Environmental distribution of *Echinococcus* and *Taenia* spp-contaminated dog faeces in Kyrgyzstan

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Abstract

Recently there have been epidemics of human cystic (CE) and alveolar echinococcosis (AE) in Kyrgyzstan. This study investigated two districts for the presence of *Echinococcus granulosus* s.l. and *E. multilocularis* eggs, species identity confirmed by PCR, in dog faeces and the level of environmental contamination with parasite eggs in 2017-2018. In the Alay district 5 villages with a high reported annual incidence of AE of 162 cases per 100,000 and 5 villages in the Kochkor district which had a much lower incidence of 21 cases per 100,000 were investigated. However the proportion of dog faeces containing *E. granulosus* s.l eggs was approximately 4.2% and 3.5% in Alay and Kochkor respectively. For *E. multilocularis*, the corresponding proportions were 2.8% and 3.2%. Environmental contamination of *Echinococcus* spp. eggs in canine faeces was estimated using the McMaster technique for faecal egg counts, weight and density of canine faeces. The level of environmental contamination with *E. multilocularis* eggs in both districts was similar at 4.4 and 5.0 eggs/metre$^2$ in Alay and Kochkor respectively. The corresponding values for *E. granulosus* s.l. were 8.3 and 7.5 eggs/metre$^2$, which was significantly greater than the estimates for *E. multilocularis*. There was no association between village or district level incidence of human AE or CE and the proportion of dog faeces containing eggs of *Echinococcus* spp. or the level of environmental contamination. An increase in the contamination of taeniid eggs in these regions occurs in the autumn, after the return of farmers with dogs from summer mountain pastures.

**Keywords:** *Echinococcus granulosus* s.l.; *Echinococcus multilocularis*; environmental contamination; McMaster method; dog faeces, *Taenia hydatigena, Taenia crassiceps, Taenia ovis*

https://doi.org/10.1017/S003118202300118X Published online by Cambridge University Press
Introduction

Alveolar and cystic echinococcosis are major zoonoses in Central Asia. Alveolar echinococcosis (AE) is caused by the parasite *Echinococcus multilocularis* whilst cystic echinococcosis (CE) is caused by *Echinococcus granulosus s.l.* Both diseases cause extensive human morbidity and alveolar echinococcosis is usually fatal if left untreated (Torgerson *et al.*, 2008). In Kyrgyzstan, there is now evidence for a major epidemic of AE (Usbahieva *et al.*, 2013; Raimkylov *et al.*, 2015; Counotte *et al.*, 2016; Paternoster *et al.*, 2020). The annual burden of echinococcosis in Kyrgyzstan for 2013 was estimated to be 11,915 [95% uncertainty interval (UI): 4,705 - 27,114] Disability Adjusted Life Years (DALY) for AE and 3,052 [95% UI: 1,508 - 5,205] DALY for CE (Counotte *et al.*, 2016).

Foxes (*Vulpes vulpes*) are one of the usual definitive hosts of *E. multilocularis*. Studies in foxes in Naryn district in Kyrgyzstan have demonstrated a prevalence of *E. multilocularis* infection of 64% (Ziadinov *et al.*, 2010). Dogs have been shown to be suitable definitive hosts for *E. multilocularis* with similar egg reproduction as in foxes (Kapel *et al.*, 2006). In dogs in Naryn district, the prevalence of *E. granulosus s.l.* has previously been reported to be approximately 18%, whilst that of *E. multilocularis* is approximately 19% (Ziadinov *et al.*, 2008). *Echinococcus* spp. have complex life cycles where carnivore hosts play an important role in the transmission in Kyrgyzstan. It is hypothesized that in Kyrgyzstan the dog plays an important role in the zoonotic risk, however the wild animal cycle including foxes functions as a reservoir for *E. multilocularis* (Ziadinov *et al.*, 2008, 2010; Mastin *et al.*, 2015; Van Kesteren *et al.*, 2017).

It has been hypothesized that an increase in human CE and AE in Central Asia may be related to the dissolution of the Soviet Union in 1991 with fundamental sociocultural, economic and structural changes (Torgerson, 2013). Furthermore, a decline in the activities of veterinary services, including a decrease or absence of veterinary control over meat.
production. The process of domestic slaughter has increased, as well as the privatization of large livestock farms. A further consequence of the crisis has been increasing human poverty (Torgerson, 2013) along with an increase in the number of dogs in both rural and urban areas (Torgerson et al., 2006).

The first case of human AE in Kyrgyzstan was diagnosed in 1996 but until 2003, only small numbers of cases were registered annually. The number of reported cases has increased significantly since 2004 (Usubalieva et al., 2013). Human AE has a prolonged latent period. The epidemic of human AE started approximately 15 years after the dissolution of the Soviet Union. Therefore, it is hypothesized that the socio-economic changes that lead to the increase in incidence of CE, also created the conditions that allowed the colonization of dogs by *E. multilocularis*. This has resulted in an onward transmission to humans due to the close contact between dogs and human and hence an epidemic of human AE (Torgerson, 2013).

In Kyrgyzstan, the national annual incidence of reported human cases of CE 25 years ago was approximately 4 cases per 100,000. In recent years it is now approximately 15 per 100,000. Of serious concern is that the much more pathogenic form of AE, has emerged as a major public health problem. During the last 20 years AE has increased to approximately 200 reported cases per year (Usubalieva et al., 2013; Raimkylov et al., 2015; Counotte et al., 2016; Paternoster et al., 2020). In certain districts, very high prevalences of human AE have been detected by ultrasound surveillance. For example, an ultrasound study in the south of Kyrgyzstan (Bebezov et al., 2018) documented a prevalence of AE of 4.2%.

The aim of this study is to investigate the environmental contamination of taeniid eggs in high and lower incidence areas for AE. We also wished to investigate seasonal associations which may give indications of transmission and if control could be targeted. We also hypothesized if there may be a link between the contamination with eggs of *E. multilocularis* in public areas and the local incidence of human AE and if dogs, are the main source of
infection for the human population.

**Material and methods**

**Study area and sample collection**

For the study, villages were selected based on human incidence data (Paternoster *et al.*, 2020). Five villages were selected in the Alay district where the reported incidence of AE between 2014-2016 was amongst the highest in Kyrgyzstan. For comparison 5 villages were selected in the Kochkor district which has a substantially lower reported incidence of human AE. Terek, Sopu-Korgon, Sogondu, Kun-Elek and Chii-Talaa villages were explored in Alay district; Mantysh, Komsomol, Don-Alysh, Chekildek and Ak-Talaa villages in Kochkor district (Figure 1). For each village, samples of all fresh dog faeces were collected and assigned a specific identification number. Fresh faeces were intact faeces that had the appearance of being recently deposited. For older faecal samples, which were dried out with some evidence of degradation, the number and position were recorded. Dog faeces were identified by morphology, size, and content. The GPS coordinates of all observed faecal samples (fresh and old) were recorded. It was only possible to access public parts of the villages for sample collection and not, for example, enclosed private gardens. The area of the parts of the village that was accessible, and samples taken was estimated using the Google Maps Area Calculator Tool (Frančula, 2020).

All samples were frozen for 5 days at minus 80 °C prior to analysis for safety reasons.

**Mapping of surgical cases**

The national surveillance system for surgical cases of human echinococcosis compiled by the State Sanitary and Epidemiological Service in Bishkek, Kyrgyzstan was used. This data has been previously reported by Paternoster *et al.* (2020). For this study the community level
incidence of AE was used to explore if there was any association with the level of *Echinococcus* spp. contamination in dog faeces and density of environmental contamination and with human incidence was used.

**Egg isolation**

The eggs were isolated according to a previously described study (Mathis *et al*., 1996) with some modifications. In brief, the samples were thawed, afterwards 2 grams of faeces were weighed into a 15 ml Falcon tube. Subsequently, a 1:4 solution of PBS-Tween-Na\(\text{N}_3\) was added, shaken vigorously for 1 minute and centrifuged for 10 minutes at 500 g. After removing the supernatant, the pellet was re-suspended in 8 ml of zinc chloride solution (density 1.45), then the samples were shaken again and centrifuged for 30 min at 400 g. Afterwards, the supernatant was passed through nylon meshes with a mesh size of 40 and 21 \(\mu\)m (Lanz-Anliker, Switzerland). A 21 \(\mu\)m nylon mesh was used to collect the taeniid eggs. The sediment was placed in a flat-walled Nunc tube and examined for the presence of taeniid eggs using an inverted microscope. Positive samples were collected and centrifuged at 500 g for 5 min.

**DNA Isolation from taeniid eggs**

An isolation of the DNA was achieved by using a method adapted from that previously described (Trachsel *et al*., 2007). In brief, the pellet was transferred into a 1.5 ml Eppendorf tube and centrifuged for 1 minute at 8000 g. The supernatant was removed, and the pelleted eggs resuspended in 200 \(\mu\)l distilled water. Then 25 \(\mu\)l of 1M potassium hydroxide (KOH) and 7 \(\mu\)l dithiothreitol (DTT) were added to the sample and vortexed, followed by an incubation at 65°C for 15 minutes.

Furthermore, 60 \(\mu\)l 2M Tris-HCL pH 8.4 and 2 \(\mu\)l concentrated HCL (12.4N/>37%)
were added. After the addition of 200 µl of buffer AL (QIAamp DNA Mini Kit, Qiagen) and 20 µl Proteinase K, the sample was incubated at 56°C for 10 minutes. 50 µl of Chelex (50%) was added and the mix was put on a rotor at room temperature between 30 minutes and 3 hours. The tubes then were centrifuged 16000 g for 1 min. and the supernatant was transferred to a fresh tube together with 200 µl pure ethanol. Everything was vortexed and spun down.

The samples were applied to Qiagen spin columns and centrifuged for 1 minute at 8000 g. The columns were washed with 300 µl buffer AW1 and centrifuged for 1 minute at 8000 g. This wash step was repeated once again. Then the columns were washed with buffer AW2 and centrifuged for 1 minute at 8000 g. After a repetition of the wash step with AW2, the columns were centrifuged empty at full speed for 3 minutes. Next, the columns were placed in a 1.5 ml Eppendorf tube, 100 µl AE elution buffer was added and incubated for 1 minute at room temperature, to elute the DNA from the membrane. As a last step, the columns were centrifuged for 1 minute at 8000 g and the flow-through was collected.

**Polymerase chain reaction (PCR)**

For the PCR a multiplex PCR adapted to a previous protocol (Trachsel et al., 2007) with a commercial kit (Qiagen Multiplex PCR Kit, Qiagen, Hilden, Germany) was used. First a mastermix containing 25 µl Qiagen Mastermix, 18 µl distilled water, both from the multiplex PCR Kit, as well as 5 µl primermix were prepared in PCR tubes. The primermix is composed of 80 µl Cest5 primers and 10 µl from Cest1,2,3,4 primers as well as 380 µl distilled water. Primers Cest 1 (5′- TGC TGA TTT GTT AAA GTT AGT GAT C-3′) and Cest2 (5′-CAT AAA TCA ATG GAA ACA ACA AG-3′) amplified a 395 bp stretch of the *nad1* gene of *E. multilocularis*, primers Cest3 (5′-YGA YTC TTT TTA GGG GAA GGT GTG GTG-3′) and Cest5 (5′-GCG GTG TGT ACM TGA GCT AAA C-3′) amplified a 267bp stretch of the *rrnS*...
of *Taenia* spp., and primers Cest4 (5′-GTG TTT GTG TGT TAC ATT AAT AAG GGT G-3′) and Cest5 amplified a 117bp stretch of the *rrnS* of *E. granulosus* (*s.l.*). Finally, 2 µl of DNA solution was added. To confirm the validity of the PCR, positive as well as negative controls were implemented.

The cycling conditions were 15 minutes at 95°C, 30 seconds at 94°C, 90 seconds at 58°C with a repetition of 40 cycles and another 10 minutes at 72°C.

**Sequencing**

For a more accurate determination of the taeniid species, all sequencing was made at Microsynth Switzerland. Therefore, the DNA was purified by using a commercial kit (MinElute PCR Purification Kit, Qiagen) and sent for sequencing.

**Density of contamination**

Estimates of the faecal egg counts were made using the McMaster technique (Deplazes and Eckert, 1988a). For estimations of the mean faecal egg count only samples with mono-infections with taeniids, with *E. granulosus s.l.*, *E. multilocularis* or *Taenia* spp. (species not determined), respectively, were used. These infections were diagnosed by PCR as described above.

To calculate the average mass of a canine faecal samples, 50 samples were collected, randomly weighed, and the mean weight was calculated. Total numbers of eggs were the eggs per gram multiplied by the average faecal weight. Density of contamination was estimated according to:

\[
\frac{epg \times weight \times px \times total}{area} \quad (1)
\]

Where,
epg = mean eggs per gramme of *E. granulosus* s.l., *E. multilocularis* or *Taenia* spp. eggs

*weight* = mean weight of a single faecal sample

*pv* = proportion of fresh faecal samples containing *Echinococcus* spp. or *Taenia* spp. (including mixed infections)

*total* = total number of faecal samples found (fresh and old)

*area* = sample area from which faeces were searched and collected.

**Statistical analysis**

For the statistical evaluation the statistic program R (R Core Team, 2022) was used. Poisson regression was used to examine if there was any association between the prevalence of *Echinococcus* spp. in the canine faecal samples or the level of contamination with parasite eggs and the incidence of echinococcosis from the national surveillance reports.

For the egg contamination per metre$^2$, the faecal egg counts were analyzed using the eggCounts package in R (Torgerson *et al.*, 2014). Uncertainty in the total egg contamination was estimated by using equation (1). Here the uncertainty in the proportion of faecal samples containing parasite eggs was estimated by using 2000 beta random variables from Beta $(a+1,b+1)$ where $a$ is the number of fresh faecal samples positive for *Echinococcus* spp. or *Taenia* spp., and $b$ are the number of fresh faecal samples that were negative. Uncertainty in the faecal egg counts was calculated by taking 2000 draws from the posterior distribution from the single sample egg count function in eggCounts. Each of these random draws were analyzed in equation (1). The 2.5%, 50% and 97.5% quantiles of the distribution of the 2000 resulting samples were estimates of the median and 95% confidence limits of the faecal egg contamination in terms of eggs per metre$^2$. All data and R code are provided as supplementary files 1&2.

https://doi.org/10.1017/S003118202300118X Published online by Cambridge University Press
Results

Taeniid egg contamination in fecal samples of dogs

A total of 2013 fresh samples of dog faeces was examined. In 53 samples *E. multilocularis* was detected (of which 10 were mixed infections with *Taenia* spp.), 54 samples with *E. granulosus s.l.* (of which 27 were mixed infections with *Taenia* spp.) and 285 samples positive for *Taenia* spp. (of which 37 were mixed infections with *Echinococcus* spp.). Results of all samples are summarized in the table 1.

A total of 7065 old samples of dog faeces were detected and recorded. The minimum number of old samples of dog faeces was in February in the Alay district, which can be explained by the large amount of snow that fell during that period. Also, the minimum number of old samples of dog faeces was in the Alay district in June, since at that time more dogs were with farmers on summer pastures in the mountains. The maximum number of old samples of dog faeces in the Alay district was found in September and November.

In the Kochkor district, fewer old dog faecal samples were found in June than in other months (September, November, and February), as at that moment more dogs were on summer pastures in the mountains with farmers.

After PCR testing of dog fecal samples, we selected 298 samples for the quantitative egg counts by the McMaster method, 26 samples with *E. granulosus s.l.*, 42 with *E. multilocularis* and 230 with *Taenia* spp. The mean eggs per gramme were 1607 (CI 1188-2144) for *E. granulosus s.l.%; 1287 (CI 1009-1623) for *E. multilocularis* and 1199 (CI 1092-1314) for *Taenia* spp. There was little variation in the proportion of samples in which *Taenia* spp. and *Echinococcus* spp. eggs were found across the 4 sampling periods. Only between September 2017 and February 2018 an apparent decrease in the proportion of samples containing *E. granulosus s.l.* eggs (p<0.01, Figure 2) was observed.

There was some heterogeneity in the level of contamination of eggs of *E.
*Echinococcus multilocularis*, which ranged from 17 eggs per metre$^2$ (CI 8.6-31) for the village of Chekildek in Kochkor district, to several villages having no *E. multilocularis* detected. There were no significant differences between the level of contamination in villages in Kochkor district compared to villages in Alay district. There was some evidence of decreased contamination of the environment in the winter months in the Alay district, whereas in the Kochkor district there was some evidence of increased levels of contamination (Figure 3).

For *E. granulosus* s.l. there was also considerable heterogeneity with the level of contamination ranging from 21 eggs per metre$^2$ (CI 9.9-40.5) for the village of Chekildek in the Kochkor district to 2.9 eggs per metre$^2$ (CI 0.9-7.2) in Chii-Talaa in the Alay district (Figure 4). There was some evidence of lower contamination rates in both districts during the winter (Figure 4).

Contamination with *Taenia* spp. eggs decreased from the autumn throughout the winter before increasing again the following autumn in the Alay district (Figure 5). In Kochkor district, there was a continuous decrease during the period of this study.

There was no association with the level of environmental contamination in public areas, and the reported village level incidence reported in the national surveillance system. There was no evidence for a difference in the proportion of dog faeces containing *E. multilocularis* eggs in the high incidence district of Alay in comparison to the lower incidence district of Kochkor. The same is true in respect to the level of contamination. There was also no evidence of an association between the proportions of dog faecal samples containing *E. granulosus* s.l. eggs and the incidence of CE in the human population ($p>0.05$). Likewise in respect to the level of contamination. Summary data together with the proportion of dog faecal samples containing *Echinococcus* spp parasite eggs isolated from dog faeces and the level of contamination is given in table 2.
Sequencing of randomly selected positive Taenia spp. faecal samples of dogs

In order for the Taenia species to be identified, 15 samples from each year were randomly selected and sequenced (Trachsel et al., 2007). Of these 30 samples, 26 were Taenia hydatigena, 3 were T. crassiceps, and 1 T. ovis.

Discussion

Widespread contamination of village public spaces with dog faeces containing eggs of *E. multilocularis*, *E. granulosus* and *Taenia* spp was demonstrated. This represents a source of infection for the human population for *Echinococcus* spp and *T. crassiceps*. However, it was not possible to detect any association between the intensity of environmental contamination and the officially reported incidence of AE or CE. The incidence of AE in the 5 villages of the study area in Alay district was over 7 times higher compared to the study villages in the Kochkor district (table 3). Yet there was a very similar proportion of dog faeces containing of *E. multilocularis* eggs and similar levels of contamination. Likewise, no differences could be detected in the proportion of faecal samples with eggs of *E. granulosus s.l.* or the level of contamination. This would support the hypothesis that a direct environmental contamination of public areas is not the main driver of this epidemic of human echinococcosis in Kyrgyzstan. However, a weakness of our approach was our method for estimating the level of contamination in the villages. We were not able to access considerable parts of the villages, such as private gardens, to collect samples and check for faecal contamination. It is possible that these areas had a different intensity of contamination compared to the areas we were able to access, and this could generate bias. Arguably, there might also be greater contact between dog faeces and humans in areas such as private gardens which might be a greater driver of transmission. However, it is worth noting that there appears to be quite considerable accumulations of dog faeces in places where local people frequent, often such as around the
village school, mosque or shops, where we were able to obtain samples (see figures in supplementary file 3). It was also assumed that the faces observed and/or collected were dog faeces based on the morphology. It is possible that some may have been faeces of foxes or other wild carnivores. Ideally DNA analysis of the samples would confirm the species origin of the samples. In Tibetan plateau village communities, which have a similarly high incidence of human AE as the villages in the present study, 13 of 155 carnivore faecal samples (8.3%) were shown to be fox faces by PCR analysis. The remainder were dog faeces (Vaniscotte et al. 2011). However, regardless of this the estimated contamination levels would be valid.

There is also a long latent period between infection and the development of the disease. Therefore, cases reported in the national surveillance system will represent infection events some time, possibly years previously. Thus, the levels of contamination may have changed between when the infection event occurred and the detection of human cases through surveillance. It is also assumed that the reporting of cases to the national surveillance system is accurate and has no under reporting in either of the two districts. Nearly all cases of AE and CE are treated in either Bishkek or Osh. Suitable medical facilities are not available in the rural areas where this study was undertaken. But the patient origin is reported from the hospital records, and this would suggest there is minimal bias in reporting, as nearly all reports originate from these main medical centers. In the Alay district an ultrasound surveillance study was previously undertaken (Bebezov et al., 2018) and reported an AE prevalence of 4.2% in that district, which is consistent with the very high reported incidence of treated cases.

Nevertheless, our data might also point to other sources of infection. The water supply has been shown to be a risk factor for human echinococcosis and a systematic review and a meta-analysis has suggested it may represent a considerable attributable fraction for human
echinococcosis (Torgerson et al., 2020). Investigations into potential contamination of the water supplies of these villages are ongoing. It is noteworthy that much of the population of these villages does not have access to a safe and sanitary water supply.

There was some, albeit inconsistent evidence for seasonal variations in the contamination of village spaces. Thus, in the Alay district, the contamination levels for both Echinococcus species and for Taenia spp. declined in the winter before recovering by the following autumn. It can be hypothesized that the large number of eggs of the parasites in the autumn period of the year is due to the fact that dogs descend with farmers from summer pastures to villages before the onset of winter. Farmers on summer pastures in the mountains slaughter sheep themselves, they feed the affected organs with echinococcosis cysts (lungs and liver) to dogs. Dogs may also prey on small mammals which are a natural reservoir of E. multilocularis and T. crassiceps. Data of the results of a previous study (Abdyjaparov and Kuttubaev, 2004) indicate the presence of AE invasion in 10 species of rodents in alpine districts in Kyrgyzstan. These included the area around Sari Tash (altitude 3170 metres), where E. multilocularis infection of 0.8% of rodents was documented. This included 4% of red marmots (Marmota caudata). In Sary Mogal (altitude 2980 metres), which is a neighbouring settlement to Sari Tash, the study by Babesov et al (2018) documented a high prevalence of human AE. The result shows that the majority of Taenia infections were T. hydatigena, a species maintained in a domestic cycle mainly with dogs and sheep and goats, ie the same as E. granulosus, on the other hand T. crassiceps has a cycle comparable with E. multilocularis. Three of 30 Taenia spp samples sequenced were shown to be T. crassiceps which is further evidence that domestic dogs are preying on small mammal species. However there were insufficient numbers of T. crassiceps samples to infer any differences in predation behaviour of dogs in different villages. T. crassiceps cysticercosis has occasionally caused severe disease in humans (Deplazes et al, 2019).
An interesting observation was that in faecal samples only eggs of *E. granulosus* s.l., had significantly higher egg count than samples containing *Taenia* spp. Gemmell (1987) demonstrated that *T. hydati digna* had a substantially higher biotic potential than *E. granulosus* s.l. Dogs infected with *T. hydatigena* producing approximately 38,000 eggs per day compared to an estimated 8,470 eggs per day for dogs infected with *E. granulosus*. The majority of the *Taenia* spp samples that were sequenced were shown to be *T. hydatigena*. Thus it could be expected that the intensity of egg contamination of faeces of dogs infected with *Taenia* spp would be far higher than those infected with *E. granulosus* s.l. This anomaly is likely explained by the fact that most *Taenia* spp eggs remain in the proglottids following expulsion from an infected dog, and the proglottids either are voided separately from the faeces or migrate from the faces following defaecation by the dog. Deplazes and Eckert (1988b) observed that only 36% of the expelled proglottids from dogs experimentally infected with *T. hydatigena* were associated with defecation. Further analyses revealed that only 16% of the total estimated number of eggs were recovered from the faeces, the remainder were excreted independently of defecation with the proglottids. Assuming this is the case in the present study then the total number of eggs voided by an infected dog would be over 6 times the numbers detected in faecal samples. This then reconciles the results of the present study to earlier studies demonstrating the much higher biotic potential of *T. hydatigena* compared to *E. granulosus* s.l.

Dogs are the main definitive host for *E. granulosus* s.l. Data suggests that, for *E. multilocularis*, they may be an important source of infection for human AE (Torgerson et al., 2020). Consequently, regular treatment of dogs with praziquantel is an important intervention to avoid human disease (Savioli et al., 2011). The efficiency of an intervention program in which dogs are medicated depends on the number of registered dogs. Furthermore, the acceptance of the community as well as the access to households are decisive points for the
efficiency of such an intervention program (Savioli et al., 2011). An aggravating factor in the control of these zoonotic diseases is the traditional movement of livestock. The farmers move their animals between the valley and the pastures for 6-8 months per year in order to find rich grazing land. With the livestock also the dogs move each season which increases the possibility of a reinfection (World Bank, 2011).

Although this study found that there is no obvious correlation between the increase in AE (and CE) cases in humans and the contamination with *E. multilocularis* (or *E. granulosus s.l.*) in dog faeces in public areas, the role of the dog cannot be excluded given the increase in human AE. To clarify this question conclusively, further parameters need to be examined. The involvement of wild definitive hosts contaminating the environment, changes in the infection cycle and in the pathogenicity of the parasite, the susceptibility of the population as well as changes in environmental factors are only a few parameters that need to be investigated in more detail.

It has been argued that humans are relatively resistant to AE. This might result in the low incidence of human AE in Switzerland despite widespread contamination of the environment with fox faeces and hence potential exposure of the population (Gottstein et al., 2015). However, there are subtle suggestions in our results that may provide evidence against this hypothesis. The contamination of the public areas with *E. multilocularis* eggs is similar in the two districts, yet there are markedly differences in the reported human incidence of disease, although different levels of contamination in private gardens can not be discounted. Both populations are ethnic Kyrgyz and it would seem unlikely that one population would be markedly more resistant to the parasite than the other. Furthermore, the level of contamination with *E. granulosus s.l.* seems to be somewhat higher that *E. multilocularis* in the Alay districts. Yet in Alay there is a considerably higher incidence of AE than CE, which may point to a greater susceptibility to AE.
Acknowledgments. The author thanks the Swiss School of Public Health, European Union's Horizon 2020 research and innovation program under grant agreement no. 801076.

Author’s contribution. Conceptualization, KA, PT; Investigation, KA, PK, IM, GP, PD, PT methodology, KA, PT; software, PT; laboratory analyses, KA, PK; formal analysis; Funding acquisition, PT, PD; resources, KA, PD, PT; writing—original draft preparation, KA, PT; writing—review and editing, KA, PK, GP, PD, PT; All authors have read and agreed to the submitted version of the article

Financial support. This research was funded by the Swiss National Science Foundation, grant number: 173131-“Transmission modelling of emergent echinococcosis in Kyrgyzstan”.

Competing interests. The authors declare there are no conflicts of interest.

Ethical standards. Not applicable

Supplementary material. The supplementary material for this article can be found at DOI
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Parasitology 20, 431-456


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Table 1. Overview of the number of positive taeniid samples in dog faeces (2017-2018)

<table>
<thead>
<tr>
<th>Name of village</th>
<th>Total old faecal samples</th>
<th>Total fresh faecal samples</th>
<th>E. multilocularis</th>
<th>E. granulosus s.l.</th>
<th>Taenia spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terek</td>
<td>494</td>
<td>183</td>
<td>8</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>Sopu-Korgon</td>
<td>589</td>
<td>184</td>
<td>0</td>
<td>11</td>
<td>59</td>
</tr>
<tr>
<td>Sogondu</td>
<td>811</td>
<td>193</td>
<td>5</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Kun-Elek</td>
<td>384</td>
<td>204</td>
<td>0</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Chii-Talaa</td>
<td>611</td>
<td>210</td>
<td>13</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Mantysh</td>
<td>764</td>
<td>197</td>
<td>3</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Komsomol</td>
<td>963</td>
<td>241</td>
<td>14</td>
<td>13</td>
<td>53</td>
</tr>
<tr>
<td>Don-Alysh</td>
<td>887</td>
<td>207</td>
<td>6</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td>Chekildek</td>
<td>725</td>
<td>195</td>
<td>8</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Ak-Talaa</td>
<td>837</td>
<td>199</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 2. Proportion of faecal samples containing *Echinococcus* spp. eggs and the level of contamination in public areas in the high and low incidence districts.

<table>
<thead>
<tr>
<th>District</th>
<th>Incidence AE(^1)</th>
<th>Incidence CE(^1)</th>
<th>Proportion (%) of samples positive for <em>E. multilocularis</em>(^2)</th>
<th>Cumulative contamination, <em>E. multilocularis</em>(^3)</th>
<th>Proportion (%) of samples positive for <em>E. granulosus</em> s.l.(^2)</th>
<th>Cumulative contamination, <em>E. granulosus</em> s.l.(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alay</td>
<td>162</td>
<td>42</td>
<td>2.8 (1.8-4.0)</td>
<td>4.4 (2.7-6.6)</td>
<td>4.2 (3.0-5.6)</td>
<td>8.3 (5.4-12.5)</td>
</tr>
<tr>
<td>Kochor</td>
<td>21</td>
<td>25</td>
<td>3.2 (2.3-4.5)</td>
<td>5.0 (3.2-7.5)</td>
<td>3.5 (2.4-4.7)</td>
<td>7.5 (4.9-11.7)</td>
</tr>
</tbody>
</table>

1. Annual incidence per 100,000 population
2. Proportion of canine faecal samples (%) (95% CI)
3. Estimated total eggs per metre\(^2\) from September 2017-November 2018 (95% CI)
Figure 1. Location of the expedition points in Kyrgyzstan and separately on top of the Kochkor (low AE incidence) district and on the left side of the Alay (high AE incidence) district.
Figure 2. Proportion of faecal samples (+/- 95% confidence intervals) containing *E. granulosus s.l.*, *E. multilocularis* and *Taenia* spp. eggs across the 4 sampling periods.
Figure 3. Estimated contamination of *E. multilocularis* eggs in canine faeces. Alay district (left), Kochkor district (right).
**Figure 4.** Estimated contamination of *E. granulosus* s.l. eggs in canine faeces. Alay district (left), Kochkor district (right).
Figure 5. Estimated contamination of *Taenia spp.* eggs in canine faeces. Alay district (left), Kochkor district (right).