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A survey on parasites in wild rodents in Xiji County, a northwestern part of China

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Abstract. Rodents act as an indicator for evaluation of environment contaminations and public health risks caused by parasites. A survey of parasites in wild rodents was conducted in 14 villages in Xijin County, where alveolar echinococcosis by *Echinococcus multilocularis* is epidemic. In total, 72 rodents including 25 mice, 16 *Citellus dauricus* (Daurian ground squirrel), 12 squirrels and 19 mole rat (*Myospalax fontanieri*) were captured. Infections (2.8%) of *Taenia taeniaeformis*, which is transmitted mainly between mice and cats, were found in mice in Wangping (WP) and Miaoping (MP) villages, but other cestodes' infections were not observed. WP and MP isolates were principally similar in morphology but, unlike WP isolate, MP isolate had no hooks on the scolex. Using 18S rRNA as a biomarker, the phylogenetic analysis showed that WP and MP isolates grouped together with European and Asian isolates and formed a separate cluster. These results highlights the prevalence of *T. taeniaeformis* in cats or/and dogs and a risk of opportunistic infections in human populations.

Wild rodents act as an intermediate or paratenic host of multiple cestodes, some of which have veterinary medical significance, and have been used as an indicator for evaluation of public health risks caused by zoonotic parasites (Reperant *et al*., 2009). A number of helminths including nematodes, trematodes and cestodes have been described in feral rodents (Panti-May *et al*., 2015; Paramasvaran *et al*., 2005; Reperant *et al*., 2009; Tung *et al*., 2013). For instance, *Taenia taeniaeformis* a canine cestode is known to be potentially zoonotic (Rossin, Malizia & Denegri, 2004); *Echinococcus multilocularis* is another canine cestode responsible for 0.3 – 0.5 million cases of alveolar echinococcosis in the world (Craig *et al*., 2007). Here parasites residing in the liver of rodents were focused on because *E. multilocularis* was previously found to be prevalent in Xiji County, located in the southwestern part of Ningxia Hui Autonomous Region, China (Yang *et al*., 2008).

Wild rodents were captured in June, 2015, by using traps set across the entire Xiji County. The animal experimental protocol was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China. All trapped animals were humanely slaughtered and the livers were carefully cut into pieces for detection of cysts. The dissected parasites were washed thrice using freshly-prepared ice-cold PBS and observed under optical microscopy (Olympus). Afterwards, parasites were immediately frozen in liquid nitrogen and grounded into powder, followed by

extraction of genomic DNA using DNAzol (Invitrogen) according to the instructions. In brief, after homogenization and centrifugation 0.5 ml of 100% ethanol were added into the supernatant, mixed well and stored overnight at -20ºC. After two washes with 75% ethanol, DNA pellets were air-dried and resuspended into nuclease-free water. DNA concentration was determined by Nanodrop (Thermo Fisher).

Polymerase chain reaction (PCR) was used for amplification of 18S rRNA using two pairs of the following primers: 5'-CGTTAAATGGATAACTGTAATAAC-3' (forward primer) and 5'-TATCACCAT GGTAGGCAGATCACC-3' (reverse primer). Reaction conditions were as follows: denaturation at 95ºC for 3min, 34 cycles of 95ºC for 30sec, 55ºC (for 18S) or 56.4ºC (for ITS1) for 30sec and 72ºC for 30sec, and a final extension 72ºC for 10min. PCR products were purified using kit (Axygen), ligated into pMD18-T vector (Takara) and transformed into DH5α competent cells. Positive transformants were sequenced (Genscript) and the sequences in this study were aligned with other 18S rRNA sequences retrieved from GenBank using Clustal W integrated in SeaView, followed by screening out the appropriate nucleotide model using FindModel with PAUP to construct an initial tree (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). A Bayesian tree was resolved by TOPALi v2.5 using GTR plus gamma with the below parameters: 5 runs, 100,000 generations, 10 sample frequency and 25% burn in (Milne *et al*., 2009). *Caenorhabditis elegans* 18S rRNA sequence was used as outgroup. The 18S sequences reported here were deposited in GenBank under the accession numbers KT721700 and KT721701.

A total of 72 wild rodents were captured in 14 villages, including 25 mice (*Rattus norvegicus* and *Mus musculus*), 16 *Citellus dauricus*, 12 squirrels (*Sciuridae*) and 19 *Myospalax fontanieri Milne*. Only one cyst was dissected from one mouse trapped in Wangping (WP) village and from one mouse in Miaoping (MP) village, respectively, and the morphology of two parasites was in general similar. Both were ~ 2.5 cm in length and had a large scolex with four suckers, pseudo-segmented tegument and a terminal large bladder filled with transparent fluid. However, the WP isolate dissected from *Rattus norvegicus* had two rows of hooks, while the MP isolate dissected from *Mus musculus* did not. Based on the above morphological traits, two isolates were primarily identified as *Taenia taeniaeformis* though they showed morphological differences compared to the previously described (Olson *et al*., 2000; Zhang *et al*., 2012). The two isolates were thus validated by use of 18S rRNA as a molecular marker. The 18S rRNA nucleotide sequence of the MP isolate was 327 bp in length and shared 94%– 99% identity with four *T. taeniaeformis* isolates from Germany, Japan, Belgium and Finland, whilst the 18S rRNA of the WP isolate was 337 bp and shared 92% – 95% identity. In the Bayesian tree based on 18S rRNA sequences, both MP and WP isolates were clustered together with *T. taeniaeformis* isolated from Germany (TtaeGermany_JQ609340.1), Japan (TtaeSRN_AB731629.1 and TtaeACR_AB731630.1), Belgium (TtaeBMM_AB731628.1) and Finland (TtaeTtaFi_AB731631.1) with high probabilities (Fig. 1). These results further confirm that both two specimens with different morphology are larvae of *T. taeniaeformis*.

In this survey, besides *T. taeniaeformis*, other cestodes including *E. multilocularis* were not found. It is appreciable because more cats have been raised in Xiji County in recent years, leading to a decrease of quantity of feral rodents near local residents, especially mice and *Citellus dauricus*. The observation of *T. taeniaeformis* infections in wild mice is consistent with the above presumption. But it is prudent to conclude the low frequency of *E. multilocularis* are due to many factors, for instance temperature, are contributed to prevalence (Burlet, Deplazes & Hegglin, 2011). Long-term and all year-long surveillance of wild rodents will be required in the future studies. *T. taeniaeformis* MP and WP isolates showed difference in hooks, and had abnormal morphology, particularly much shorter in length than the previously reported (Zhang *et al*., 2012). It can be explained by

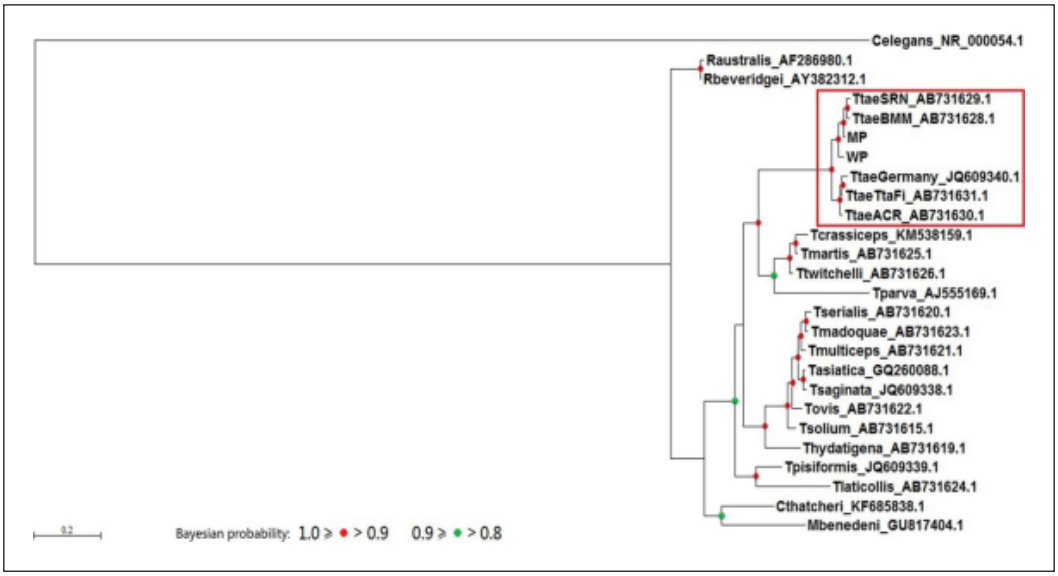

Figure 1A. Bayesian tree of cestodes using 18S rRNA sequences. Two WP and MP isolates constituted a separate clade with other isolates from Europe and Asia, which was boxed in red. GenBank accession numbers for each 18S rRNA were shown behind '_' and Bayesian probabilities more than 0.80 were indicated on each node. *C. elegans* 18S rRNA sequence was used as outgroup. Celegans: *Caenorhabditis elegans*, Raustralis : *Raillietina australis*, Rbeveridgei: *Raillietina beveridgei*, TtaeSRN: *Taenia taeniaeformis*_SRN, TtaeBMM: *Taenia taeniaeformis*_BMM, MP: MP isolate, WP: WP isolate, TtaeGermany: *Taenia taeniaeformis*_Germany, TtaeTtaFi: *Taenia taeniaeformis*_TtaFi, TtaeACR: *Taenia taeniaeformis*_ACR, Tcrassiceps: *Taenia crassiceps*, Tmartis: *Taenia martis*, Ttwitchelli: *Taenia twitchelli*, Tparva: *Taenia parva*, Tserialis: *Taenia serialis*, Tmadoquae: *Taenia madoquae*, Tmulticeps: *Taenia multiceps*, Tasiatica: *Taenia asiatica*, Tsaginate: *Taenia saginata*, Tovis: *Taenia ovis*, Tsolium: *Taenia solium*, Thydatigena: *Taenia hydatigena*, Tpisiformis: *Taenia pisiformis*, Tlaticollis: *Taenia laticollis*, Cthatcheri: *Cathetocephalus thatcheri*, Mbenedeni: *Moniezia benedeni*.

that parasites may be at different developmental stages or that the micro-environments of different rodent species are different in a support of parasite growth and development, as indirectly reflected by discrepancy of infectivity of *T. taeniaeformis* in different rats and mice (Brandt and Sewell, 1981).

Infection of strobilocerci in wild rodents in the study is an informative indicator of prevalence of *T. taeniaeformis* in cats or dogs. Although the infection rate is extremely low, *T. taeniaeformis* has the capacity of infecting humans (Rossin, Malizia & Denegri, 2004). The prevalence of *T. taeniaeformis* in wild rodents poses infection risks in humans, especially those who are in close contact with pets, and it is necessary to enhance management and control of diseases in local cats and dogs.

Conflict of interest statement

The authors declare no conflict of interest.

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