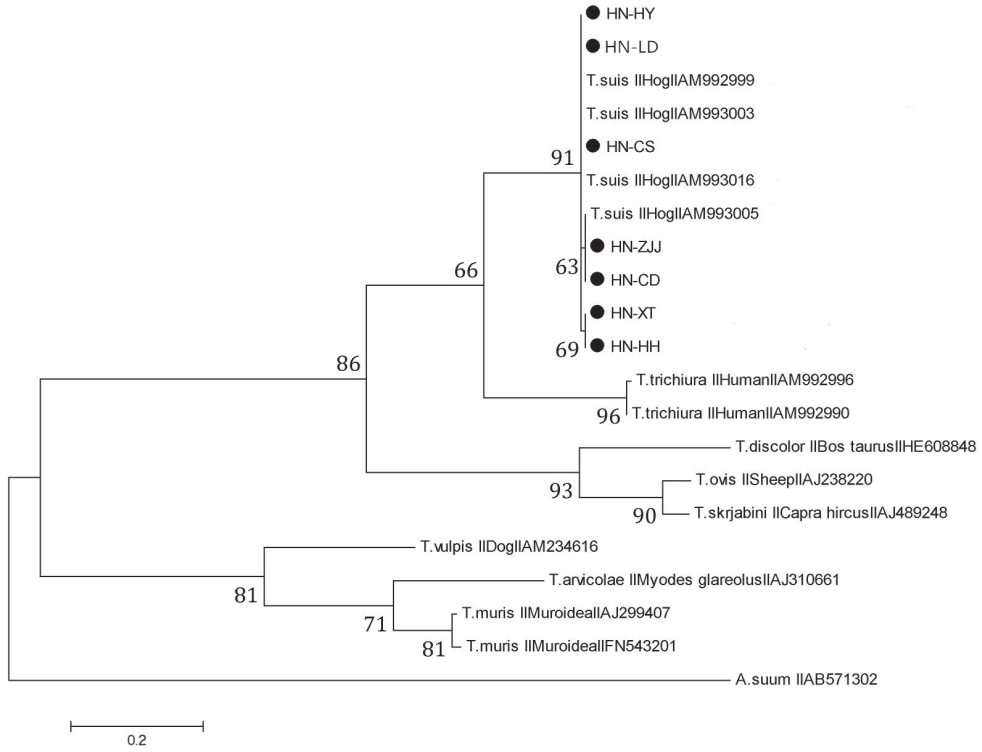

Fig. 2. Phylogenetic tree was generated using maximum likelihood (ML) method to show genetic relationships among examined *Trichuris* species based on ITS-2 sequences. *Ascaris suum* (AB571302) was employed as the out-group.

ity than that of previous reports [5], while growing evidence indicated that nucleotide variation in ITS-2 rDNA sequence in *T. suis* was higher than other nematodes [5,15].

In order to ascertain the evolutionary relationships between *T. suis* and other whipworm species, phylogenetic tree based on ITS-2 rDNA sequence was reconstructed using ML method in MEGA5. All isolates collected in the present study and *T. suis* available in Genbank generated a monophyletic clade, and these isolates were randomly distributed (Fig. 2), which indicated that in the process of evolution, there was no existence of geographical isolates of *T. suis* [16]. The result of this phylogenetic tree supported that *Trichuris* in pigs were more similar to *Trichuris* in humans compared with other *Trichuris* species, and obvious inter-species nucleotide variations in ITS-1 and

ITS-2 rDNA sequences among those 2 *Trichuris* species were observed [5,15]. It was suggested that *Trichuris* gained from pigs and humans represented 2 discrepant populations, consistent with the results of the previous studies [15,17]. The results revealed that *Trichuris* from different hosts belonged to 3 distinct groups, in agreement with previous study of evolutionary relationships in *T. suis* and other whipworm species based on nuclear and mitochondrial genes [18,19].

Historically, *T. suis* has universally been considered as an unimportant intestinal parasite in pigs owing to limited reports available in China. In the present study, high infection rate (8.91%) of *T. suis* in pigs was detected in Hunan province. The prevalence may be mainly related to geographical regions, seasons, raising systems and growth stages of pigs. Therefore, effective integrated strategies and measures should be taken to control *T. suis* infection in pigs. For instance, pig farmers should make regular anti-*T. suis* treatment project and improve feeding condition and management for pigs. Phylogenetic analysis indicated that all *T. suis* isolates collected in this study and those in Genbank database generated a monophyletic clade. This is the first exhaustive, comprehensive survey of *T. suis* infection from intensive and extensive farms in Hunan province, subtropical China.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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