Modelling the age variation of larval protoscoleces of *Echinococcus granulosus* in sheep

P.R. Torgerson, I. Ziadinov, D. Aknazarov, R. Nurgaziev, P. Deplazes

**Abstract**

In autumn 2006, a study of the age-dynamics of *Echinococcus granulosus* cyst abundance was undertaken from an abattoir study of 1081 sheep slaughtered in Naryn Province in central Kyrgyzstan, an area endemic for echinococcosis. The results demonstrated approximately 64% of sheep were infected with the prevalence increasing markedly with age. The mean abundance was 3.8 cysts per sheep. From established models, an infection pressure of 1.33 cysts per year was estimated. In addition all cysts were recovered from infected sheep and the numbers of protoscoleces was evaluated in each cyst. A new model was developed that examined the variation in numbers of protoscoleces per infected sheep with age. This demonstrated that young sheep aged 1–2 years had very few protoscoleces, but there was a massive increase as the sheep aged. The best-fitting model assumed that the number of protoscoleces in a sheep was proportional to the volume of the cysts. In this model, the radius of the individual cyst increased linearly with the age of the cyst and hence the volume increased with the cube of the cyst age. This combined with the linear increase in numbers of cysts with age resulted in a massive accumulation of protoscoleces with the age of sheep. When the model was parameterised it demonstrated that 80% of protoscoleces were present in sheep aged 4 years and older and this represented just 28% sheep slaughtered. An average sheep at 6 or more years of age has an abundance of over 9700 protoscoleces, whilst in a young sheep of 1 year of age an average of just 16 protoscoleces is expected. The average for the sample population across all ages was 1562 protoscoleces per sheep. The maximum number of protoscoleces in a single cyst was just 482 for sheep aged 1 year rising to 92,000 for sheep aged 6 years or older. The mean volume of cysts containing protoscoleces increased from approximately 0.7 ml at 1 year of age to 8.8 ml at 6 years of age. Cysts containing protoscoleces ranged from a diameter of 0.5–8 cm with a volume of fluid ranging from 0.2 to 50 ml. It is hypothesised that removal of old sheep through a culling programme could substantially improve the control of cystic echinococcosis.

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1. Introduction

The family Taeniidae includes important cestode parasites of the genus *Echinococcus* and *Taenia*. Some of these parasites are notable zoonoses whilst others can have an adverse effect on animal health. Earlier work has shown that the biotic potential of *Taenia* spp. in dogs, such as *Taenia hydatigena*, is high with an infected dog producing 38,000 eggs per day. In contrast a dog infected with *Echinococcus granulosus* only produces approximately 8500 eggs per day (Gemmell, 1990). However, in the intermediate host, the metacestode of *T. hydatigena* will only produce one scolex and hence has the potential to produce just one adult in the final host.

In contrast, the metacestode of *E. granulosus* produces protoscoleces asexually and has the capability to produce many adults in the final host as each protoscolex has the potential to develop into one adult parasite. Thus the asexual reproduction exhibited by *Echinococcus* has a potential unsurpassed by other tapeworms (Thompson, 1995). However, there have been few studies quantifying the extent of this asexual reproduction in the intermediate host and this is an important objective of this study.

To date, mathematical models of the transmission of *E. granulosus* in sheep have described a linear relationship between the age of sheep and the numbers of cysts. This finding has been robust across a number of studies from different countries such as New Zealand, China, Jordan, Uruguay, Peru and Kazakhstan (Roberts et al., 1986; Ming et al., 1992; Cabrera et al., 1995; Torgerson et al., 1998, 2003a; Duerger and Gilman, 2001). However an important question that has not been addressed in any of these studies is the variation in numbers of protoscoleces and how this affects the
transmission dynamics. Therefore this study not only looked at the variation in cyst numbers with age but also examined the variation in numbers of protoscoleces and proposes a mathematical model which will improve the model description of the transmission of *Echinococcus* in sheep.

Finally, human cystic echinococcosis (CE) caused by *E. granulosus* is a major public health problem throughout the world. The disease results in serious socio-economic effects (Eckert and Deplazes, 2004; Budke et al., 2006) and is a re-emerging disease in Kyrgyzstan following the socio-economic changes resulting from the collapse of the Soviet Union (Torgerson et al., 2003b, 2006). The annual surgical incidence across the country is approaching 20 cases per 100,000 or more. Although a high prevalence of *E. granulosus* has been found in dogs in Naryn province (Ziadinov et al., 2008), there is little information regarding the transmission dynamics of *E. granulosus* in sheep in this country. Such information is important to define baseline levels of infection pressure which is vital for devising intervention strategies (Torgerson and Heath, 2003).

2. Materials and methods

2.1. Study area and animals

A total of 1081 sheep were necropsied in Naryn city abattoir, Kyrgyzstan in autumn 2006. All sheep originated from Naryn province. The age of each sheep was estimated by careful examination of its dentition. Any ambiguity was resolved by carefully questioning the animal’s owner. The liver, lungs and internal organs of each sheep were examined visually and palpated for the presence of cystic lesions. All hydatid cysts that were found were carefully excised and taken to the laboratory for further examination. The total number of cysts from each sheep was recorded. The external diameter of each cyst was recorded. The laminated membrane, germinal layer and cyst contents including cyst fluid and protoscoleces were carefully removed. Cyst fluid, including protoscoleces, was removed by aspiration and fluid volume measured. The remaining laminated membrane including the germinal layer was carefully washed to recover any remaining protoscoleces. Protoscoleces were allowed to sediment and then counted. For small cysts, total protoscolece count was determined by examination of the cyst contents and counting under a binocular microscope. Only fully developed, invaginated protoscoleces were counted. For larger cysts, a proportion of the total sediment was examined and total protoscoleces were estimated by extrapolation to the entire sediment volume. All cyst collection was undertaken by the same individuals. All laboratory analyses of the cysts were undertaken by one person who also participated in the cyst collection.

2.2. Analysis

The variation in cyst number with age was modelled according to methods described previously (Torgerson et al., 2003b). To analyse the variation in the numbers of protoscoleces with the age of sheep additional models were developed.

Assuming the cysts were spherical, it can be supposed that the radius \( r \) of a cyst of age \( t \) will vary according to the age of the cyst by some function:

\[
  r_t = f(t)
\]

Therefore the volume \( V_t \) of a cyst (assumed to be spherical) of age \( t \) will vary according to:

\[
  V_t = \frac{4}{3} \pi (f(t))^3
\]

If it is assumed that the numbers of protoscoleces \( n_t \) in a cyst of age \( t \) is dependent on the volume of the cyst,

\[
  n_t = P \frac{4}{3} \pi (f(t))^3
\]

where \( p \) is some unknown constant. By gathering all the constants and taking the cube root and adding to \( f(t) \) it can be seen that the number of protoscoleces \( n_t \) in cysts of age \( t \) will be related to the cube of an as yet unknown function of the age \( g(t) \):

\[
  n_t = (g(t))^3
\]

An individual sheep is exposed to infection pressure \( h \) cysts per year and the number of cysts \( X_{ct} \) in a sheep of age \( T \) is given by:

\[
  X_{ct} = hT \text{ (Roberts et al., 1986)}
\]

This assumes that cysts, once acquired, never disappear.

Thus the total number of protoscoleces \( P_t \) in a sheep at age \( T \) will be:

\[
  P_t = h \sum_{t=1}^{T} |g(T)|^3 + h|g(T-1)|^3 + \ldots + h|g(1)|^3
\]

For example, a sheep of 3 years of age exposed to an infection pressure of two cysts per year will have: 2\[|g(3)|^3\] protoscoleces from cysts acquired in the first year of life, 2\[|g(2)|^3\] from cysts acquired in the second year and 2\[|g(1)|^3\] from cysts acquired in the third year as the cysts had 3, 2 and 1 years to grow, respectively.

Eq. (3) can be more simply written as:

\[
  P_t = h \sum_{t=1}^{T} |g(T)|^3
\]

The nature of the function \( g(t) \) was explored by analysing the total number of protoscoleces per sheep, i.e., \( P_t \). First the maximum likelihood estimate (MLE) of the infection pressure \( h \) in Eq. (1), assuming aggregation of the cysts according to a negative binomial distribution (Torgerson et al., 2003b), was found. Using this MLE of \( h \), various functions of \( g(t) \) were compared. These included a function where the radius increased at an exponential rate according to age, a logistic function, an asymptotic growth function and a linear growth function. The model with the lowest Akaike Information Criterion (AIC) (Akaike, 1974) was selected for further analysis. Once the model with the lowest AIC was determined a Bayesian analysis was undertaken to better define the parameters in the model and their credible intervals (CIs). For this analysis, the variation of protoscoleces with age was assumed to be a two-stage process. Sheep could be infected or not infected. Conditional on being infected the cysts may contain protoscoleces. Thus the model was a two-stage conditional model. The probability of observation \( O_j \) having 0 cysts in sheep of age \( T \), assuming the probability distribution is a negative binomial distribution (Torgerson and Heath, 2003) is given by:

\[
  p(O_j = 0) = \left( \frac{k_T}{k_T + X_{ct}} \right)^{O_j}
\]

where \( k_T \) is the negative binomial constant for the distribution of cysts at age \( T \), or age-dependent aggregation parameter. This is the proportion of zero counts that is given by a negative binomial distributed variable of mean \( X_{ct} \) (from Eq. (1)) with \( k_T \) negative binomial constant. As the degree of aggregation of cysts within sheep decreases with age (Lahmar et al., 1999) the negative binomial constant was modelled to vary with age as:

\[
  k_T = \gamma T
\]

where \( \gamma \) is a constant. By substituting Eq. (5) into 4, the likelihood for observing zero cysts at age \( T \) is:

\[
  p(O_j = 0) = \left( \frac{\gamma T}{1 + X_{ct}} \right)^{O_j}
\]

The expected number of protoscoleces \( P_t \), in an individual sheep \( i \) of age \( T \) known to be infected with hydatid cysts is:

\[
  P_t = \frac{P_t}{1 - p(O_j = 0)}
\]
Table 1

The prevalence and abundance of hydatid cysts and abundance of protoscoleces stratified according to age for sheep from Naryn Oblast, Kyrgyzstan.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number sheep</th>
<th>Number infected</th>
<th>% Sheep infected (exact binomial confidence intervals)</th>
<th>Mean abundance of cysts</th>
<th>Mean numbers of protoscoleces per sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>205</td>
<td>92</td>
<td>44.9 (37.9–52.0)</td>
<td>2.6</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>264</td>
<td>140</td>
<td>53.0 (46.8–59.2)</td>
<td>3.11</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>280</td>
<td>165</td>
<td>58.9 (52.9–64.8)</td>
<td>2.5</td>
<td>711</td>
</tr>
<tr>
<td>4</td>
<td>188</td>
<td>155</td>
<td>82.5 (76.2–87.6)</td>
<td>4.61</td>
<td>1726</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>93</td>
<td>97.9 (92.6–99.7)</td>
<td>7.1</td>
<td>6899</td>
</tr>
<tr>
<td>&gt;6</td>
<td>49</td>
<td>49</td>
<td>100 (92.8–100)</td>
<td>10.28</td>
<td>9774</td>
</tr>
<tr>
<td>Mean across all age groups</td>
<td>1081</td>
<td>694</td>
<td>64.2 (61.3–67.1)</td>
<td>3.81</td>
<td>1562</td>
</tr>
</tbody>
</table>

Table 2

Data on cysts of Echinococcus granulosus containing protoscoleces including proportion of fertile cysts, range of protoscoleces in cysts and volume and size of cysts.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of cysts containing protoscoleces per sheep</th>
<th>Protoscoleces per fertile cyst (range)</th>
<th>Mean volume of cysts containing protoscoleces (ml) (range)</th>
<th>Size range (in cm) (largest external diameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>215 (16–482)</td>
<td>0.7 (0.3–1)</td>
<td>0.5–2</td>
</tr>
<tr>
<td>2</td>
<td>0.11</td>
<td>870 (297–3100)</td>
<td>1.3 (0.2–5)</td>
<td>0.5–3</td>
</tr>
<tr>
<td>3</td>
<td>0.34</td>
<td>2095 (500–9120)</td>
<td>1.7 (0.8–12)</td>
<td>0.5–3</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>3061 (168–16222)</td>
<td>2.1 (1.5–18)</td>
<td>0.5–5</td>
</tr>
<tr>
<td>5</td>
<td>0.84</td>
<td>8192 (1700–19000)</td>
<td>4.8 (0.5–12)</td>
<td>0.5–6</td>
</tr>
<tr>
<td>&gt;6</td>
<td>0.78</td>
<td>12603 (120–92000)</td>
<td>8.8 (0.5–50)</td>
<td>0.5–8</td>
</tr>
</tbody>
</table>

As \( P_T \) is the expected number of protoscoleces in sheep regardless of infection status – see Eq. (2) the expected number of protoscoleces \( P_{T_1} \) in an infected sheep of age \( T \) can be calculated by substituting Eqs. (3) and (6) into Eq. (7) and is given by

\[
P_{T_1} = \frac{h^Y \pi}{1 - \left( \frac{1}{\pi} \right)^Y} \tag{8}
\]

As the variance of the numbers of protoscoleces was much greater than the mean, the likelihood function was also considered to be a negative binomial. Therefore the probability of the number of observed protoscoleces \( S_n \) for each observation \( O_i \) is

\[
p(O_i = S_n) = \frac{\Gamma(k_p + S_n)}{\Gamma(k_p) S_n^p} \left( \frac{P_{T_1}}{k_p + P_{T_1}} \right)^S_n \left( \frac{k_p}{k_p + P_{T_1}} \right)^{n - S_n}
\]

where \( k_p \) is the aggregation constant for the negative binomial distribution of protoscoleces. The total likelihood of the data given the model,

\[
l(D/m) = \prod_{i=1}^{n} p(O_i = S_n) \times \prod_{j=1}^{n} p(O_j = 0)
\]

For the Bayesian analysis of CIs of the parameters, the model was set up as a spreadsheet model in Excel. Initial starting values of the parameters were used based on the MLE estimates previously calculated when comparing different model fits. Uniform non-informative priors were used for parameters. A macro was written in Excel in order to execute a Metropolis–Hastings algorithm (Ziadovitch et al., 2008). Following a burn-in of 5000 iterations, the distribution of the parameters of interest were sampled from the resultant Markov Chain. Convergence was assessed visually and compared to chains originating with different starting values (although the same MLE value of \( h \) was used in each case). Autocorrelation was also investigated by analysing sub-samples of the chain. The median and 95% CIs were sampled from 10,000 iterations of the stationary Markov chain.

3. Results

3.1. Summary data

The variations in cyst prevalence and abundance and the variation in numbers of protoscoleces with age are given in Table 1. The overall prevalence of infection was 64%.

![Fig. 1. The mean number of protoscoleces observed in each age class of sheep (solid bars). The open bars show the fitted model results together with their 95% credible intervals.](image)

Data regarding the mean number of protoxole-containing cysts, the numbers of protoscoleces they contained and their volume are given in Table 2. Both absolute numbers of cysts and mean cyst volume appeared to increase with age (Fig. 1, Table 1). Numbers of protoscoleces in fertile cysts increased with age. However, the lower range recorded did not show this dramatic increase with just 120 protoscoleces in the smallest cysts in old animals. However, the numbers of protoscoleces in the largest cysts increased dramatically with age, reaching 92,000 in the oldest sheep. Likewise the external diameters of cysts increased with age. In young sheep, the maximum external diameter was 2 cm, increasing to 8 cm in old sheep. The largest amount of fluid recovered from a single cyst was 50 ml.

3.2. Best fitting model

The model that provided the best fit was that where the radius of the cyst increased linearly with the age of the cyst and the number of protoscoleces was directly proportional to the volume (Table 3). This took the form:

\[
P_T = h \sum_{T=1}^{Y} \pi T^3
\]

where \( P_T \) is mean number of protoscoleces at age \( T \), \( h \) is the infection pressure in terms of numbers of cysts per year and \( \pi \) is a constant. There were two further parameters: \( \gamma \), relating to the variation of

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Table 3
Akaike Information Criterion (AIC) for the competing models for the increase in size of the cyst.

<table>
<thead>
<tr>
<th>Model for increasing cyst size [g(t)]</th>
<th>Number of free parameters</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear increase in the radius</td>
<td>4</td>
<td>3307.2</td>
</tr>
<tr>
<td>Logistic increase in the radius</td>
<td>7</td>
<td>3312.8</td>
</tr>
<tr>
<td>Exponential increase in the radius</td>
<td>5</td>
<td>3313.8</td>
</tr>
<tr>
<td>Asymptotic increase in the radius</td>
<td>5</td>
<td>3321.1</td>
</tr>
</tbody>
</table>

Fig. 2. The proportion of the total number of protoscoleces in the sheep population found in each age class of animals together with their 95% Confidence Intervals obtained by bootstrap iteration.

Table 4
Bayesian median values and their 95% credible intervals (CI) of model parameters for sheep from Kyrgyzstan.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median value</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>1.33</td>
<td>1.22</td>
<td>1.43</td>
</tr>
<tr>
<td>γ</td>
<td>0.25</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>a</td>
<td>2.56</td>
<td>2.19</td>
<td>3.07</td>
</tr>
<tr>
<td>kp</td>
<td>0.013</td>
<td>0.010</td>
<td>0.014</td>
</tr>
</tbody>
</table>

cyst aggregation with age (Eqs. (4) and (5)), and \( k_p \), the negative binomial constant related to the degree of aggregation of protoscoleces within cysts. These parameters and their 95% CIs are given in Table 4.

The numbers of cysts increased with age as did the prevalence of infection (Table 1). The numbers of protoscoleces in sheep increased dramatically with age. Sheep 1 year of age had a mean abundance of just 215 protoscoleces per sheep. This increased to 9774 in the oldest age group (Table 1). The actual data compared with the model fit is shown in Fig. 1. Approximately 80% of protoscoleces were recovered from sheep aged 4 years or older (Fig. 2). However, these sheep represented just 28% of the animals presented for slaughter.

4. Discussion

This study has developed the modelling of echinococcosis in the intermediate host further by modelling the numbers of protoscoleces in sheep. This is important as these represent the number of prospective adult *Echinococcus* that can be transmitted to dogs and hence is potentially more useful than analysing only cyst numbers. The very small numbers of protoscoleces in young sheep and the very high numbers in older sheep are a result of both increased numbers of cysts in old sheep and the time which acquired cysts have had to develop. Evidence for continuous development of cysts is in the data. In the oldest group of sheep, the lower limit of protoscoleces numbers per fertile cyst is low: only 120 (Table 2). This is lower than the mean number of protoscoleces per fertile cyst in 1-year-old sheep. However, the upper limit of protoscoleces increases relentlessly with age. Thus in 1-year-old sheep, the maximum number of protoscoleces found in a single cyst was just 482. This had increased to 92,000 by the time the sheep were 6 years or older. Likewise the lower limit of the volume for individual fertile cysts was approximately 0.2–0.5 ml in every age group, whilst the maximum volume was 1 ml in young sheep increasing to 62 ml in old sheep. Similar patterns were seen with the range of external cyst diameters. There is little other comparable data regarding the yield of protoscoleces from cysts and relative cyst size. Early work by Dew (1926) suggested that the diameters of cysts ranged up to 10 cm in old animals. In pigs, the size range for fertile cysts has been reported as between 1.5 and over 6.0 cm (Hutchinson and Lidetul, 2007). As these pigs were feral pigs from Australia they are likely to be infected with the sheep G1 strain of *E. granulosus*. In Kyrgyzstan, the G1 strain also appears to be the most important strain circulating between dogs and sheep (Ziadnov et al., 2008). Data from Turkey indicated that fertile liver cysts from sheep had a mean of 12,000 protoscoleces, with those from the lung had 5800 (Yildiz and Gurcan, 2003). This is consistent with the figures reported in the present study. A study in Greece reported a mean of 297 protoscoleces per animal (Himonas et al., 1994) which is somewhat lower than our findings of 1562 per animal. This study demonstrates how *E. granulosus* can compensate in terms of its biotic potential, for the relatively low egg output in infected dogs. Thus a single egg will result in a cyst containing 215 protoscoleces within 1 year, increasing to at least 12,000 by the time the cyst is 5–6 years of age, with an upper limit of at least 92,000.

Assuming the linear model of cyst acquisition, sheep aged 4 years and older have most of the infectious protoscoleces in the population. If it is assumed that the sampled population in the abattoir is representative of the age structure of sheep in this district in Kyrgyzstan then sheep aged 4 years and older have approximately 80% of the total protoscoleces, but constitute only 28% of the total sheep population. The bias introduced by the sampling technique could suggest that old sheep will contain an even higher proportion of protoscoleces. This result supports a theoretical control programme that would involve culling old sheep with controlled disposal of the resultant offal. Periodic treatment of dogs with praziquantel is a tried and successful method of control of echinococcosis. However such a programme requires many years of sustained intervention to be successful because of the substantial reservoir of *Echinococcus* in sheep which were infected before control started (Torgerson and Heath, 2003). These sheep can provide a continuous source of new infection for dogs for many years. If culling of older sheep was undertaken then a huge amount of infectious biomass could be removed from the system with the loss of a relatively small proportion of the sheep population. This would reduce transmission to dogs very quickly. Other studies such as that of Duerger and Gilman (2001), who found no viable protoscoleces in sheep less than 3 years of age provide further support for such an intervention programme. Selective culling is practiced for other zoonoses such as brucellosis (Minas, 2006). Such test and cull methods, however need a careful economic assessment of the likely effects. In the former Soviet Union, a strategy of dermatological testing sheep aged 5 years or older with a protoscoleces antigen was developed. Positive sheep were tagged, segregated and slaughtered as quickly as possible (Shaikenov, 2004). It was only possible to retain negative sheep in the flock. Because the prevalence in old sheep is very high, general testing of old sheep would not result in significantly fewer sheep being culled and would require a reasonably accurate diagnostic test. However for individual, valuable breeding animals it would be worth considering if such a programme was implemented. Modelling studies are being under-
taken to understand how such a culling programme would reduce transmission and if it would improve cost effectiveness by reducing the time required for control and if this would offset the likely higher initial costs.

It is not known how sensitive our method of palpation and visual examination was for the detection of hydatid cysts. It is possible that small cysts could have been overlooked in the initial examination at the abattoir. In this case this study might underestimate the numbers of small cysts that may contain few if any protoscoleces. Consequently in terms of the numbers of protoscoleces per sheep the present data may only slightly underestimate the true numbers. The resources to recover every liver and undertake a detailed examination including thinly slicing the livers was not available. Another possible source of bias may have occurred since the cyst analysis was not undertaken blindly: i.e., the individual who undertook the laboratory examination was also involved in cyst collection. However, all investigators are veterinarians and the individual undertaking the laboratory analysis had also received training in the relevant parasitological techniques so any such bias should have been ameliorated. Finally, the convenience of an abattoir sample rather than a true random sample was used in the study. The bias that this introduces is likely to be with the sample size of each age group presented as older animals are more likely to be slaughtered at home and young animals are more likely to fetch a good price at market. To acquire a true random sample would have entailed purchasing sheep randomly selected from farms and resources were not available for such a study.

Acknowledgements

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References


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