SHORT REPORT: INADEQUACY OF YAKS AS HOSTS FOR THE SHEEP DOG STRAIN OF *ECHINOCOCCUS GRANULOSUS* OR FOR *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 (*Echinococcus granulosus* in yaks, sheep, and goats) and multilocular (alveolar) (*E. multilocularis*) hydatids are common in western Sichuan in the People’s Republic of China. Humans and dogs are equally infected with both species. Many yaks (*Bos grunniens*) were found with multilocular cysts that visually were deemed to be *E. multilocularis*. However, a histologic and molecular study showed that they were actually *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 *Echinococcus granulosus* and also for *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 (*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 *Echinococcus granulosus* and 50% to *E. multilocularis*.1

To initiate a demonstration of hydatid control for the area, 100 four-year-old yaks from the four townships of the Datangba Flats 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 *E. multilocularis*) or unilocular (certainly *E. granulosus*). Often, both types of cysts were found in the same animal (Table 1). Qiu and others2 had previously described atypical *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 *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 *E. multilocularis*. This might be expected because dogs in this area have been shown to be heavily infected with both *Echinococcus* species.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 *E. granulosus*.3 As emphasized by Xiao and others,3 the identification of echinococcal lesions in yaks as *E. granulosus* rather than *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 *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 *cox1* gene have been described.4,5 The *cox1* sequences obtained from cysts were aligned with published sequences for various *E. granulosus* genotypes, *E. multilocularis*, and other *Echinococcus spp.*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 *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 *E. granulosus*; there were 36 nucleotide differences between the sequences and the published *E. multilocularis* sequence.

The analysis of unilocular and multilocular cysts thus showed that both types were caused by the sheep strain of *E. granulosus* and not *E. multilocularis*. This was supported by examination of hematoxylin and eosin-stained histologic sections. The convoluted laminated membranes in the multilocular cysts were often found in the same host.

### Table 1

<table>
<thead>
<tr>
<th>Townships</th>
<th>Cha Zha</th>
<th>Cha Long</th>
<th>Ka Long</th>
<th>Da De</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of multilocular cysts</td>
<td>48%</td>
<td>50%</td>
<td>57%</td>
<td>67%</td>
</tr>
<tr>
<td>Prevalence of unilocular cysts</td>
<td>28%</td>
<td>67%</td>
<td>67%</td>
<td>78%</td>
</tr>
<tr>
<td>Cyst intensity (average number per infected animal)</td>
<td>4.7</td>
<td>11.5</td>
<td>6.0</td>
<td>9.7</td>
</tr>
</tbody>
</table>

* Both multilocular and unilocular cysts were often found in the same host.
lar cysts were lined on the inside by germinal membrane, but showed no budding to the exterior, and there were no pro-
toscoleces 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 ger-
minal membranes continue to proliferate within a confined
space. The unilocular cysts also had no evidence of pro-
toscoleces 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 inci-
dence 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 vaccinating6
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 Da-
tangba Flatlands yaks, sheep, and goats could all produce
cysts that would be able to reinfect 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 hy-
datid cysts.

Emerging technology has shown that not all types of graz-
ing animals are involved in the transmission of cystic hydatid
disease caused by *E. granulosus* to dogs.7,8 Zhang and others8
reviewed previous work in China showing the predominance
of the sheep strain (G1 genotype) and a report of this geno-
type 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 contrib-
uting 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,4 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 infect-
tive to humans.5

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

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REFERENCES

P, 2000. Epidemiological survey on hydatidosis in Tibetan re-
gion of Western Sichuan, II. Infection situation among domes-
alveolaris Echinococcus infection in yaks and sheep in Shiqu
3. Xiao N, Qiu J, Nakao M, Nakaya K, Yamasaki H, Sako Y, Ma-
muti W, Schantz PM, Craig PS, Ito A, 2003. Short report:
Identification of *Echinococcus* species from a yak in the Qing-
445–446.
the genus *Echinococcus* identified by mitochondrial DNA se-
tochondrial genomic markers confirm the presence of the camel
strain (G6) genotype of *Echinococcus granulosus* in
of hydatidosis using vaccination - a review of formulation and
delivery of the vaccine and recommendations for practical use
7. McManus DP, 2002. The molecular epidemiology of *Echinococ-
cus granulosus* and cystic hydatid disease. *Trans R Soc Trop