Echinococcus multilocularis and other zoonotic parasites in red foxes in Estonia

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SUMMARY

Red fox (*Vulpes vulpes*) is the most widely distributed canid in the world and an important source of multiple zoonotic pathogens capable of causing life-threatening diseases, such as rabies and alveolar echinococcosis. Informing general public of potential risks related to foxes is becoming more important since the fox densities have increased in many countries and the species is colonizing urban areas in Europe and around the world with increasing pace, bringing zoonotic pathogens to the immediate neighbourhood of humans and their companion animals. The aim of this study was to examine the parasite fauna of red foxes in Estonia. We found in Estonian foxes a total of 17 endoparasite taxa, including ten zoonotic species. All the analysed individuals were infected and the average parasite species richness was 6·37. However, the infection rates varied to a very large extent for different parasite species, ranging from 0·9 to 91·5%. Of zoonotic species, the highest infection rate was observed for *Alaria alata* (90·7%), *Eucoleus aerophilus* (87·6%) and *Uncinaria stenocephala* (84·3%). The prevalence of tapeworm *Echinococcus multilocularis*, a causative agent for alveolar echinococcosis, was also relatively high (31·5%), presenting a potential risk to human health.

Key words: *Mesocestoides*, *Metorchis bilis*, *Pearsonema plica*, sarcoptic mange, *Toxascaris leonina*, *Toxocara canis*, *Vulpes vulpes*, zoonoses.

INTRODUCTION

The effects exerted by predators in ecosystems can have profound consequences for species of conservation importance, but also for human and animal health. The red fox (*Vulpes vulpes*; hereafter 'fox') is the most widely distributed wild terrestrial carnivore in the world with the species range of approximately 70 million km^2 (Macdonald and Reynolds, 2008) due to its high ecological plasticity. Foxes influence other species primarily in two ways: via predation and through transmission of pathogens. Considering these and the relatively high numbers, fox can be regarded as one of the most influential mesopredators in the Holarctic. Together with other mesopredators such as the raccoon dog (*Nyctereutes procyonoides*) they are highly effective vectors of multiple zoonotic diseases, including rabies, alveolar echinococcosis and sarcoptic mange, posing significant risk for humans and domesticated animals (Smith *et al*. 2003; Deplazes *et al*. 2004; Letkova *et al*. 2006; Kauhala and Kowalczyk, 2011; Süld *et al*. 2014; Laurimaa *et al*. 2015*a*, *b*).

The parasite fauna of the fox has been of considerable scientific interest in Europe, largely because the species harbours zoonotic parasites (Smith *et al*. 2003; Letkova *et al*. 2006). However, informing the general public of potential risks related to foxes is becoming more and more important since fox density appears to have increased in many countries of Europe partly as a consequence of highly successful vaccination of wildlife against rabies (Vos, 1995; Gloor *et al*. 2001). Moreover, foxes are colonizing urban areas in Europe and around the world with increasing pace (Harris and Rayner 1986; Gloor *et al*. 2001; Bateman and Fleming, 2012), bringing zoonotic pathogens to the immediate neighbourhood of humans and their companion animals (Deplazes *et al*. 2004; Davidson *et al*. 2012; Laurimaa *et al*. 2015*a*; Umhang *et al*. 2015). The same trend has also been observed among Estonian foxes that have over the last decade colonized urban areas in Estonia (Plumer *et al*. 2014).

The parasite fauna of Estonian red foxes was investigated about a decade ago, when a pilot study based on examination of 17 animals revealed 16 endoparasite taxa, including the small fox tapeworm *Echinococcus multilocularis* (Moks *et al*. 2005; Moks, 2008). This tapeworm is probably the most studied parasite of foxes in Europe (Conraths and Deplazes, 2015; Knapp *et al*. 2015; Vuitton *et al*. 2015), including the Baltic States (Marcinkute *et al*. 2015), as it can cause a potentially fatal disease in humans (Eckert *et al*. 2001). Considering the relatively small number of animals examined by Moks (2008), it is important to evaluate the fox parasite fauna in Estonia by including significantly

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larger set of samples. Moreover, as approximately a decade has passed since the last study by Moks *et al*. (2005), it would be of considerable interest to evaluate the changes in the prevalence of *E. multilocularis*.

MATERIALS AND METHODS

Sample collection

In total, 111 red fox carcasses provided by hunters were examined for internal parasites. Legally hunted red foxes were collected during the hunting seasons 2010/11 and 2011/12. All the animals originated from the mainland of Estonia (eight counties; Fig. 1).

Sex, sampling region and time were recorded for each animal. All animals collected with fur ($n = 99$) were examined for sores and patches of thick crusty skin as clinical signs of sarcoptic mange. After weighing the carcasses, internal organs were removed and kept at −80°C for at least 5 days before parasitological examination for safety precautions (Eckert *et al*. 2001), since highly dangerous tapeworms *E. multilocularis* (Moks *et al*. 2005; Laurimaa *et al*. 2015*a*, *b*) and *E. granulosus* (Moks *et al*. 2006, 2008; Laurimaa *et al*. 2015*c*) have been found in Estonia. Lungs, gall bladder and urinary bladder were studied using established washing and sieving techniques for helminth detection (Parre, 1985). Briefly, the respective organ was cut open and the lumen was washed with tap water through 200 μm mesh sieve to reveal helminth infection. The small and large intestines were separated and examined by sedimentation and counting technique (Hofer *et al*. 2000). Up to 200 specimens were counted per helminth species, since continuing to very large numbers (often thousands) would have been too laborious. Parasites were stored in 95% ethanol.

Parasite identification

Trematodes, cestodes and nematodes were identified according to their morphology after Kozlov (1977). Cestodes from genera *Echinococcus* and *Mesocestoides* were further identified after Abuladze (1964) and Hrčkova *et al*. (2011), respectively.

As the scoleces of tapeworms from the genus *Taenia* were deformed and lacking some of the features required for morphological identification (e.g. hooks and gravid proglottids), these samples were submitted to genetic identification. PCR-based genetic identification of *Taenia* species was conducted as described in Laurimaa *et al*. (2016). Essentially, DNA was extracted using the High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's instructions and primers CesCox1F and CesCox2R were used to amplify a fragment of *cox1* gene of mitochondrial DNA with total length of 506 base pairs. PCR was performed using a touchdown protocol described in Laurimaa *et al*. (2016). Eventually, samples were purified and sequenced using the same primers as used in the primary PCR.

Statistical analyses

For statistical analysis, collected animals were divided between two seasons: autumn (September–November) and winter (December–March). We used nonparametric Mann–Whitney *U* test to analyse variation in the number of helminth species between animal gender, the two seasons and sarcoptic mange, and to measure if male foxes weighed more than females. The same analysis was used to determine whether sarcoptic mange or animal gender influenced the overall intensity of helminth infection. Intensity of parasitic infection in each fox was determined as a sum of the number of observed parasite specimens from different species (varying between 1 and 784). However, as the upper limit in counting the parasite specimens from one species was set to 200, this should be considered as relative intensity.

A multivariable analysis was used to study the abundance of different parasite species in red foxes with respect to gender, sampling season (autumn/winter), and infection with scabies. Initially, the mathematical distribution (Poisson or negative binomial) that produced the best description of the abundance data was determined for the most abundant parasite taxa. Parasite species found in < 20 foxes were excluded from the analysis due to low variability. The final analyses using negative binomial generalized linear model examined the factors gender, season and scabies as fixed effects. Multivariable analyses were performed using package 'MASS' (Venables and Ripley, 2002) in software R (R Development Core Team, 2014). In addition, chi-square was calculated to compare parasite infection between the sexes.

To assess the co-occurrence of different parasite species, we calculated the *C*-score (Stone and Roberts, 1990) for all pairs of parasite species as described in Süld *et al*. (2014). Essentially, we generated 999 random matrices with fixed row and column occurrence (e.g. if parasite A has a prevalence of 20% in the raw data, this level of prevalence will remain the same in all randomized datasets) and recalculated all pairwise *C*-scores for each matrix. Observed *C*-scores were standardized and the significance of effect was estimated from the number of randomized *C*-scores more extreme than the observed value. The co-occurrence analysis was carried out using package 'vegan' (Oksanen *et al*. 2015) in software R.

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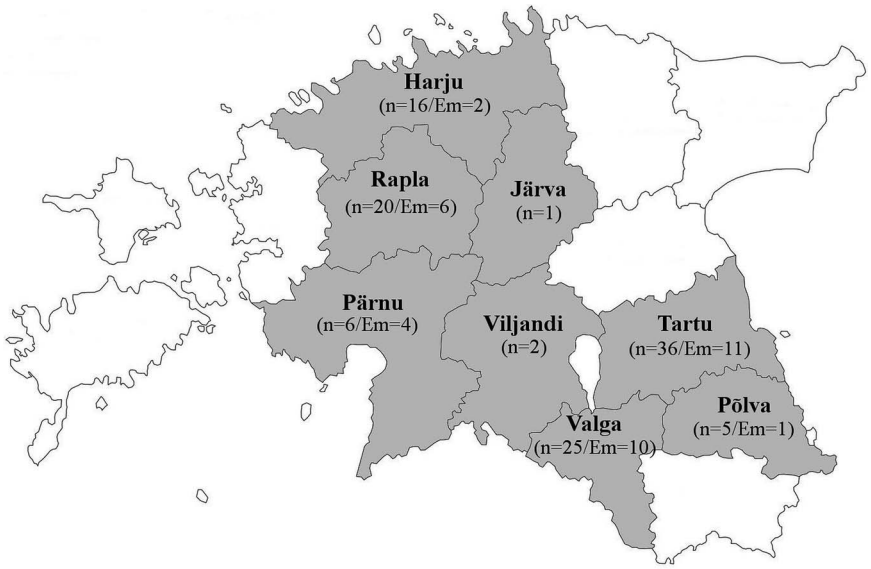

Fig. 1. Map of red fox carcasses collected in Estonia. The number of carcasses examined is given in parenthesis; Em – the number *Echinococcus multilocularis* positive animals.

RESULTS

Red fox parasite fauna in Estonia

Due to decomposition or hunting damage some samples were excluded. In total, 108 small intestine, 105 lung and 106 urinary bladder samples were examined. Gall bladders were examined for 15 animals, though parasites detected in this organ were not included to statistical analyses due to the small sample size.

We identified 16 helminth species: nine nematode species, three trematode species, four cestode species and one additional cestode taxon that was determined at the genus level (Table 1). Examined red foxes were highly infected with *Pearsonema plica* (91·5%), *Alaria alata* (90·7%), *Eucoleus aerophilus* (87·6%), *Uncinaria stenocephala* (84·3%) and *Mesocestoides* spp. (77·8%). Infection with other helminth species was lower (Table 1). In autumn, foxes had on average more helminth species than in winter (mean number of species 7·7 *vs* 6·3; Mann–Whitney *U* test: $z = -2{\cdot}36$; $P = 0{\cdot}02$). However, the number of helminth species varied more in winter period. To support the occurrence of higher parasite abundance in autumn, factor winter was associated with lower infection risk with *U. stenocephala* and *Toxocara canis* in the multivariable analysis (Table 2). We detected zoonotic tapeworm *E. multilocularis* infection in 31·5% ($n = 34$) of the animals and ectoparasitic *Sarcoptes scabiei* infection in 21·2% ($n = 21$) of the animals. Two *Mesocestoides* species (*M. litteratus* and *M. lineatus*) were present in foxes, but we were not able to determine the species in most of the cases due to the absence of mature proglottids. Although two morphotypes of *Taenia* species could be differentiated on the basis of scolex morphology, the genetic identification revealed the presence of only *T. polyacantha*.

All the analysed animals were infected. Average parasite species richness was 6·37 species (95% CI 5·99–6·75). Considering animals whose all organs were examined (intestines, lungs and urinary bladder; $n = 98$), majority of red foxes (75·5%) were infected with five to eight species (Table 3). Scabied animals were infected with an average of 7·33 (95% CI 6·32–8·35) parasite species. The highest number of different parasites in the internal organs of a single fox was 12.

Scabies

Considering animals whose all organs were examined (intestines, lungs and urinary bladder; $n = 98$) we found that scabied animals were parasitized with significantly higher number of parasite specimens (mean number of specimens 468 *vs* 259; Mann–Whitney *U* test: $z = -3{\cdot}61$; $P < 0{\cdot}01$). However, no statistically significant difference was found for the number of helminth species between scabied and healthy foxes (Mann–Whitney *U* test: $z = -1{\cdot}87$; $P = 0{\cdot}06$). The multivariable analysis showed no significant interactions between risk factors, but detected sarcoptic mange as a significant factor in estimating infection risk with three nematode species: *U. stenocephala, T. canis* and *E. aerophilus* (Table 2). Positive regression parameters indicate increased risk of infection with a certain parasite species due to the presence of analysed risk factor (e.g. male for *U. stenocephala*), while the negative regression parameters indicate that the opposite factors (e.g. autumn and presence of scabies for *U. stenocephala*) are associated with increased risk of

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Table 1. Prevalence of helminth species in Estonian red foxes

Helminth species	Prevalence (95% CI)	No. pos/exam.	No. of helminths (min–max)	Mean intensity[a]
Trematoda	**90·7**			
*Alaria alata**	90·7 (85·2–96·2)	98/108	1,…,>200	103
*Metorchis bilis**	60·0 (35·2–84·8)	9/15	1–28	9
Isthmiophora melis	0·9 (0–2·7)	1/108	3	3
Cestoda	**90·7**			
Mesocestoides spp. (*M. lineatus** and *M. litteratus*)	77·8 (70·0–85·6)	84/108	1,…,>200	75
Taenia spp. (*T. polyacantha*[b])	70·4 (61·8–79·0)	76/108	1–130	22
*Echinococcus multilocularis**	31·5 (22·7–40·3)	34/108	3,…,>200	87
Diphyllobothrium sp.*	1·9 (0–4·5)	2/108	1–4	3
Nematoda	**100**			
Pearsonema plica	91·5 (86·2–96·8)	97/106	1–147	35
*Eucoleus aerophilus**	87·6 (81·3–93·9)	92/105	1–127	16
*Uncinaria stenocephala**	84·3 (77·4–91·2)	91/108	1,…,>200	33
Crenosoma vulpis	53·3 (43·8–62·8)	56/105	1,…,>200	38
*Toxocara canis**	29·6 (21·0–38·2)	32/108	1–20	5
*Toxascaris leonina**	5·6 (1·3–9·9)	6/108	1–4	3
Molineus patens	8·3 (3·1–13·5)	9/108	1–3	1
Angiostrongylus vasorum	2·9 (0–6·1)	3/105	1–17	6
*Aonchotheca putorii**	2·8 (0–5·9)	3/108	1	1

Asterisks (*) mark the species/taxa of zoonotic potential.
[a] Note that the upper limit in counting was 200 and therefore the numbers are indicative.
[b] Single specimens identified genetically as *Taenia polyacantha*, see also Laurimaa *et al*. 2016.

Table 2. Risk factors for parasite infection in Estonian red foxes

Parasite	Factor: parameter	Regression parameter (S.E.)	IRR (95% CI)	*P*-value
Alaria alata	Gender: Male	−0·003 (0·29)	1·00 (0·57–1·74)	0·990
n = 90	Season: Winter	−0·08 (0·41)	0·92 (0·37–1·95)	0·842
	Scabies: Healthy	−0·51 (0·35)	0·60 (0·29–1·14)	0·148
Mesocestoides spp.	Gender: Male	−0·03 (0·39)	0·97 (0·45–2·12)	0·945
n = 77	Season: Winter	−0·15 (0·57)	0·86 (0·23–2·44)	0·793
	Scabies: Healthy	−0·52 (0·48)	0·59 (0·20–1·45)	0·275
E. multilocularis	Gender: Male	−0·26 (0·80)	0·77 (0·16–3·73)	0·749
n = 31	Season: Winter	−1·17 (1·16)	0·31 (0·03–3·04)	0·316
	Scabies: Healthy	−0·72 (0·99)	0·49 (0·07–3·36)	0·466
Taenia spp.	Gender: Male	−0·17 (0·40)	0·85 (0·38–1·92)	0·671
n = 69	Season: Winter	−0·75 (0·57)	0·47 (0·12–1·33)	0·188
	Scabies: Healthy	−0·07 (0·49)	0·94 (0·32–2·34)	0·893
Uncinaria stenocephala	Gender: Male	0·40 (0·30)	1·49 (0·82–2·72)	0·190
n = 81	**Season: Winter**	−1·12 (0·44)	0·33 (0·12–0·73)	**0·010**
	Scabies: Healthy	−1·01 (0·37)	0·37 (0·17–0·72)	**0·006**
Eucoleus aerophilus	Gender: Male	−0·15 (0·27)	0·86 (0·51–1·46)	0·584
n = 87	Season: Winter	−0·67 (0·39)	0·51 (0·22–1·03)	0·083
	Scabies: Healthy	−1·03 (0·33)	0·36 (0·18–0·66)	**0·002**
Pearsonema plica	Gender: Male	0·14 (0·25)	1·15 (0·70–1·90)	0·578
n = 80	Season: Winter	0·31 (0·36)	1·36 (0·63–2·63)	0·400
	Scabies: Healthy	0·11 (0·31)	1·11 (0·58–2·01)	0·725
Crenosoma vulpis	Gender: Male	0·09 (0·54)	1·09 (0·38–3·24)	0·869
n = 52	Season: Winter	0·04 (0·78)	1·04 (0·15–4·01)	0·956
	Scabies: Healthy	−1·25 (0·66)	0·29 (0·06–0·96)	0·059
Toxocara canis	Gender: Male	0·49 (0·48)	1·63 (0·61–4·42)	0·306
n = 28	**Season: Winter**	−2·13 (0·59)	0·12 (0·03–0·37)	**<0·001**
	Scabies: Healthy	−2·17 (0·52)	0·11 (0·04–0·31)	**<0·001**

IRR, incidence rate ratio.
Significant factors are shown in bold typeface.

infection. The incidence rate ratio (IRR in Table 2) provides the estimated rate ratio of parasite abundance in the presence or absence of a specific risk factor when all other variables are constant [e.g. male fox sampled in winter with no signs of scabies would have 0·11 times the abundance rate of *T. canis* compared with that of a scabied male fox sampled in winter (a 89% decrease)].

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Table 3. The number of endoparasite taxa in red foxes ($n = 98$). *Taenia* and *Mesocestoides* species were included as one species, respectively. Gall bladder parasites were omitted

No. of parasite taxa	No. of foxes (%)
One	2 (2·0)
Two	2 (2·0)
Three	5 (5·1)[1]
Four	4 (4·1)
Five	15 (15·3)[3]
Six	16 (16·3)[1]
Seven	32 (32·7)[6]
Eight	11 (11·2)[2]
Nine	9 (9·2)[3]
Ten	0 (–)
Eleven	1 (1·0)[1]
Twelve	1 (1·0)[1]

Numbers in superscript mark the number of animals infected with sarcoptic mange.

Variation between males and females

The analysed males weighed significantly more than females (Mann–Whitney U test: $z = 4{\cdot}33$; $P < 0{\cdot}01$). Mean weights were 5·26 and 4·52 kg for males and females, respectively. There was no significant relationship between gender and both the number of helminth species and infection intensity [Mann–Whitney U test: (1) $z = 1{\cdot}25$; $P = 0{\cdot}21$; (2) $z = 1{\cdot}34$; $P = 0{\cdot}18$]. Neither did the multivariable analysis detect gender as significant factor estimating parasite infection intensity (Table 2). However, the chi-square test (χ^2) detected significant effects in parasite species infection between the sexes: infection with nematode *Molineus patens* occurred more often in females (2·3 *vs* 15·1%; $\chi^2 = 4{\cdot}56$; $P = 0{\cdot}03$), and *T. canis* infection was more prevalent in males (44·2 *vs* 17·0%; $\chi^2 = 8{\cdot}50$; $P < 0{\cdot}01$).

Co-occurrence analysis

The co-occurrence analysis between different parasite species did not reveal any significant relationships (Supplementary Table S1).

DISCUSSION

Parasite fauna of the red fox in Estonia

The foxes examined in this study were highly parasitized: all examined animals were infected (100% infection rate). Similar results, with all the animals being infected, have previously been reported in Germany (Schöffel *et al*. 1991) and Ukraine (Zvegintsova *et al*. 2007).

We identified 16 endoparasite species and one additional cestode taxon at the genus level (Table 1). Although based on a rather limited number of fox samples ($n = 17$) Moks (2008) reported a similar number of endoparasite taxa ($n = 16$) from the same internal organs. However, there are some differences in the species composition between these two studies. We found four nematode species that have previously not been identified in Estonian foxes: *T. leonina, M. patens, A. vasorum* and *A. putorii*. In contrast, while only a single cestode species from genus *Taenia*, namely *T. polyacantha*, was identified (genetically) in this study, Moks (2008) reported three species (*T. polyacantha, T. pisiformis* and *T. serialis*). There were two other parasites recorded by Moks (2008) but not found in this study: *Spirocerca lupi* and an unidentified acanthocephalan. Taking into account the results of these two studies, Estonian red foxes seem to harbour a minimum of 21 helminth species, which is very similar with the results from a Danish study ($n = 21$; Saeed *et al*. 2006), but less than found in Iberia ($n = 34$; Segovia *et al*. 2004) and Belarus ($n = 32$; Shimalov and Shimalov, 2003).

Despite the smaller number of animals examined from the autumn period ($n = 11$) than from winter ($n = 87$), we found that foxes sampled in autumn harboured on average more helminth species (mean number of helminths 7·7 *vs* 6·3). This finding is supported by the fact that the availability of different food categories is higher in autumn: all the crops ripen during that period, amphibians are in move to find hibernating places and the abundance of small rodents is the highest. Thus, all these food categories represent potential sources of different helminth infections.

Variation between males and females

Sexual dimorphism, with males being larger than females, has previously been reported in red foxes by Cavallini (1995) and Heptner and Naumov (1998). In this study, we also found that males weighed significantly more than females. Although we did not detect any significant relationship between the animal sex and the number of helminth species, male foxes harboured on average more *T. canis*, whereas *M. patens* occurred more often in females. The difference in the infection between the sexes could be associated with different food habits: while the females consumed more rodents, the remains of larger mammals were more prevalent in the stomach of male foxes in Estonia (E. Soe, personal communication).

Co-occurrence analyses

In principle, a parasite species could facilitate or prevent the infection of another parasite species. However, we did not detect any significant competitive or facilitative interaction (Supplementary Table S1) regardless that four of the most prevalent parasite species (*A. alata, U. stenocephala, E. aerophilus* and *P. plica*) frequently appeared

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together in one host. It is important to note, however, that as the analysis determines whether the level of co-occurrence is higher or lower than what would be expected at random, abundant species will also occur with high co-occurrence at random.

Since the parasite fauna of an animal is intimately related to its feeding habits (most parasites must be ingested in order to infect the host organism), and as the fox diet has been studied in the same animals as used in this study (Soe *et al*. unpublished observation), it was also possible to assess the co-occurrence of parasite species and food categories. For this analysis identifiable material obtained from stomachs of hunted foxes was sorted into six food categories: (1) Birds, (2) Fish, (3) Invertebrates, (4) Plants (fruit and cereal), (5) Ungulates (wild boar, roe deer carrion) and (6) Small mammals (rodents). However, the co-occurrence analysis between parasite species and food categories did not reveal any significant relationships (not shown), although we expected to find some, e.g. between 'Small mammals' and *E. multilocularis*, as this parasite is assimilated by consuming the mentioned food category. One of the reasons for not detecting any significant effects could be that the stomach content analysis reflects animal's food consumption during a short time period (basically the last meal). Significantly larger sample size could potentially help to overcome this limitation.

Red fox as a vector for zoonotic parasites in Estonia

Of the 17 endoparasite taxa recorded in this study, ten are of zoonotic importance (Table 1), capable of causing serious health problems to humans, but also to domesticated animals. *Echinococcus multilocularis*, a tapeworm causing potentially fatal disease – alveolar echinococcosis – in humans (Eckert *et al*. 2001; Vuitton *et al*. 2015), is probably the most important zoonotic agent found in this study. Typical transmission cycle of *E. multilocularis* in Europe is wildlife-based, involving red foxes as predominant definitive host and small rodents as intermediate host. In addition to red foxes, several other canid species, e.g. the arctic fox (*Vulpes lagopus*), raccoon dog, golden jackal (*Canis aureus*) and grey wolf (*Canis lupus*), can act as definitive hosts for *E. multilocularis* in Europe (Eckert *et al*. 2001; Fuglei *et al*. 2008; Szell *et al*. 2013; Marcinkute *et al*. 2015). Humans are considered as accidental hosts for this parasite and they can be infected by ingesting parasite eggs via direct contact with a definitive host or through contact with contaminated water, soil or food (Eckert *et al*. 2001). Upon infection, the parasite causes tumour-like cysts in internal organs of humans, mainly in the liver, but can also spread to other distant organs.

During a previous pilot study of Estonian red foxes about a decade ago, *E. multilocularis* was found in 29·4% (5/17) of red foxes (Moks *et al*. 2005). As only a small number of carcasses were examined in that study, we aimed to specify the abundance of *E. multilocularis* by involving considerably higher number of fox samples. Nonetheless, we found a rather similar share of infected animals (31·5%), confirming the relatively high infection rate of *E. multilocularis* among Estonian red foxes. Even higher prevalence rates have been reported for foxes in Lithuania (58·7%; Bružinskaite-Schmidhalter *et al*. 2012) and in Kyrgyzstan (63·6%; Ziadinov *et al*. 2010). The highest number of *E. multilocularis* specimens counted earlier in Estonian red fox was 927 (Moks *et al*. 2005). Although we stopped parasite counting at 200 in this study, two samples likely included more than 1000 specimens. Moks *et al*. (2005) identified *E. multilocularis*-positive foxes from all three counties examined (Hiiu, Põlva, Tartu), whereas the infected animals from this study originated from six counties (Harju, Pärnu, Põlva, Rapla, Tartu and Valga; Fig. 1). However, the parasite is likely to exist all over the country and only due to low sample size the infection was not found in animals from counties Järva ($n = 1$) and Viljandi ($n = 2$). Estonia has the lowest human echinococcosis rate of the three Baltic States (Marcinkute *et al*. 2015): altogether 13 cases of unspecified echinococcosis have officially been registered in Estonia since 1986. However, considering the rapid increase in fox numbers during 2006–2010 (Plumer *et al*. 2014), the number of human cases could grow in coming years as the incubation period for *E. multilocularis* is usually 5–15 years.

One of the reasons why the proportion of infected foxes has remained high could be related to supplementary feeding sites. While these sites are intended for wild boars (*Sus scrofa*), they are also known to attract many non-target species, e.g. rodents, red foxes and raccoon dogs (Oja, 2011). Rodents, as the intermediate host species for *E. multilocularis*, can easily become infected with the tapeworm by eating contaminated substances at the supplementary feeding sites. Rodents, however, constitute a large share of red foxes diet in Estonia (Süld *et al*. 2014). Supplementary feeding sites therefore represent potential hot-spots for the spread of *E. multilocularis* and other zoonotic pathogens in Estonian wildlife.

While susceptible to *E. multilocularis*, the red fox seems 'immune' to another species of this genus, namely the *Echinococcus granulosus*, although the parasite can infect the red fox in nature (Jenkins and Craig, 1992; Segovia *et al*. 2004; Keidans *et al*. 2005). Moreover, no significant difference in growth, segmentation or maturation of the parasite has been detected between dogs and foxes (Thompson, 1983). *Echinococcus granulosus* has been recorded in Estonia in other canid species,

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such as the grey wolf (Moks *et al*. 2006) and domestic dog (Laurimaa *et al*. 2015*c*), but never in foxes. Both moose (*Alces alces*; Moks *et al*. 2008) and roe deer (*Capreolus capreolus*; Marcinkute *et al*. 2015) have been reported as intermediate host species for the parasite in Estonia, but foxes apparently do not consume internal organs of these ungulate carcasses available in nature. On the other hand, the susceptibility to infection could depend on the genotype of *E. granulosus*: while the infection in foxes has been proven with genotype G1 (Thompson, 1983; Jenkins and Craig, 1992), this genotype has in Estonia been reported only in few dogs (Laurimaa *et al*. 2015*c*), whereas the most abundant genotypes in the country appear to be G8 and G10 (Moks *et al*. 2008; Marcinkute *et al*. 2015). As G1 is evolutionarily highly divergent from G8 and G10 (Saarma *et al*. 2009; Knapp *et al*. 2011), it could result in variations in infectivity between different *E. granulosus* genotypes.

Shortly after the oral vaccination campaign against rabies was imposed to Estonian wildlife in 2005, the number of foxes started to increase rapidly (Veeroja and Männil, 2015). With eradicating rabies from Estonia (Pärtel, 2013), one of the most important factors regulating the fox population size was lost. However, after the harsh winter of 2010/2011 sarcoptic mange started to spread extensively and the estimated number of foxes has since been continuously decreasing (Veeroja and Männil, 2015). Sarcoptic mange has been reported to be the main cause in reducing population densities of red foxes and raccoon dogs in Estonia (Süld *et al*. 2014, Laurimaa *et al*. 2016) and elsewhere in Europe (Kauhala and Kowalczyk, 2011). Scabies (mange) is a contagious disease that can infect domestic animals, notably dogs and the disease has been reported from urban foxes in Estonia (Plumer *et al*. 2014). Animal scabies can also occasionally infect humans, causing severe itching as it burrows into the skin, but this infection is usually short-lived (Arlian, 1989; Heukelbach and Feldmeier, 2006). Although the prevalence of sarcoptic mange in Estonian foxes is 21·2%, the actual proportion of infected animals could be lower. It could be suggested that the skinned animals ($n = 12$) provided by the hunters were also scabies-free, since the hunters only collect the skin of healthy animals (fur of wild fox is inexpensive and the costs of hunting and tannery are high).

Animals infected with scabies are frequently undernourished (Newman *et al*. 2002), and are therefore constantly in search of food, encountering potentially a wider range of different parasites than healthy animals. As the animals examined in this study were from the period when mange became widespread in Estonia (2010–2012), comparison of parasite fauna in scabied and healthy animals was of considerable interest. In this study, we showed that although there was no statistically significant difference in the number of helminth species between scabied and healthy foxes ($P = 0{\cdot}06$), the infected foxes appeared to harbour more helminth species (mean numbers 7·33 and 6·18, respectively). Scabied animals were also parasitized with higher number of parasite specimens. In addition, we found that parasite infection was more intense among scabied animals in case of three nematode species: *U. stenocephala, T. canis* and *E. aerophilus* (Table 2). *Uncinaria stenocephala* and *T. canis* are typical geohelminths and animals could have got the infection by simply cleaning their fur, which is already in bad condition due to the sarcoptic mange. It is not clear why such relation was found with *E. aerophilus*. This nematode is located in respiratory organs of the definitive host, suggesting that there might be an association with scabies and lung parasites. It is likely that infection with this species occurred more frequently in scabied animals because earthworms, the intermediate hosts for *E. aerophilus*, are relatively easy to catch.

Although the most prevalent parasite species identified in this study *P. plica* (91·5%) is not considered as zoonotic, the three other species (*A. alata, E. aerophilus, U. stenocephala*) with both high prevalence rate and mean intensity (Table 1) can be considered as parasites with high zoonotic potential. Clinical symptoms of the lung worm *E. aerophilus* are mainly pulmonary (Laloševic *et al*. 2008). The nematode is mostly transmitted with earthworms, but humans can probably be infected directly by ingesting the parasite eggs with inadequately washed vegetables. *Uncinaria stenocephala* and *A. alata* can occasionally infect humans and cause severe diseases that are characterized by migrating larvae (Tamminga *et al*. 2009; Wasiluk, 2009). Infectivity to humans and the observed high prevalence rates in Estonia make *E. aerophilus, U. stenocephala* and *A. alata* together with *E. multilocularis* the pathogens representing a considerable public health risk. Lithuania is the only country, where *P. plica, A. alata, E. aerophilus* and *E. multilocularis* are even more abundant among red foxes than in Estonia (Bružinskaite-Schmidhalter *et al*. 2012; Marcinkute *et al*. 2015), making the Baltic region hyperendemic for these four parasite species.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0031182016001013.

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