SHORT REPORT: INADEQUACY OF YAKS AS HOSTS FOR THE SHEEP DOG STRAIN OF \textit{ECHINOCOCCUS GRANULOSUS} OR FOR \textit{E. MULTILOCULARIS}

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Abstract. Hydatid disease (echinococcosis) has a two-host cycle involving the domestic dog and grazing animals. Humans are also infected by the dog. Both unilocular (\textit{Echinococcus granulosus} in yaks, sheep, and goats) and multilocular (alveolar) (\textit{E. multilocularis} in hares and rodents) hydatids are common in western Sichuan in the People’s Republic of China. Humans and dogs are equally infected with both species. Many yaks (\textit{Bos grunniens}) were found with multilocular cysts that visually were deemed to be \textit{E. multilocularis}. However, a histologic and molecular study showed that they were actually \textit{E. granulosus}. No infective cysts were found in 125 necropsied yaks. We conclude that the yak is an inadequate and dead-end host for the sheep dog (G1) strain of \textit{Echinococcus granulosus} and also for \textit{E. multilocularis}.

Hydatid disease (echinococcosis) is widespread and endemic in western parts of China, particularly among Tibetan pastoral herders inhabiting grazing lands above 3,800 meters. In Tibet, animal infection rates of 54% in yaks (\textit{Bos grunniens}) and 81% in sheep have been recorded. Human hydatid infection rates in exclusively stock-raising areas were 8–27%, of which 50% were due to \textit{Echinococcus granulosus} and 50% to \textit{E. multilocularis}.\textsuperscript{1}

To initiate a demonstration of hydatid control for the area, 100 four-year-old yaks from the four townships of the Datangba Flatlands in Ganzi County, Sichuan, People’s Republic of China were necropsied to provide baseline data (Table 1). An additional 25 older yaks slaughtered for meat and identified as originating from Datangba were examined at slaughter. Visually, cysts found in any of the 125 yaks investigated were classified as multilocular (probably \textit{E. multilocularis}) or unilocular (certainly \textit{E. granulosus}). Often, both types of cysts were found in the same animal (Table 1). Qiu and others\textsuperscript{2} had previously described atypical \textit{E. multilocularis} infections in the livers of yaks and sheep. These cysts did not contain brood capsules or protoscoleces, but had a strong resemblance to \textit{E. multilocularis} infections in humans. If the multilocular cysts contained infective protoscoleces, this would be the first record of large grazing animals acting as an intermediate host for \textit{E. multilocularis}. This might be expected because dogs in this area have been shown to be heavily infected with both \textit{Echinococcus} species.\textsuperscript{3} An echinococcal lesion in the liver of a yak from a neighboring region (Shiqu County) in the Qinghai-Tibet plateau region was shown by mitochondrial DNA typing to be the result of infection by the G1 genotype of \textit{E. granulosus}.\textsuperscript{3} As emphasized by Xiao and others,\textsuperscript{3} the identification of echinococcal lesions in yaks as \textit{E. granulosus} rather than \textit{E. multilocularis} should be confirmed by molecular typing of a larger number of samples, which is what we now report.

The study was a joint research program between the Chinese government and the New Zealand government. It was instituted by the New Zealand Ministry of Foreign Affairs and Trade and administered by their agency (Landcare Research New Zealand Limited). The research program was reviewed and approved by the Beijing Ministry of Foreign Affairs and Economic Cooperation, their provincial and prefectural counterparts, and the Veterinary and Human Health administrators of Ganzi County. Yaks were humanely killed by their owners in the traditional Buddhist manner. All work on \textit{Echinococcus} at the Queensland Institute of Medical Research was reviewed and approved by the Queensland Institute of Medical Research (Bancroft Centre) Ethics Committee.

Cysts were cut open and then dissected from liver or lung tissue. They were then fixed immediately in 95% (v/v) ethanol. Portions of unilocular and multilocular cysts (Figure 1) were sent to the Australian laboratory for genotyping. Methods for genomic DNA isolation and purification, and polymerase chain reaction amplification, automatic sequencing, and alignment analysis of fragments of the mitochondrial \textit{cox1} gene have been described.\textsuperscript{3,5} The \textit{cox1} sequences obtained from cysts were aligned with published sequences for various \textit{E. granulosus} genotypes, \textit{E. multilocularis}, and other \textit{Echinococcus} spp.\textsuperscript{4,5}

Sequences were obtained from nine liver or lung cysts from individual yaks and one liver and one lung cyst from individual Tibetan sheep (no protoscoleces present). In addition, sequence was obtained from a New Zealand sheep cyst (protoscoleces present) for comparison. The \textit{cox1} sequences for the unilocular and multilocular yak cysts and the sheep material were all identical to that of the common sheep strain (G1 genotype) of \textit{E. granulosus}; there were 36 nucleotide differences between the sequences and the published \textit{E. multilocularis} sequence.

The analysis of unilocular and multilocular cysts thus showed that both types were caused by the sheep strain of \textit{E. granulosus} and not \textit{E. multilocularis}. This was supported by examination of hematoxylin and eosin–stained histologic sections. The convoluted laminated membranes in the multilocu-

\begin{table}[h]
\centering
\caption{Prevalence of \textit{Echinococcus} spp. in 100 four-year-old yaks from Datangba Flatlands, Ganzi County, Ganzi Prefecture, Sichuan, People’s Republic of China*}
\begin{tabular}{|c|c|c|c|}
\hline
Townships & Cha Zhu & Cha Long & Ka Long & Da De \\
\hline
Prevalence of multilocular cysts & 48\textsuperscript{%} & 50\textsuperscript{%} & 57\textsuperscript{%} & 67\textsuperscript{%} \\
Prevalence of unilocular cysts & 28\textsuperscript{%} & 67\textsuperscript{%} & 67\textsuperscript{%} & 78\textsuperscript{%} \\
Cyst intensity (average number per infected animal) & 4.7 & 11.5 & 6.0 & 9.7 \\
\hline
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\* Both multilocular and unilocular cysts were often found in the same host.

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lar cysts were lined on the inside by germinal membrane, but showed no budding to the exterior, and there were no protoscoleces present. These multilocular cysts were probably a manifestation of an immune response to *E. granulosus* that walls off the developing cyst so that the laminated and germinal membranes continue to proliferate within a confined space. The unilocular cysts also had no evidence of protoscoleces or developing brood capsules.

Although the liver and lung cysts from the 25 necropsied older yaks were often 2–3 times the diameter of those from 4-year-old yaks, there was no increase in prevalence or incidence of cysts. It is tempting to conclude that the continued exposure to *E. granulosus* eggs in this environment, in which no hydatid control has been practiced until now, results in the stimulation and maintenance of immunity to reinfection.

A Ganzi Hydatid Control and Community Health Project in Sichuan is intended to provide guideline information for the development of future hydatid control programs in China, and is focused on interrupting the lifecycle of the hydatid parasite by dosing dogs with praziquantel and vaccinating the animals that host the cystic stage of the tapeworm. An understanding of the tapeworm life cycle and how people can avoid becoming infected with hydatid disease is included as part of the community and health education activities.

At the beginning of the project, it was thought that Datangba Flatlands yaks, sheep, and goats could all produce cysts that would be able to reinfest dogs with the parasite. Vaccination of all these animals would prevent infections from becoming established and reduce the chances of dogs becoming reinfected by eating animal organs containing hydatid cysts.

Emerging technology has shown that not all types of grazing animals are involved in the transmission of cystic hydatid disease caused by *E. granulosus* to dogs. Zhang and others reviewed previous work in China showing the predominance of the sheep strain (G1 genotype) and a report of this genotype in a sample of hydatid material from yaks.

We have now shown that this local Datangba sheep strain of *E. granulosus* usually produces protoscoleces in sheep and goats, and not in yaks. There are reports of yaks contributing to human hydatid disease in a population of yaks around Qinghai Lake in Qinghai and in cattle in Xinjiang. The Qinghai yaks and Xinjiang cattle are now thought to actively host a different (G5) genotype, which does produce cysts that are infective for dogs, but this cycle may not occur elsewhere in western China. The G5 (cattle-dog strain) has been shown by genotyping human cyst material to be infective to humans.

We are now proposing to collect human hydatid cyst material from people in Datangba undergoing hydatid surgery at the Ganzi Hospital to determine the infective hydatid genotype. If it is solely or predominantly G1, as we predict, the control of hydatid disease caused by *E. granulosus* will concentrate there on sheep and goats, while putting less emphasis on yaks.

References