The impact of socio-cultural factors on transmission of *Taenia* spp. and *Echinococcus granulosus* in Kosovo

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SUMMARY

Echinococcus granulosus sensu latu (s.l.) and *Taenia hydatigena* are common parasites of ruminant intermediate hosts in the Balkans. Transmission is linked mainly to home slaughtering and the feeding of infected organs to dogs. In Kosovo, many old sheep are slaughtered particularly during Eid al-Adha (Feast of Sacrifice). To determine whether this tradition could affect parasite transmission, we compared the probability of 504 dogs to contract taenid infections after deworming during one period before Eid al-Adha and a similar period beginning with this event. Initially, taeniid eggs were detected in 6·2% (CI 4·2–8·6) of the dogs. The prevalence before Eid al-Adha was significantly lower (1·2%, CI 0·4–2·6) as compared with the prevalence after the event (4·3%, CI 2·6–6·3). A comparable trend was apparent at species level for *T. hydatigena* and *E. granulosus*. These results indicate that the pronounced increase of taeniid infections, including *E. granulosus* s.l., after Eid al-Adha is linked to traditional home slaughtering that occurs during this celebration. This particular epidemiological situation provides an opportunity for implementing focussed control activities.

Key words: Taenia, *Echinococcus granulosus*, echinococcosis, dogs, control.

INTRODUCTION

Cystic echinococcosis (CE) and cysticercosis are diseases caused by the larval stages of *Echinococcus granulosus* sensu lato (s.l.) and *Taenia* spp. Dogs and other carnivores are definitive hosts for a variety of taeniid species, while a large number of herbivores and omnivores are intermediate hosts. CE represents a persistent, emerging or re-emerging zoonosis in many rural areas worldwide, especially where humans cohabit with dogs and sheep (Craig *et al.* 2015). Epidemiological factors associated with an increased risk of *E. granulosus* s.l. and *Taenia* spp. infections in dogs are feeding on raw viscera, home slaughtering of livestock without meat inspection, inadequate systems for the elimination of by-products, free access of dogs to raw carcasses and offal of fallen livestock or wild animals, lack of anthelminthic treatment of dogs and owners' poor health education (Craig and Larrieu, 2006; Otero-Abad and Torgerson, 2013). CE is considered endemic in different Balkan countries (Todorov & Boeva, 1999; Sotiraki & Chaligiannis, 2010; Calma *et al.* 2011; Pilaca *et al.* 2014) but only limited information is available about the situation in Kosovo. However, based on the reporting of 163 CE patients from all over Kosovo (75% living in rural and 25% in urban areas) treated at the University Clinical Center of Prishtina between 1999 and 2001, a minimal average annual incidence of 2·7/100 000 inhabitants can be calculated (F.T. Hoxha, personal communication). This incidence probably underestimates the real epidemiological situation.

According to the Kosovo Agency of Statistics, 2014 (available from http://ask.rks-gov.net/en/ag-2014), Kosovo has relatively low populations of sheep (183 584), cattle (261 689) and goats (28 430) and has an estimated number of around 55 000 owned dogs and an unknown number of ownerless dogs. Besides professional slaughtering in abattoirs, home slaughtering of sheep and cattle for private meat consumption is traditionally performed not only by farmers but also by a large part of the population in rural and in some urban areas.

In Kosovo, several religious communities coexist (Islamic 95·6%, Christian 3·9%; the Kosovo Agency of Statistics, 2011, available from http://ask.rks-gov.net/en/census-2011). An important celebration of the Muslim community is the Eid al-Adha (Feast of Sacrifice). The celebration honors the willingness of the prophet Ibrahim, to sacrifice his only son, Ismail, as an act of submission to God's command but God stopped Ibrahim and gave him a ram to sacrifice instead. To celebrate this festival,

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commonly sheep are sacrificed but, sometimes, also cattle, goats or camels. The sacrificed animals must meet quality standards and the sheep or goat has to be at least 1-year old. In Kosovo, the meat of animals slaughtered for this event is traditionally divided into three parts; the family retains one-third of the share, another third is given to relatives, friends, or neighbours and the remaining third is given to poor and needy people. Approximately 1000 cattle and 30 000 sheep are slaughtered during Eid al-Adha, and this event represents an excellent opportunity for sheep farmers to sell their animals, especially older sheep not suitable for further lamb production. The slaughtering is predominantly performed at home, very often without veterinary control and proper elimination of offal. As old sheep have been shown to harbour the majority of the *E. granulosus* protoscolex burden in their cysts (Torgerson *et al*. 2009), they represent the major source of infection for the owned and ownerless dog population in Kosovo.

In Kosovo dogs are kept as companion animals for protection, guarding or hunting and they are very common in rural areas, and are kept especially by sheep breeders. Whether or not dog ownership or contact with dogs are risk factors for human CE is controversial. Some studies could not find any significant associations (Carmona *et al*. 1998; Dowling and Torgerson 2000; Rafiei *et al*. 2007; Harandi *et al*. 2011), while other studies did (Campos-Bueno *et al*. 2000; Moro *et al*. 2008). In Kosovo, as in the neighbouring Balkan countries, the dog populations are not registered and free roaming and ownerless dogs easily have access to uncooked offal and carcasses. Furthermore, the lack of knowledge on CE and the absence of strategic deworming of dogs contribute to the persistence of the infection pressure even in hunting and pet dogs. In 2003/04, the prevalence of *Taenia* spp. and *E. granulosus* (sheep strain, G1–G3) in naturally infected pet dogs, sheepdogs and hunting and ownerless dogs was 7·5% (CI 4·8–11·1) and 1·3% (CI 0·4–3·3), respectively, in Kosovo (Sherifi *et al*. 2011). The occurrence of CE in slaughtered cattle from all over the country was high (72%), indicating a high environmental contamination with *E. granulosus* s.l. eggs (Hamidi *et al*. 2010).

The objective of the present study was to determine the risk of transmission of *Taenia* spp. and *E. granulosus* in dogs associated with the high slaughtering activity during Eid al-Adha and to develop a strategy for the monitoring and of controlling taeniid infections in dogs.

METHODS

Study area

Kosovo is located in the central Balkan peninsula in Southeastern Europe, has continental and Mediterranean climate zones, covers 10 908 km^2 and had a human population of 1 739 825 in 2011 (the Kosovo Agency of Statistics, ASK, 2011, available from http://ask.rks-gov.net/en/census-2011).

To assess the occurrence of *Taenia* spp. and *E. granulosus* s.l. in dogs, fecal samples were collected in 21 randomly selected municipalities (out of a total of 38) with comparable dog keeping conditions and practices in the regions of Prishtina, Mitrovica, Prizren and Ferizaj. These four regions cover 4884 km^2 or 44·8% of the total country's surface.

Sampling

As the dog population is not officially registered, private veterinary practices, which are licensed by the government, were contracted to recruit dog owners in randomly selected villages. The veterinarians were supervised by the two first authors of this study and supplied with questionnaires, sterile plastic cups, sterile wooden spatulas, gloves and praziquantel tablets. Fresh fecal samples of chained dogs were collected from the ground within the area accessible to them or from inside their cages or, in the case of unchained single dogs, from their defecation places pinpointed by the owners.

The study was carried out between January 2012 and March 2013. To determine whether the Eid al-Adha event could affect parasite transmission, we compared the probability of 504 dogs from 272 owners to contract taenid infections after deworming during one period before Eid al-Adha (pre-event period) and a similar period beginning with this event (event period). For practical reasons the sampling periods lasted between 73 and 110 days. After each collection, all dogs were orally treated with praziquantel tablets (5 mg kg^{-1} body weight, 50 mg tbl^{-1}. Prazikvantel®, Veterinarski Zavod, Zemun), provided by the Kosovo Food and Veterinary Agency.

Baseline samples were collected when dogs were dewormed for the first time between 31 January and 20 May 2012 (baseline period). Samples of the pre-event period were collected roughly 5–7 months (mean 182 days, SD ± 27 days, range 114–249 days) after the initial deworming from 30 August to 17 October 2012 (pre-event period) when dogs were dewormed for the second time. The Eid al-Adha event was celebrated on the 26 October 2012 and the event samples were collected roughly $4^{1/2}$–6 months after the second deworming (mean 162 days, SD ± 20 days, range 92–215 days) from the 11 January to 25 March 2013 (s. Table 1). Thus, the time between deworming and sample collection was always considerably longer than the prepatency periods of *T. hydatigena* (57–71 days, Deplazes *et al*. 1990) or *E. granulosus* s.l. (34–58 days; Thompson, 2017).

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Table 1. Time line of the sampling periods and the Eid a-Adha event

Periods/event	Mean date	Sampling from	to	Duration (days)
Baseline period	11.03.2012	31.01.2012	20.05.2012	110
Pre-Eid al-Adha period	08.09.2012	30.07.2012	12.10.2012	74
Eid al-Adha event	26.10.2012[a]	(no sampling)	(no sampling)	3
Post-Eid al-Adha	21.02.2013	15.01.2013	29.03.2013	73

[a] Eid al-Adha lasts 3 days. However, usually sheep are slaughtered on the first day of the celebration.

Sample testing

Flotation technique for detection of taeniid eggs. Private veterinarians working in the investigated areas collected fecal samples (around 5–10 g) in plastic tubes. After collection the samples were natively stored in refrigerators at 4–8 °C and sent weekly to the Laboratory of Parasitology, Faculty of Agriculture and Veterinary in Prishtina. For biosafety reasons (killing of eggs of *Echinococcus* spp.), all samples were deep-frozen at −80 °C for 5 days and were thereafter kept at −20 °C. Helminth eggs, including taeniid eggs, were detected by a modified flotation technique using the Ovatec® system (Zoetis, Germany). Two grams of feces were added to Ovatec® tubes containing 12 mL flotation solution (density 1·24; 8 litre of tap water containing 2·88 kg NaCl and 1·60 kg normal sugar) and mixed thoroughly with sterile wooden spatulas. The tubes were completely filled with flotation solution until a positive meniscus was formed and covered with a 2×22 mm^2 cover slip for 30 min. Thereafter, cover slips were carefully removed and transferred to glass slides for microscopic examination (magnification 100–400×). Ovatec® tubes were washed and decontaminated by storing them overnight in 4·3% sodium hypochlorite solution before reusing.

Flotation and sieving techniques for isolation of taeniid eggs. All taeniid egg positive samples plus 250 randomly selected negative samples from all three sampling phases were analysed at the Institute of Parasitology, University of Zurich, with the flotation and sieving method (F/Si) described by Mathis *et al.* (1996), using 2 g of feces.

DNA analyses

DNA isolation from the F/Si purified taeniid eggs was done as described by Stefanic *et al.* (2004). A multiplex polymerase chain reaction (PCR), targeting mitochondrial DNA of *Taenia* spp., *E. granulosus* s.l. and *E. multilocularis,* was carried out as described by Trachsel *et al.* (2007). *Echinococcus granulosus* s.l. positive samples were subjected to an additional PCR specific for *E. granulosus* sensu stricto genotypes (Stefanic *et al.* 2004). For species identification, *Taenia* spp. specific amplicons were purified using the MinElute PCR purification kit (Qiagen, Hilden, Germany) and sent for direct sequencing to Synergene Biotech GmbH (Zurich, Switzerland) using $\text{Cest5}_{\text{seq}}$ primer (Trachsel *et al.* 2007).

Validation of the diagnostic procedure

The diagnostic strategy for the detection of taeniid-eggs using a simple flotation test (Ovatec® modified) was validated against the well-established F/Si procedure. Using the same amount of feces, 55 of the 58 samples tested as taeniid eggs positive by Ovatec® system were also positive by the F/Si method. In the three Ovatec® positive but negative F/Si samples, the multiplex PCR was negative in two cases, the third sample contained DNA of *Mesocestoides litteratus* as confirmed by direct sequencing. Furthermore, three out of 250 samples negative for taeniid eggs by Ovatec® showed taeniid-like eggs with the F/Si-method. The multiplex PCR revealed that two samples had bands corresponding to *Taenia* spp., while the other one was positive for *E. granulosus*. Direct sequencing of the first two amplicons revealed 99 and 98% homology with sequences registered in GenBank as *T. hydatigena* (LC107783) and, *M. litteratus* (JN088186), respectively.

Data analysis

A logistic regression analyses were used to investigate the effect of sex (female *vs* male), age (in months), the number of owned dogs per owner, the urbanization (rural, suburban and urban), the region (Prishtina, Mitrovica, Prizren and Ferizaj) and the dog type (sheepdog *vs* other dogs) in the untreated population (baseline period).

The differences of the prevalence rates between the three periods were tested with the Cochran-*Q* test. If significant difference between the three periods were detected, the Mc-Nemar test was used as a post-hoc test to determine which periods have significant different prevalence rates. Exact binomial 95% confidence intervals (CI) for means of binomial variables, were calculated according to the method of Clopper and Pearson (1934).

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Table 2. Overall prevalence rates (% pos) and 95% confidence intervals (95% CI) of sheep dogs, other dogs and all dogs for taenid eggs, *Taenia hydatigena*, *Taenia ovis* and *Echinococcus granulosus* over the three study periods

		Baseline		Pre-event		Event period	
Dog type	Species	N pos	% pos (95% CI)	N pos	% pos (95% CI)	N pos	% pos (95% CI)
Sheepdogs ($N = 138$)	Taenid eggs[a]	20	14·49 (9·08–21·49)	4	2·90 (0·80–7·26)	14	10·14 (5·66–16·44)
	T. hydatigena	18	13·04 (7·92–19·83)	4	2·90 (0·80–7·26)	12	8·70 (4·57–14·70)
	T. ovis	1	0·72 (0·02–3·97)	0	0·00 (0·00–2·15)	0	0·00 (0·00–2·15)
	E. granulosus[b]	4	2·90 (0·80–7·26)	0	0·00 (0·00–2·15)	1	0·72 (0·02–3·97)
Other dogs ($N = 366$)	Taenid eggs[a]	11	3·01 (1·51–5·31)	2	0·55 (0·07–1·96)	7	1·91 (0·77–3·90)
	T. hydatigena	10	2·73 (1·32–4·97)	1	0·27 (0·01–1·51)	5	1·37 (0·45–3·16)
	T. ovis	0	0·00 (0·00–0·82)	1	0·27 (0·01–1·51)	1	0·27 (0·01–1·51)
	E. granulosus[b]	2	0·55 (0·07–1·96)	0	0·00 (0·00–0·82)	2	0·55 (0·07–1·96)
All dogs ($N = 504$)	Taenid eggs[a]	31	6·15 (4·22–8·62)	6	1·19 (0·44–2·57)	21	4·17 (2·60–6·30)
	T. hydatigena	28	5·56 (3·72–7·93)	5	0·99 (0·32–2·30)	17	3·37 (1·98–5·35)
	T. ovis	1	0·20 (0·00–1·10)	1	0·20 (0·00–1·10)	1	0·20 (0·00–1·10)
	E. granulosus[b]	6	1·19 (0·44–2·57)	0	0·00 (0·00–0·59)	3	0·60 (0·12–1·73)

[a] Confirmed by the flotation and sieving test.
[b] In six cases double infection with *T. hydatigena* (four in sheepdogs and two in other dogs).

RESULTS

In 31 of the 504 investigated dogs taeniid eggs were identified during the baseline sampling before the first treatment (6·15%, 95% CI: 4·22–8·62%). The analyses of these eggs revealed six infections with *E. granulosus* s.l. (1·19%, CI: 0·44–2·57%), one infection with *T. ovis* (0·20%, CI: 0·00–1·10%) and 28 with *T. hydatigena* (5·56%, CI: 4·05–8·39%). The proportion of dogs excreting taeniid eggs during the pre-event sampling was significantly lower (1·19%, CI: 0·44–2·57) and increased thereafter to an intermediate level in the event period (4·17%, CI: 2·60–6·30%).

According to a logistic regression analysis of the baseline data, sex, age, number of dogs per owner, urbanization and area did not affect the likelihood for an infection. The only significant factor was dog type (Wald statistics 14·5, df 1, $P < 0·001$). Sheepdogs were much more likely to be infected with *T. hydatigena* than other dogs (Odds ratio 5·5, CI: 2·3–13·2).

Considering the different infection levels of sheepdogs and other dogs, prevalence rates for the different taeniid species are shown separately for these two groups (Table 2). Although infection levels in sheepdogs were generally higher, changes of the levels between the three sampling phases were similar in both groups.

Statistical analyses for the temporal changes were only made for the most abundant species. The prevalence rates of *T. hydatigena* differed significantly between the three time points for both dog groups (sheep dogs: Cochran-Q 14·8, df 2, $P = 0·001$; other dogs: Cochran-Q 8·1, df 2, $P = 0·017$). It decreased from 13·0 to 2·9% (McNemar-Test: $P = 0·001$) and, thereafter, increased to 8·7% ($P = 0·021$) for sheepdogs. In other dogs, only the decrease from 2·7% (baseline period) to 0·27% (pre-event period) was statistically significant ($P = 0·012$).

The relatively high prevalence rates for *T. hydatigena* detected in the samples of the event-period, as compared with the pre-event samples, implies that the force of infection was lower between the first and the second sampling period than between the second and third. We therefore modelled two scenarios that could explain the observed prevalences. The first scenario assumes constant forces of infection for the periods between sampling 1 and 2 and between 2 and 3, whereas the second scenario assumes a change of infection pressure during the Celebration (Fig. 1). Model 2 predicts that 6·3% of the sheep dog population got infected during Eid al-Adha in 2012.

A *T. hydatigena* infection detected in the baseline samples was a very good predictor for later taenid infections, e.g. nine out of the 18 sheepdogs that were infected with *T. hydatigena* during the baseline period were also found infected at another sampling phase (50·0%, CI: 26·0–74·0%) and two of these nine dogs were even infected during all three periods. On the other hand, only five out of 120 sheepdogs with no prior *T. hydatigena* infection were infected at a later stage with this tapeworm (4·2%, CI: 1·4–9·5%). Also, *E. granulosus* infections were linked with *T. hydatigena* infections: 37 out of all dogs were at least once infected with *T. hydatigena*; seven out of these 37 dogs were also infected with *E. granulosus* (18·9%, CI: 8·0–35·2%); and only two out of the 467 dogs that were never infected with *T. hydatigena* had an *E. granulosus* infection (0·4%, CI: 0·1–1·5%). No *Echinococcus multilocularis* infections were identified in this study.

DISCUSSION

This study was designed to assess the impact of a socio-cultural factor, the slaughtering of high numbers of sheep on occasion of Eid al-Adha, on

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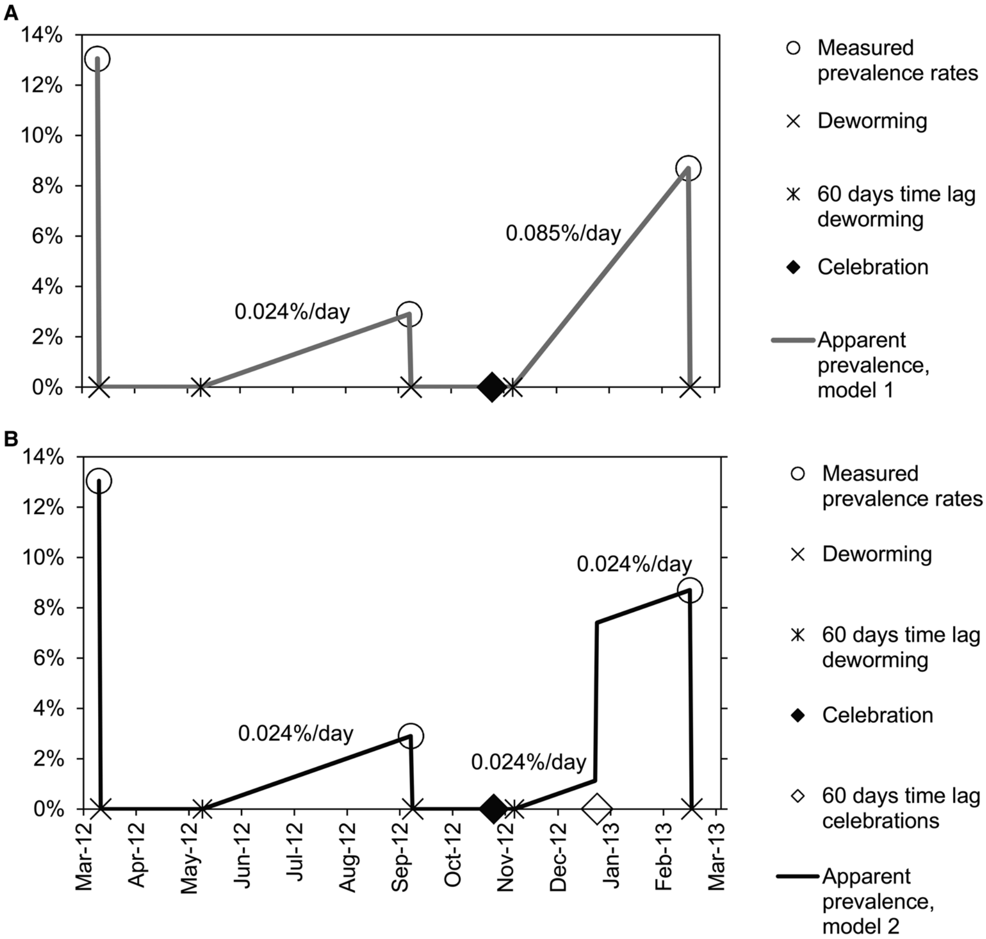

Fig. 1. Modelled prevalence over time for patent *Taenia hydatigena* infections in the sheep dog population in Kosovo. The measured prevalences are plotted in the *x*-axis at the mean date when the infections of the dogs were measured (Table 2). The inclinations of the connecting lines between the apparent prevalence rates represent the calculated daily incidence rates. It is assumed that the prevalence after the first deworming in spring 2012 went down to zero and patent infections started to increase 60 days after the deworming continuously until the second deworming procedure in autumn 2012. (A) In model one, we assumed a continuous increase of the apparent prevalence starting from 60 days after the 2nd deworming procedure (prepatent period) up to the prevalence measured in spring 2013. This increase is much stronger than between the 1st and the 2nd deworming procedure to achieve the prevalence measures in early 2013. (B) In model two we assumed that the prevalence rates are determined, on the one hand, by the same continuous infection pressure throughout the whole year (0·024%/day) but also by a strong infection boost during the celebration days. Accordingly the apparent prevalence rate should increase with the same inclination 60 days after each deworming procedure throughout the year until 60 days after the celebrations, when the infections from the EC-FS [Eid al-Adha (Feast of Sacrifice)] became patent. Only when the apparent prevalence jumps from 1·1 to 7·4% during this time, can the end prevalence of 8·7% in spring 2013 be reached.

the transmission of *Taenia* spp. and *E. granulosus* to dogs in Kosovo. The outcomes of this study indicate that this event was associated with an increased infection rate of dogs with *Taenia* spp. and *E. granulosus*, with a significantly lower infection rate during the period before Eid al-Adha (prevalence 1·2%, CI 0·4–2·6) as compared with the period after this event (4·2%, CI 2·6–6·3). Since all dogs had been treated shortly before the event and, since the post event sampling started after the prepatent period of these parasites (5–10 weeks), these infections must have been acquired during the time of the celebration and it is very unlikely that other seasonal factors could be reponsable for the observed increase within this very short time frame. The baseline infection rates (6·2%, 2–4 months after Eid al-Adha 2011) were very similar to the infection rate in dogs determined in 2005 (7·5%) in a cross-sectional study (Sherifi, 2006) and, therefore, we assume that it represents the general infection level in dogs.

Almost 62% (34 of 55) of all infected dogs lived in rural areas, while only seven (12·7%) were kept in urban and 14 (25·5%) in suburban areas. Also, 35 of 55 (63·6%) of all infected dogs were sheepdogs, which cohabit with sheep flocks as guard dogs and are very often ‘rewarded’ with viscera and raw meat from home slaughtered sheep by their owners.

Nearly 24% (14 of 55) of infected dogs were pet dogs. In rural and suburban areas, pet dogs usually live unchained in house yards. These dogs most

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likely become infected through the ingestion of viscera and raw meat after the private slaughtering of animals by their owners, who purchase sheep from different farmers, without meet inspection. These uncontrolled feeding of raw viscera has been identified as major risk factor for the transmission of CE in many different settings (Otero-Abad and Torgerson, 2013).

Activities or habits based on religious beliefs or cultural traditions have been observed to be linked with risk factors for CE (Schwabe, 1986). Several studies document different infection risks for members of different but coexisting religious communities or ethnicities in different regions of the world. This has been shown in California, where people of Basque origin had 1340 times higher risk for CE than other residents, or in New Zealand, where Maoris had a 6·4 times higher risk than other ethnicities (Schwabe, 1986). Similarly, Greek Cypriots had a greater risk than Turkish Cypriots and in Cyprus and Lebanon, the surgical incidence rate for CE was higher in the Christian than the Muslim community (Schwabe, 1986). A direct correlation between slaughtering of animals in winter and high levels of infections in dogs with *Echinococcus* has been shown previously in the semi-nomadic pastoral community of the Tibetan plateau (Wang *et al*. 2001) and the impact of socio-cultural aspects on the perpetuation of echinococcosis transmission has been discussed (Macpherson, 2005).

It can be assumed that the Eid al-Adha contributes to an increased annual incidence of canine echinococcosis in Kosovo, as old sheep are sold to private persons all over the country for home slaughtering prior to this event. During the other periods of the year such sheep are slaughtered more locally, being infection sources for a more limited number of dogs. However, the home slaughtering tradition during this event contributes to a temporal concentration of transmission. Therefore, this particular epidemiological situation provides a valuable opportunity for implementing improved control activities including information of the population and deworming of dogs. For more than 10 years, Kosovo Veterinary Services has offered free Praziquantel tablets to deworm dogs. The treatment is carried out once a year together with vaccination of dogs against rabies. However, the deworming strategy appeared to have had little effect since prevalence rates of *Taenia* spp. and *E. granulosus* in dogs have remained unchanged since 2003/04 (Sherifi, 2006; Sherifi *et al*. 2011). Based on the findings of this study, the control strategy designed by the Kosovo Veterinary Services includes the treatment of all dogs 2–4 weeks after the celebration of Eid al-Adha. Furthermore, it is recommended to deworm the relatively small sheepdog population at least four times a year and, on this occasion, to inform farmers how to correctly dispose of slaughter waste and carcasses.

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