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PROTOZOOLOGY - SHORT COMMUNICATION

First report of *Giardia duodenalis* genotypes in Zangxiang pigs from China

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Abstract

Giardia duodenalis is an important zoonotic intestinal protozoan of animals and humans. We collected 450 faecal specimens from four age groups (pre-weaned piglets, weaned piglets, juveniles, adults) of Zangxiang pigs from Shaanxi and Qinghai provinces, to investigate the prevalence and genetic diversity of *G. duodenalis* at the *β-giardin* (*bg*), *triosephosphate isomerase* (*tpi*) and *glutamate dehydrogenase* (*gdh*) loci using nested PCRs in the present study. A total of 28 faecal samples were positive for presence of *G. duodenalis*, with an overall prevalence of 6.2%. *Giardia duodenalis* was detected in pigs from all age groups and in both investigated provinces. Significant differences ($P < 0.0001$) in prevalence were observed among the four age groups with prevalence decreasing with age. Sequence analysis indicated existence of genetic diversity of *G. duodenalis* isolates from Zangxiang pigs, with 4, 2 and 4 haplotypes at the *bg*, *tpi* and *gdh* loci, respectively. Two assemblages were identified, including the zoonotic assemblage B and assemblage E, with the latter as the predominant assemblage found in both locations and all age groups except adults. The present study expanded the host range of *G. duodenalis* and provided fundamental data for controlling *G. duodenalis* infection in Zangxiang pigs.

Keywords *Giardia duodenalis* · Zangxiang pigs · Prevalence · Assemblage · China

Introduction

Giardia duodenalis (syn. *Giardia intestinalis* and *Giardia lamblia*), an important zoonotic protozoan, can inhabit in the intestines of a range of animal hosts and humans (Feng and Xiao 2011). The infection rate of *G. duodenalis* ranges from 0.9 to 93.1% in livestock, and from 3 to 66.7% in humans (Asher et al. 2014; Torres-Romero et al. 2014; Ramírez et al. 2015; Azcona-Gutiérrez et al. 2017; Omarova et al. 2018; Yin et al. 2018). The faecal-oral route was reported as one of the important transmission pathways for *G. duodenalis*, and the cysts excreted from hosts with faeces would be potential infection sources of other individuals (Feng and Xiao 2011). Although asymptomatic and self-limited infections are common, *G. duodenalis* can lead to clinical symptoms including diarrhoea, bloat and malabsorption, especially in young or immunocompromised hosts (e.g. patients with acquired immune deficiency syndrome) (Hunter and Thompson 2005; Feng and Xiao 2011; Song et al. 2016).

Zangxiang pig is an important economic pig breed mainly distributed in the Qinghai-Tibetan Plateau and other alpine areas at altitudes of 2800–3500 m (Li 2018). This breed has a strong resistance to harsh environments and its meat is rich in proteins, essential amino acids (EAAs) and fatty acids (EFAs), and abundant minerals (Song et al. 2011). Recently, artificial breeding for this breed was introduced into more than 15 provinces (Song et al. 2011; Yang et al. 2014; Xu and Zhang 2015). Due to changes in habitats and food sources, several pathogens unknown for the original breed in Qinghai-Tibetan Plateau, including Pseudorabies virus (PRV), Porcine circovirus (PCV), *Actinobacillus pleuropneumoniae*, *Trichuris* spp. and *Cysticercus cellulosae*, were introduced into the new Zangxiang pig populations (Tang 2012; Bian 2013; Jiang et al. 2014). No published articles on *G. duodenalis* infection are yet available in Zangxiang pigs,

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but this parasite is present in domestic pigs and wild boars from other locations or countries, with prevalences up to 66.4% (Farzan et al. 2011; Siwila and Mwape 2012; Fava et al. 2013; Minetti et al. 2014; Schär et al. 2014; Petersen et al. 2015; Rodriguez-Rivera et al. 2016; Vasco et al. 2016; Wang et al. 2017). Of the eight assemblages (A-H) within *G. duodenalis*, six assemblages have been identified in pigs and wild boars, including 4 (assemblages C, D, E and F) which are found in animals and 2 (assemblages A and B) which are zoonotic assemblages (Farzan et al. 2011; Fava et al. 2013; Minetti et al. 2014; Rodriguez-Rivera et al. 2016). To understand the infection status of *G. duodenalis* in translocated Zangxiang pigs, we investigated the prevalence and genetic diversity of *G. duodenalis* in Qinghai and Shaanxi provinces, northwestern China, using a multilocus genotyping (MLG) tool based on the *β-giardin* (*bg*), *triosephosphate isomerase* (*tpi*) and *glutamate dehydrogenase* (*gdh*) loci.

Materials and methods

Specimen collection

From October to December 2017, a total of 450 faecal specimens were collected from Zangxiang pigs from Tongchuan city in Shaanxi province and Haidong city in Qinghai province (Fig. 1 and Table 1). Zangxiang pigs from Qinghai province were reared in an intensive farm on a mountain, while animals from Shaanxi province were bred in a free-range facility on a hillside. There is no human activity or other animals residing within 8 km of the farms. Four age groups, pre-weaned piglets (< 1 month), weaned pigs (1–4 months), juveniles (4–12 months) and adults (> 12 months), were examined. No obvious clinical signs were observed in the examined Zangxiang pigs. Each faecal specimen was directly collected from the rectum of the animal and each pig was sampled only once. All faecal specimens were collected into disposable plastic bags, marked with sampling sites, age groups and codes, then transported to the laboratory immediately and stored in 2.5% potassium dichromate at 4 °C.

Genomic DNA extraction and PCR amplification

After washed with distilled water to remove the potassium dichromate, genomic DNA was extracted from each faecal specimen using a commercial E.Z.N.A® Stool DNA kit (Omega Bio-Tek Lnc., GA, USA) according to the manufacturer's instructions. The extracted genomic DNA was stored at − 20 °C for further analysis.

The presence of *G. duodenalis* was determined by using nested PCR targeting the *bg*, *gdh* and *tpi* genes as previously described (Wang et al. 2017; Yin et al. 2018). Both positive and negative controls were included in each PCR reaction. The PCR amplicons were identified by using electrophoresis in 1% (*w*/*v*) agarose gels with ethidium bromide staining.

Sequence analysis

All positive amplicons were sent to Xi'an Qingke (Biological Co., Ltd. Xi'an, China) for sequencing using an ABI PRISM 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were checked and edited using DNAStar 5.0 (Burland 2000) and Clustal X 1.81 (Thompson et al. 1997). The amended sequences at each gene locus were aligned with the sequences available in GenBank within the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) to identify the assemblages and subtypes of *G. duodenalis*.

Statistical analysis

The differences in prevalence among age groups and locations were compared using the *chi-square* test (SPSS 20.0 Inc., Chicago, IL, USA), and differences were considered to be significant when the *P* value was < 0.05.

Results and discussion

G. duodenalis has been widely reported in a wide variety of hosts, including artiodactyla (e.g. goats, cattle), rodents (e.g. bamboo rats, rabbits), poultry (e.g. greylag geese, mute swans) and humans (Elmberg et al. 2017; Faria et al. 2017; Ma et al. 2018; Naguib et al. 2018; Zhang et al. 2018; Zhong et al. 2018a, b). In the present study, the overall prevalence of *G. duodenalis* in Zangxiang pigs was 6.2% (28/450) (Table 1), with a significant difference ($\chi^2 = 24.50$, $df = 3$, $P < 0.0001$) detected among age groups. The highest prevalence (26.8%) was found in pre-weaned pigs, and the infection rate decreased with increasing age (Table 1). Our results were inconsistent with prevalence from previous studies in wild boars (Castro-Hermida et al. 2011; Rodriguez-Rivera et al. 2016) and domestic pigs (Armson et al. 2009; Siwila and Mwape 2012; Petersen et al. 2015; Wang et al. 2017). For example, Castro-Hermida et al. (2011) found a higher prevalence in adult (> 1 year old) wild boars than in juveniles (< 1 year old) from northwestern Spain, and Rodriguez-Rivera et al. (2016) reported that the prevalence increased with age in feral pigs. These results may indicate different susceptibility of these animals to *G. duodenalis*. Furthermore, detection procedures would be also responsible for the difference. For example, the presence of *G. duodenalis* in pigs in Cambodian was investigated using conventional microscopy (Schär et al. 2014). Another study in Ontario, Canada, first used an immunofluorescent assay to detect *G. duodenalis* in pooled faecal

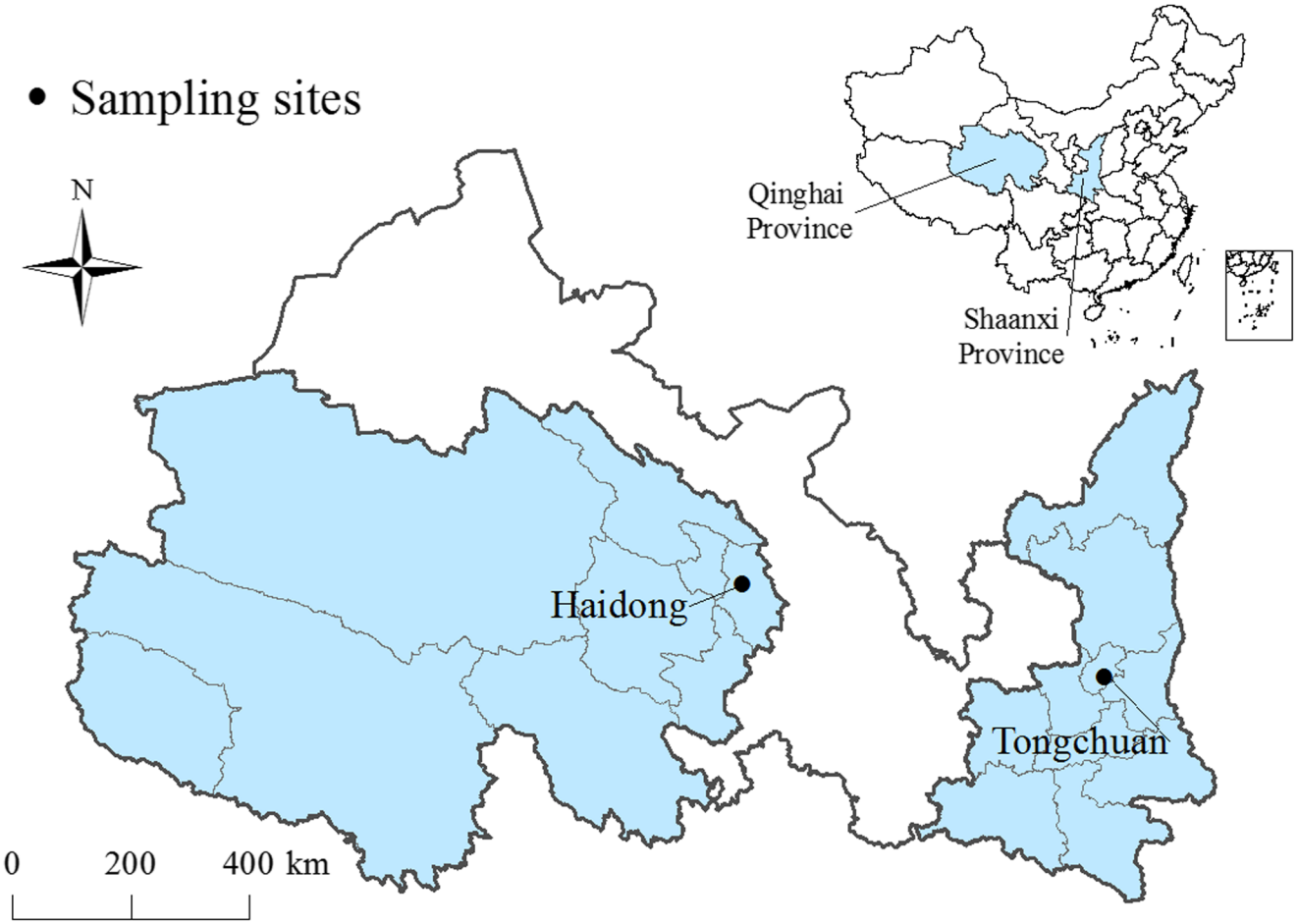

Fig. 1 Sampling sites in this study

samples from pigs and the positive samples were then analysed further using molecular techniques targeting *bg* and the small subunit (SS) rRNA (*SSU rRNA*) loci (Farzan et al. 2011). In our study, all samples were directly detected by nested PCRs targeting the *bg*, *tpi* and *gdh* loci, respectively. Compared with previous studies which used traditional

Table 1 Intra-assemblage substitutions in *bg*, *tpi* and *gdh* sequences of assemblage E

Subtype (number)	Nucleotide positions and substitutions			GenBank ID
bg	57	162	242	
Ref. sequence	T	A	A	KU668892
bgE1 (10)	T	A	A	MK313793
bgE2 (1)	C	G	G	MK313794
bgE3 (1)	C	A	T	MK313795
bgE4 (1)	C	A	A	MK313796
tpi	144	347	456	
Ref. sequence	A	G	G	KJ668136
tpiE1 (1)	G	A	A	MK313802
tpiE2 (2)	A	G	G	MK313801
gdh	104	149	218	
Ref. sequence	T	T	T	MF034657
gdhE1 (7)	T	T	T	MK313798
gdhE2 (3)	T	C	C	MK313799
gdhE3 (2)	C	C	G	MK313800

microscopy (Schär et al. 2014), the molecular technology used in our study would improve the detection limit of *G. duodenalis* in Zangxiang pigs.

Six assemblages (A-F) have previously been reported in wild boars and domestic pigs (Farzan et al. 2011; Fava et al. 2013; Minetti et al. 2014; Rodriguez-Rivera et al. 2016). In the present study, assemblage E was identified as the predominant assemblage (78.6%, 22/28) distributed across both geographical regions examined and in all age groups except the adults (Table 1). Assemblage E was previously thought to be host-specific but has been reported in humans in several studies (Foronda et al. 2008; Helmy et al. 2014; Abdel-Moein and Saeed 2016; Fantinatti et al. 2016; Scalia et al. 2016; Zahedi et al. 2017) and therefore should also be considered potentially zoonotic. Therefore, the zoonotic potential of *G. duodenalis* in Zangxiang pigs should be further investigated. Additionally, this assemblage was also found to be prevalent in domestic pigs from Ontario in Canada (Farzan et al. 2011), Western Australia (Armson et al. 2009), Minas Gerais in Brazil (Fava et al. 2013), Ogun state in Nigeria (Akinkuotu et al. 2019) and wild boars from Sichuan province in China (Li et al. 2017) and Texas in the USA (Rodriguez-Rivera et al. 2016). Furthermore, assemblage B was also found in five pre-weaned piglets and one adult Zangxiang pig from Shaanxi province. Assemblage B commonly infect humans (Feng and Xiao 2011; Cacciò et al. 2018) and has been linked with acute symptomatic giardiasis (Johargy et al. 2010).

In the present study, different amplification efficiencies for *bg*, *tpi* and *gdh* gene loci were found. Of the 28 *G. duodenalis*-positive samples from Zangxiang pigs, 13, 3 and 18 samples were successfully amplified for the *bg*, *tpi* and *gdh*, respectively. Sequence comparison revealed 4 haplotypes at the *bg* locus, including one known subtype (named as bgE1) that has 100% identity to sequences of *G. duodenalis* isolates from wild boars (KU668892), Tibetan sheep (KY633473), Xiang pigs (KU668882) and humans (AYO72729) in China, and lambs (EU726983) in Spain (Cacciò et al. 2002), and three novel ones (named as bgE2-bgE4) (Table 2). BLAST analysis showed that subtypes bgE2, bgE3 and bgE4 have 99% identities to reference sequences from pre-weaned dairy calves (MK252649) and Tibetan sheep (KY633466) in China, and lambs in Ethiopia (KT922250), respectively (Feng et al. 2019; Jin et al. 2017). Additionally, 2 and 4 haplotypes of *G. duodenalis* were also identified in Zangxiang pigs at the *tpi* and *gdh* loci in the present study (Table 2), but they all have been reported previously in domestic pigs and other animals.

In conclusion, *G. duodenalis* was detected in Zangxiang pigs, and but its prevalence is significantly affected by age. Genetic analysis identified both assemblage B and E in these Zangxiang pigs, both of which are potentially zoonotic and genetic diversity among *G. duodenalis* isolates from these animals was also identified. To the best of our knowledge, this is the first study of *G. duodenalis* infection in Zangxiang pigs, and the findings will support control strategies on *G. duodenalis* infection in these animals.

Table 2 Intra-assemblage substitutions in *bg*, *tpi* and *gdh* sequences of assemblage E

Subtype (number)	Nucleotide positions and substitutions			GenBank ID
bg	57	162	242	
Ref. sequence	T	A	G	KU668892
bgE1 (10)	T	A	A	MK313793
bgE2 (1)	C	G	G	MK313794
bgE3 (1)	C	A	T	MK313795
bgE4 (1)	C	A	A	MK313796
tpi	144	347	456	
Ref. sequence	A	G	G	KJ668136
tpiE1 (1)	G	A	G	MK313802
tpiE2 (2)	A	G	A	MK313801
gdh	104	149	218	
Ref. sequence	T	T	T	MF034657
gdhE1 (7)	T	T	T	MK313798
gdhE2 (3)	T	C	C	MK313799
gdhE3 (2)	C	C	G	MK313800

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study including the collection of faecal samples were permitted by the farm owners. The protocols in this study were approved by the Guidance of Laboratory Animal Care and Use of Chinese Ministry of Health, China, and the Research Ethics Committee of Northwest A&F University.

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