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

P.R. Torgerson [a,c,*], I. Ziadinov [a,b], D. Aknazarov [b], R. Nurgaziev [b], P. Deplazes [a]

[a] *Institute for Parasitology, University of Zürich, Winterthurerstrasse 266a., CH-8057 Zürich, Switzerland*
[b] *Kyrgyz Research Veterinary Institute Bishkek, Kyrgyzstan*
[c] *Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre, Saint Kitts and Nevis*

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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 could be found. The average for the sampled 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.

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

* Corresponding author. Address: Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre, Saint Kitts and Nevis. Tel.: +1 869 465 4161; fax: +1 869 465 1203.
E-mail address: p.torgerson@rossevt.edu.kn (P.R. Torgerson).

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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 protoscolex 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_t 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:

$$X_{cT} = hT \text{ (Roberts et al., 1986)} \tag{1}$$

This assumes that cysts, once acquired, never disappear.

Thus the total number of protoscoleces P_T in a sheep at age T will be:

$$P_T = h[g(T)]^3 + h[g(T-1)]^3 + \ldots + h[g(1)]^3 \tag{2}$$

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. (2) can be more simply written as:

$$P_T = h\sum_{T=1}^{T}[g(T)]^3 \tag{3}$$

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 Oj having 0 cysts in sheep of age T, assuming the probability distribution is a negative binomial distribution (Torgerson and Heath, 2003) is given by:

$$p(O_j = 0) = \left(\frac{k_T}{k_T + X_{cT}}\right)^{k_T} \tag{4}$$

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:

$$K_T = \gamma T \tag{5}$$

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

$$p(O_j = 0) = \left(\frac{\gamma T}{\gamma T + X_{cT}}\right)^{\gamma T} \tag{6}$$

The expected number of protoscoleces P_{Ti} in an individual sheep i of age T known to be infected with hydatid cysts is:

$$P_{Ti} = \frac{P_T}{1 - p(O_j = 0)} \tag{7}$$