Journal of Infection and Public Health xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Prevalence and risk factors of *Giardia duodenalis* in domestic rabbbits (*Oryctolagus cuniculus*) in Jilin and Liaoning province, northeastern China

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ARTICLE INFO

Article history:
Received 6 October 2017
Received in revised form 21 January 2018
Accepted 21 February 2018

Keywords:
Domestic rabbits
Prevalence
Genotyping
Northern China

ABSTRACT

Background: Giardia duodenalis is one of the most important intestinal parasites that can infect virtually all animals, including rabbits and humans. However, there is little information regarding the prevalence and genotypes of *G. duodenalis* in domestic rabbits in China.
Methods: A total of 426 rabbit fecal samples (136 from Shenyang City, 174 from Changchun City, and 116 from Jilin City) were examined by Lugol's iodine staining with microscopy analysis, and the positive samples were genotyped at the triosephosphate isomerase (*tpi*) and the beta giardin (*bg*) gene loci using nested PCR.
Results: Forty-two (9.86%) out of 426 rabbit fecal samples were *G. duodenalis*-positive under microscopy analysis, and the highest *G. duodenalis* infection rate was 23.08% on farm 6. The prevalence of *G. duodenalis* in rabbits from different cities ranged from 1.47% to 14.37%. Among different age groups, *G. duodenalis* prevalence in rabbits ranged from 5.41% to 12.58%. The prevalence of *G. duodenalis* in outdoor rabbits and indoor rabbits was 14.29% and 6.77%, respectively. In the present study, region and farming mode were highly correlated with *G. duodenalis* infection in rabbits. All 42 *G. duodenalis* isolates were successfully amplified and sequenced at the *tpi* and *bg* loci, and only *G. duodenalis* assemblage B were identified.
Conclusion: This study not only further confirmed the dominance of *G. duodenalis* assemblage B in rabbits, but also further improved the foundation data concerning the distribution of *G. duodenalis* assemblages in China.

Introduction

Giardiasis is caused by *Giardia duodenalis* (syn. *Giardia intestinalis*, *G. lamblia*), a widespread enteric parasite of mammalian species [1–4]. Giardiasis is transmitted through the fecal-oral route [5], and shows symptoms of chronic diarrhea [4]. However, *G. duodenalis* coinfection with immune-compromised patients can cause significant morbidity and mortality [6]. In general, giardiasis affects around 2.0×10^8 people worldwide each year [7]. A wide range of animals and humans were reported as hosts for *G. duodenalis* [8]. Phylogenetic analysis revealed that eight assemblages (A–H) of *G. duodenalis* could be identified worldwide at present [1,9]. Among them, *G. duodenalis* assemblages A and B are responsible for the majority of human infections [10]. More importantly, some assemblages of *G. duodenalis* (e.g., assemblages A and B) can infect both humans and animals, raising public health concerns.

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https://doi.org/10.1016/j.jiph.2018.02.008

Table 1
Summarized the prevalence and assemblages of *Giardia duodenalis* in rabbits in the world (available data).

Regions	No. tested	No. positive	Prevalence	Assemblages	References
Central China	955	80	8.4%	B and E	[4]
Heilongjiang	378	28	7.41%	B	[14]
Europe	528	40	7.6%	B	[18]
Australia	97	1	1.03%	A	[19]
Ecuador	20	4	20%	NA	[20]
Germany	232	0	0%	–	[28]
California	4	0	0%	–	[29]

Recently, studies have suggested that rabbits are an important reservoir for many pathogens [11–13], but only a small number of genotypes/assemblages of *G. duodenalis* in rabbits were reported around the world (listed in Table 1), especially in China. In China, information regarding the prevalence and genotypes of *G. duodenalis* infection in rabbits was only reported in Heilongjiang Province [14,15] and Henan province [4]. However, information concerning the prevalence and genotypes of *G. duodenalis* in rabbits in Jilin and Liaoning Province remains unclear. The present study aimed to investigate the prevalence of *G. duodenalis* in rabbits and to identify their genotypes/assemblages. We also aimed to estimate the zoonotic potential of *G. duodenalis* from rabbits to humans.

Methods

Specimen collection and preparation

The study population comprised of 426 rabbits collected from three cities in China, in which nearly 3,437,000 rabbits were raised in 2014. The samples collected from nine farms, raising more than 3000 rabbits. According to the reported prevalence of *Giardia* in rabbit populations of 7.41% in 2012 [14], the expected prevalence was 7.5% (P) with an accepted deviation of the true prevalence of 5% (d) and a confidence level of 95% ($z = 1{\cdot}96$). The sample size was calculated as 107 [according to $n = P(1-P)z^2/d^2$]. This study was carried out in Jilin province (41°–46°N, 122°–131°E) and Liaoning province (38°–43°N, 118°–125°E), Northeast China, between May 2015 and June 2015. A total of 426 rabbit fecal samples were randomly collected from the ground after defection using sterile gloves, and were then put into box containing ice, immediately. We only used fresh and clean samples from the middle of the fecal sample for further analysis. Information regarding region, farming mode, and age were acquired by a questionnaire filled in by the farmers.

Collection and preparation of feces samples

Giardia cysts in the fecal materials were detected with Lugol's iodine staining. All *Giardia*-positive fecal specimens were washed three times, and genomic DNA was extracted using an E.Z.N.A. Stool DNA kit (OMEGA Bio-tek, Inc., Norcross, GA, USA) according to the manufacturer's procedures. The extracted DNA samples were stored at −20 °C until PCR analysis.

PCR amplification

G. duodenalis assemblages were determined by nested PCR of an approximately 530 bp fragment of the triosephosphate isomerase (*tpi*) locus [16] and a 511 bp fragment of the beta giardin (*bg*) gene locus [17]. The PCR reactions for all genes were performed in 25-μl PCR reaction composed of 1 × Ex *Taq* buffer (Mg^{2+} free), 2 mM $MgCl_2$, 200 μM each dNTP, 0.4 μM each primer, 0.625 U of Takara Ex *Taq* DNA polymerase (Takara Bio Inc., Shiga, Japan), and 2 μl of DNA. The cycling conditions were: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 60 s; followed by 72 °C for 10 min. Both positive (*Giardia*-positive isolates collected from cattle and stored at the laboratory) and negative controls were included in each test. All the secondary PCR products were examined by electrophoresis in 1.5% agarose gels and observed under UV light after GoldView™ (Solarbio, Beijing, China) staining.

Sequencing and phylogenetic analyses

All of the positive secondary PCR products were sent to the Genscript Company (Nanjing, China) for bidirectional sequencing. Meanwhile, genotypes that produced sequences with mutations, such as single nucleotide substitutions, deletions, or insertions, and that were confirmed by the DNA sequencing of at least two PCR products, were considered as novel genotypes. The genotypes/assemblages of *G. duodenalis* were determined by alignment with reference sequences available in GenBank using the computer program Clustal X 1.83 and BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

Statistical analysis

Statistical analysis of the prevalence of *G. duodenalis* infection in rabbits from different region ($x1$), farming mode ($x2$), and age ($x3$) was performed a Chi-square test in SAS (SAS Institute Inc., Cary, NC, USA, Version 9.1). In the multivariable regression analysis, each of these variables was included in the binary Logit model as an independent variable. The best model was judged by Fisher's scoring algorithm. All tests were two-sided, and the results were considered statistically significant if $P < 0.05$. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were provided to explore the strength of the association between *G. duodenalis*-positivity and the conditions investigated.

Accession numbers for nucleotide sequences

All the representative nucleotide sequences obtained in this study were deposited in GenBank with following accession numbers: KT372238 for the *tpi* gene, and KT715813 for the *bg* gene.

Results

A total of 42 (9.86%) out of 426 rabbit samples were detected as *G. duodenalis*-positive (Table 2), and the highest *G. duodenalis* infection rate was 23.08% on farm 6 (Table 3). Rabbits collected from Changchun City had a highest *G. duodenalis* infection rates compared with those collected from Jilin City and Shenyang City (Table 2). Moreover, in different age groups, the *G. duodenalis* prevalence in rabbits ranged from 5.41% to 12.58% (Table 3), and *G. duodenalis* prevalence in outdoor rabbits and indoor rabbits was 14.29% and 6.77%, respectively. All 42 *G. duodenalis* isolates were successfully amplified and sequenced at the *tpi* and *bg* loci. Sequencing analyses of the *tpi* and *bg* genes revealed that only *G. duodenalis* assemblage B was identified in this study.

Please cite this article in press as: Jiang J, et al. Prevalence and risk factors of *Giardia duodenalis* in domestic rabbbits (*Oryctolagus cuniculus*) in Jilin and Liaoning province, northeastern China. J Infect Public Health (2018), https://doi.org/10.1016/j.jiph.2018.02.008