



Native and introduced squirrels in Italy host different *Cryptosporidium* spp.

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Abstract

The present study was undertaken to describe *Cryptosporidium* spp. infection in tree squirrels from 17 locations in Northern Italy. A total of 357 squirrels were examined, including species native to Europe (*Sciurus vulgaris*; n = 123), and species introduced from North America (*Sciurus carolinensis*; n = 162) and Southeast Asia (*Callosciurus erythraeus*; n = 72). Faecal samples of all squirrels were examined for the presence of *Cryptosporidium* infection by microscopy (flotation method) and PCR/sequence analysis of the *Cryptosporidium* 18S rRNA, actin, and gp60 genes. Despite the overlapping ranges of native and introduced tree squirrel species in the study area, they host different *Cryptosporidium* spp. *Sciurus vulgaris* were exclusively infected with *Cryptosporidium* ferret genotype (n = 13) belonging to three novel gp60 subtypes, VIIIb–VIII d. *Sciurus carolinensis* hosted *C. ubiquitum* subtype XIIb (n = 2), *Cryptosporidium* skunk genotype subtype XVIa (n = 3), and chipmunk genotype I subtype XIVa (n = 1). *Cryptosporidium* chipmunk genotype I subtype XIVa was also found in two *C. erythraeus*. Comparing data from this and previous studies, we propose that *Cryptosporidium* skunk genotype and possibly *C. ubiquitum* subtype XIIb were introduced to Europe with eastern grey squirrels. *Cryptosporidium* chipmunk genotype I and ferret genotype were associated with high intensity infections, but there was no association with diarrhoea.

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Introduction

Cryptosporidium is a genus of apicomplexan parasites that infect epithelial cells in the microvillus border of the gastrointestinal tract of all classes of vertebrates (Ryan and

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Table 1. *Cryptosporidium* spp. identified in tree squirrels worldwide.

Host (scientific name)	<i>Cryptosporidium</i> taxa	Locality	Reference
American red squirrel (<i>Tamiasciurus hudsonicus</i>)	<i>C. ubiquitum</i> chipmunk genotype I deer mouse genotype III skunk genotype	North America	Stenger et al. (2015), Ziegler et al. (2007a), Ziegler et al. (2007b)
Eastern grey squirrel (<i>Sciurus carolinensis</i>)	<i>C. baileyi</i> <i>C. muris</i> <i>C. parvum</i> ^a <i>C. ubiquitum</i> chipmunk genotype I deer mouse genotype III skunk genotype	North America	Feng et al. (2007), Stenger et al. (2015), Sundberg et al. (1982), Ziegler et al. (2007a), Ziegler et al. (2007b)
Eurasian red squirrel (<i>Sciurus vulgaris</i>)	<i>C. parvum</i> ^a chipmunk genotype I [#] ferret genotype ^{#,§}	Europe [#] and Asia [§]	Bertolino et al. (2003), Feng et al. (2007), Kváč et al. (2008), Lv et al. (2009)
Fox squirrel (<i>Sciurus niger</i>)	<i>C. ubiquitum</i> , <i>Cryptosporidium</i> sp.	North America	Current (1990), Stenger et al. (2015)

^aDetermined based on oocyst size by microscopy.

Xiao 2014) and cause the diarrheal disease cryptosporidiosis. Human cryptosporidiosis is primarily caused by the zoonotic *Cryptosporidium parvum* and anthroponic *Cryptosporidium hominis*, but there are an increasing number of reports describing human cryptosporidiosis caused by *Cryptosporidium* originating from wildlife animals, including squirrels (Elwin et al. 2012a; Koehler et al. 2014; Kváč et al. 2009; Kváč et al. 2014b; Ortega and Kváč 2013; Rašková et al. 2013; Robinson et al. 2008; Stenger et al. 2015; Waldron et al. 2010). The squirrel, family *Sciuridae*, is one of the most diverse and widely distributed families of mammals, and their members host several different *Cryptosporidium* species and genotypes (Kváč et al. 2014a). Tree squirrels, a polyphyletic tribe in the squirrel family, include hundreds of species. *Cryptosporidium parvum* has been reported in the eastern grey squirrel (*Sciurus carolinensis*; hereafter grey squirrel) and fox squirrel (*Sciurus niger*) in the US (Current 1990; Sundberg et al. 1982) and in the Eurasian red squirrel (*Sciurus vulgaris*; hereafter red squirrel) in Italy (Bertolino et al. 2003). However, these studies were performed before the advent of molecular tools, when oocysts were identified based on their morphology and frequently described as *C. parvum* (smaller, spherical oocysts) or *C. muris* (larger, ovoid oocysts). Based on the current knowledge that species and genotypes of *Cryptosporidium* cannot be reliably identified from morphological data, many of the isolates identified as *C. parvum* may have been other taxa (Kváč et al. 2014a; Robertson et al. 2014). Molecular studies on *Cryptosporidium* in tree squirrels have been performed mostly in North America and Europe, with only two studies from Asia and no studies from South America and Africa (Feng et al. 2007; Kváč et al. 2008; Lv et al. 2009; Stenger et al. 2015; Ziegler et al. 2007a,b). Tree squirrels are known to host four *Cryptosporidium* species and three genotypes (Table 1). Stenger et al. (2015) showed that some *Cryptosporidium* spp. are broadly distributed across squir-

rel tribes, while others have a narrow host and geographic range. *Cryptosporidium* deer mouse genotype III and skunk genotype are frequently detected in tree squirrels in North America, but have not been detected in animals from Europe or Asia (Table 1), with the exception of a single report of the skunk genotype in a North American raccoon (*Procyon lotor*) from Poland (Lesnianska et al. 2016). Based on the knowledge that squirrels are considered among the most invasive mammalian taxa and that several squirrel species have been introduced into Europe (Bertolino 2009; Wauters et al. 2005), we hypothesized that *Cryptosporidium* species and genotypes infecting alien hosts would be more typical of the native region of those hosts. In Northern Italy, the range of native red squirrels extensively overlaps that of grey squirrels, which were introduced from North America during the last century, and, to a lesser extent, overlaps that of Pallas's squirrels (*Callosciurus erythraeus*), which were introduced from Southeast Asia in the last decade (Bertolino et al. 2014; Martinoli et al. 2010; Mazzamuto et al. 2016). Squirrels that were introduced to Italy represent a threat to red squirrels, which are distributed throughout continental Europe, and the recently described Calabrian black squirrel (*Sciurus meridionalis*), which is endemic to Southern Italy (Wauters et al. 2005, 2017).

We undertook the present study to describe the presence of *Cryptosporidium* spp. in native (*S. vulgaris*) and introduced (*S. carolinensis* and *C. erythraeus*) tree squirrels in Northern Italy. These data were used to determine whether there is interspecific transmission of *Cryptosporidium* spp. among the three host species, and to evaluate the role of tree squirrels as a reservoir of human pathogenic *Cryptosporidium* spp. Additionally, we explored host factors (i.e. population density, age, and sex) potentially affecting the distribution of *Cryptosporidium* in these hosts.

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