First prevalence survey of parasitic helminths of goats along the Han River in Hubei Province, China

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Abstract

Diseases caused by parasitic helminths cause considerable production and economic losses in livestock worldwide. Understanding the epidemiology of these parasites has important implications for controlling them. The main purpose of the present study was to estimate the prevalence of key parasitic helminths in goats along the Han River in Zhanggang, Hubei Province (from January to December 2014). We used faecal flotation and sedimentation techniques as well as PCR-based DNA sequencing to detect and identify helminths. Results showed that the prevalence of helminths was high throughout the year, particularly for gastrointestinal nematodes. These first findings provide useful baseline information for goat helminths in Zhanggang, and a starting point for the implementation of control programs. With increased expansion of the goat industry in China, the findings also emphasise the need to undertake prevalence surveys in other regions of China where extensive farming practices are used.
Parasitic helminths are common in goats and often lead to considerable economic losses due to associated diseases and/or death (Perry et al., 1999; Vercruysse et al., 2001). Moreover, some helminths of goats, such as *Fasciola hepatica* and *Schistosoma japonicum*, are zoonoses and also pose an indirect threat to human health (Mansoorlakooraj et al., 2011; Kheirandish et al., 2012). To date, the prevalence of helminths of goats has been reported from many countries, including Brazil (Vieira et al., 2014), China (Yang, 1988; Guo et al., 2003; Yang, 2005; Wang et al., 2006; Feng et al., 2008; Ma et al., 2014), Denmark (Holm et al., 2014), Ethiopia (Sissay et al., 2007), India (Gupta et al., 1987; Khajuria et al., 2013), Italy (Zanzani et al., 2014), Malaysia (Chandrawathani et al., 2009), Mongolia (Sharkhuu et al., 2001), Netherlands (Borgsteede et al., 1996), Norway (Domke et al., 2013), Pakistan (Khan et al., 2010; Ayaz et al., 2013), Papua New Guinea (Koinari et al., 2013), Switzerland (Murri et al., 2014), with gastrointestinal strongylid nematodes being very common. However, prevalence and distribution of helminths can vary considerably among different countries as a consequences of differences in environment and management (Ma et al., 2014).

Hubei Province, with its more than one thousand lakes and rivers, is located in central China. The abundance of water provides a suitable habitat for many parasites, particularly gastrointestinal worms, to thrive. Although Shen et al. (2004) reported that many parasites are found in livestock in Hubei, there is no published report of the prevalence of helminths of goats in the lake area of this province. To address this knowledge gap, we undertook this first survey along the Han River in Hubei to provide a foundation for future control efforts of helminths of goats.

**Materials and methods**

*Study area, animals and sample collection*

The study was conducted in Zhanggang, Hubei province, China, from January to December in 2014. Zhanggang is located in the middle of Hubei province (112°33˚ E, 30°22˚ N). The Han River flows through this region and provides water to rich pasture lands in Zhanggang for grazing animals. Zhanggang has a subtropical monsoon climate. In Zhanggang, goats usually feed on grasses and shrubs along the Han River, but farmers also supplement with peanut stalks in winter. In the present study, 741 faecal samples were collected directly from the rectum of goats from eight flocks along the Han River (6-12 months of age) from January to December 2014. Fresh faecal samples were transported to the Laboratory of Parasitology, Huazhong Agricultural University, under cooled and anaerobic conditions.
Conventional faecal examination

All the faecal samples were stored at 4 °C under anaerobic conditions until examination (Nielsen et al., 2010). All the samples were examined within seven days to ensure that the larvated eggs did not hatch (Nielsen et al., 2010). Samples were examined using standard flotation and sedimentation techniques (Wang, 2013). In brief, two aliquots of 2 g of faeces were homogenised in 20 ml of saturated sodium nitrate and tap water, respectively, separately sieved through a tea strainer (mesh size: 250 µm) and then transferred to separate tubes to concentrate eggs by flotation (sodium nitrate) and sedimentation (water), respectively. Helminth eggs were identified to as far as possible (genus) using morphological characteristics using a light microscope (Nikon ECLIPSE 80i) (Taylor et al., 2007).

Molecular diagnosis

A PCR-based sequencing approach was also employed for the specific detection of selected strongylids. In brief, total genomic DNA was isolated directly from 500 mg of faeces from each sample using a kit (OMEGA Soil DNA Kit, USA), according to the manufacturer’s protocol. For *H. contortus*, part (265 bp) of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA was PCR-amplified using the species-specific primer HAE (5′-CAAATGGCATTTGTCTTTTAG-3′) and a conserved primer NC2 (5′-TTAGTTTTCTTCTCCTCCGCT-3′) (Bott et al., 2009). For *Trichostrongylus* species, part (267-268 bp) of ITS-2 was amplified using a genus-specific primer TRI (5′-TCGAATGGTCATTGTCAA-3′) and NC2 (5′-TTAGTTTTCTTCTCCTCCGCT-3′) (Bott et al., 2009). Each PCR was conducted in a volume of 50 µl containing GoTaq Flexi buffer (Promega, USA), 3.0 mM of MgCl₂, 200 µM of each deoxynucleotide triphosphate, 25 pmol of each primer and 1 U of GoTaq (Promega) DNA polymerase (Bott et al., 2009). Known test-positive, test-negative and no-template controls were included in each set of PCRs. The cycling protocol in a conventional thermal cycler (Eastwin Life Sciences, Inc., China) was: 94 °C for 5 min (initial denaturation), followed by 35 cycles of 94 °C for 45 sec (denaturation), 50 °C for 45 sec (annealing) and 72 °C for 1 min (extension), with a final extension of 72 °C for 10 min. Subsequently, the size and intensity of all amplicons were assessed by electrophoresis (7 V/cm) in 1.5% agarose gels using TBE (65 mM Tris-HCl, 27 mM boric acid, 1 mM EDTA, pH 9; Bio-Rad, USA) as the buffer. Following electrophoresis, gels were stained with ethidium bromide and their size estimated by comparison to Phi-X174-HaeIII (Promega, USA) markers. Aliquots (5 µl) of individual amplicons were purified using mini-columns (Wizard PCR-Preps, Promega), and then subjected to direct, automated sequencing (BigDye Terminator v.3.1 chemistry, Applied Biosystems, USA) in both directions using the same primers as employed for PCR amplification.
3. Results and discussion

In the 741 goats examined, 592 (79.9%) excreted eggs of one or more helminths. The prevalences were higher than those in Shaanxi (78.57%), but lower than those found in Hunan (86%), Chongqing (100%) and Sichuan (99.5%). Of the helminths detected, strongylids were common, particularly *H. contortus*; this finding is in agreement with surveys in Hunan (Ma et al., 2014), Shanxi (Guo et al., 2003; Feng et al., 2008), Chongqing (Yang, 2005), Sichuan (Yang, 1988) and Heilongjiang (Wang et al., 2006), but the prevalence of trematodes in Hubei was higher than that of other provinces, except Heilongjiang (Wang et al., 2006). The differences between our and investigation in other provinces might relate to differences in number of samples examined, climate, rainfall, management and other factors (Ma et al., 2014).

The estimated prevalence of helminths of goats in Zhanggang in 2014 are given in Fig. 1. In general, the prevalence of nematodes was high throughout the year, with four peaks in February, May, September and December. The prevalence of trematodes was lower than those of nematodes, and there was one infection peak in April. The excretion of tapeworms eggs did not appear to follow a pattern.

PCR-based sequencing of ITS-2 amplicons derived from a representative subset of samples was used to establish the specific identity of *Haemonchus* and *Trichostrongylus*. To do this, genomic DNA was extracted from 128 of the 741 faecal samples for subsequent PCR using primer pairs HAE-NC2 (*H. contortus*) and TRI-NC2 (*Trichostrongylus*) (Bott et al., 2009). In total, 116 of 128 samples (90.6%) were test-positive for *H. contortus* (Fig. 1), 10 amplicons were sequenced, the sequences were 265 bp in length; the resultant sequences (231bp) were identical to *H. contortus* (GenBank accession no. AB908961.1). On the other hand, 43 of the same 128 samples (33.6%) were also test-positive for *Trichostongylus*. Four amplicons were randomly selected and sequenced, the sequences were 267 bp in length; all four sequences were identical (over 221 bp) to ITS-2 of *Trichostrongylus colubriformis* (accession no. KC521378.1).

In the present study, the prevalence of nematodes was the highest throughout the year. In Zhanggang, farmers usually graze their goats on permanent pastures, and seldom change pastures. Thus, goats are likely readily re-infected continually. Notably, *H. contortus* is the commonest nematode. In Zhanggang, the warm and humid climate throughout the year (mean annual temperature is 15.8 °C; average annual precipitation: 1.2 m) is perfectly suited for the survival and development of *H. contortus*, explaining its high prevalence in Zhanggang. For the trematodes, the prevalence of gravid worms in goats peaked in April. In Zhanggang, the intermediate host snails were common on the pastures along the Han River, thus providing a suitable environment for transmission via the intermediate snail hosts. The peak development of snails is between April and September, which is in accord with the prevalence peak/s for trematodes.

This is the first survey of parasitic helminths of goats along the Han River in Hubei, China. Importantly, it was found that the prevalence of helminths was very high, even though farmers use anthelmintics (including levamisole, benzimidazoles...
and/or ivermectin) at up to 2-5 times the recommended dosage (personal communication). Given the high prevalence of strongylid nematodes, and the common over-usage and -dosage of these anthelmintics, it is possible that a resistance problem is already looming. This issue needs to be investigated in the very near future.

With increasing living standards in China and a need for more milk, meat and leather, there has been a massive expansion in the livestock industries, which includes goat farming. The requirement for these products has accelerated the breeding of goats, which has extended into many new regions (such as the Han River region) in the past decade to boost local economies. However, parasitic helminths significantly restrict economic productivity. To date, helminths have been reported in goats and sheep in 24 provinces of China, but there is still very limited information on the prevalence and burdens of these parasites in goats generally. For the last decade, the goat farming has rapidly developed along Han River, which is a branch from Yangtze River and flows through two provinces (Shanxi and Hubei), covering an area of 159,000 square kilometers. Currently, this region is estimated to carry 10 million goats. The present study now provides useful baseline information on goat helminths in Zhanggang, and a starting point for the implementation of monitoring and control programs in this region. Moreover, with massive expansion of the goat industry in China, the present findings also emphasise the need to undertake prevalence surveys in other regions of China where extensive farming practices are used, in order to provide crucial information to underpin future control efforts.

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**Conflict of interest**

None

**References**


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Figure legends

Fig. 1. The prevalence of parasitic helminthes in goats in 2014 along the Han River in Zhanggang.
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