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Endemic *Toxoplasma gondii* Genotype II Causes Fatal Infections in Animal Hosts in Europe – Lessons Learnt

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1. Introduction

Toxoplasma gondii is a successful protozoan parasite of domestic animals, wildlife, and humans [1]. Despite this parasite is capable of causing disease and even killing its host, majority of infections are subclinical or asymptomatic. These latent, chronic infections are beneficial for the parasite: while the host is unaware of even ever acquiring the infection, the parasite stays dormant in the tissues of the host waiting for the host to be eaten by another host.

The latent *T. gondii* infections can be detected by measuring the antibody responses raised by the host against the parasite [1]. For most host species, the seroprevalence numbers are clearly higher than incidence of clinical and fatal cases. One exception is the European brown hare (*Lepus europaeus*), a host species that appears very susceptible to the infection [2]. It is worth emphasizing that if the infection proves fatal, it is not good for the parasite, either.

For this parasite, any nucleated cell of a warm-blooded animal will do, and the intestines of Felids are the place for sexual reproduction [1]. Humans are usually nothing but a dead-end host for *T. gondii*. Animal hosts clearly outnumber human hosts living on this planet and are more important for the spread and surviving of the parasite – Felids are shedding the oocyst reservoir, migrating animals are introducing the parasite to new areas, and prey animals are harboring the parasite in their tissues ready to infect the predators and scavengers. Investigating the infections in animal hosts can provide relevant clues needed for better understanding the parasite and its epidemiology, which has implications for public health also. The larger animal host population provides more options for epidemiologic studies.

Currently, one of the major issues in human toxoplasmosis research is evaluating the effect of some characteristics of the parasite, such as its genotype, on the outcome of the infection. Little is known of this effect in many animal hosts. Applying currently available methods to genetically characterize the parasite strains that cause clinical, and at worst fatal, toxoplasmosis in different host species can provide valuable new information to further understand the interactions of *T. gondii* and its various hosts: humans, domestic animals and wildlife.

In addition, free-ranging animal hosts and pet animals sharing the urban environment with humans can be regarded as sentinels for the *T. gondii* strains present in a specific area – the ones humans may encounter there as well. Characterization of both the *T. gondii* strains that are waiting to be eaten in the tissues of animals raised for human consumption and especially the possibly more virulent strains that had killed their animal hosts following natural infection in an area may thus provide important information for human health care professionals and public health decision making. Monitoring the situation assists in rapid detection of emergence of strains new to an area and changes in infection pressure. Molecular methods also allow tracing the infection sources and following the spread of an outbreak.

Majority of the *T. gondii* strains isolated from humans and animal hosts from Europe belong to genotype II, which typically only causes chronic infections if inoculated into mice (nonvirulent in mice) [9]. This is in sharp contrast to what appears to be the case in other areas, especially South America, where high level of genetic diversity is seen in *T. gondii* [9]. Fatal toxoplasmosis has been reported sporadically among individuals of both domestic and wild animal species examined postmortem [1,9], but published genotyping results of the parasites causing the severe, fatal infections are scarce. Data on the genotypes causing the fatal infections is particularly interesting from an area where the predominant genotype is considered to be of low virulence, such as Europe where type II is endemic.

This chapter describes recently published results from genetic characterization of *T. gondii* strains that proved fatal to their animal hosts following naturally acquired infection in Europe, and discusses the lessons learnt from them.

2. Summary of recent results

The special interest of our group has been genotyping the *T. gondii* strains causing fatal infections in various host species, thus far in Finland [2-4]. Recently, our group has retrospectively searched the records of European brown hares (*Lepus europaeus*), mountain hares (*Lepus timidus*) [2], and Eurasian red squirrels (*Sciurus vulgaris*) [3] examined postmortem in 2006-2009 at the only wildlife pathology laboratory in Finland, Evira, for cases of fatal toxoplasmosis. In addition, diagnosed cases of fatal toxoplasmosis in pet cats (*Felis catus*) that were necropsied at the University of Helsinki, Finland, in 2008-2010 have been thoroughly investigated [4]. The cases were confirmed with immunohistochemical staining of sections of formalin-fixed, paraffin-embedded tissue samples; the automated IHC staining protocol is described in [2]. In these studies of ours, naturally acquired toxoplasmosis was the confirmed cause of death of 14 (8.1%) of 173 European brown hares, 4 (2.7%) of 148 mountain hares [2], 3 (15.8%) of 19 Eurasian red squirrels [3], and 6 (3.1%) of

193 cats [4]. It is, indeed, not a particularly rare cause of death in these host species. However, it is important to bear in mind that this is not a good measure of disease incidence because these numbers are strongly affected by the material submitted for examination. The material available for investigations like these studies cannot be regarded as truly representative of the host animal populations of the area. This is especially the case in wild animals submitted for post-examination: only the dead animals found by active citizens before scavengers reach the wildlife pathology laboratory. The animals that had died near human settlements are very likely overrepresented.

For the genotyping of the *T. gondii* strains, we have extracted DNA from various tissue samples of the animals that had died from the infection: both formalin-fixed paraffin embedded samples, and fresh or frozen samples if available. Although most tissues have been rich in parasites in these cases, liver has become our tissue of choice. We use direct genetic characterization of the parasites in the tissues, without a bioassay step that could have a selective effect especially in case of mixed infections with several strains. Thus far two strains have been successfully isolated directly into cell cultures and cryopreserved, and from those, the genotyping analysis has been repeated from cell culture harvested parasites. The genotyping method we use is a multilocus method based on length polymorphism of seven microsatellite markers [2, 5, 6]. Six of the markers (B18, TUB2, TgM-A, W35, B17, and M33) are used for genotyping, and one additional marker (M48) for further characterization [5, 6].

As shown in Table 1, the genotyping results of the *T. gondii* strains causing the death of the animal hosts have been consistent with type II in all the 27 fatal cases examined from Finland thus far. Very similar results have been reported from other areas in Europe:

In Switzerland, a cat died from toxoplasmosis and the causative strain was genotyped using polymerase chain reaction-restriction fragment length polymorphism method with nine genetic markers (SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico) [7]. The analysis revealed type II alleles at all loci except one, Apico, which displayed a type I allele. This result is identical to the results one would obtain with this method from, for example, the reference strain PRU [1]. This commonly used genotype II reference strain was originally isolated from human fetal tissues in France and gives results that are fully consistent with genotype II with the method our group uses.

T. gondii parasites that had killed four arctic foxes (*Vulpes lagopus*) in the remote arctic archipelago Svalbard – this is very interesting from the geographical point of view - were genotyped at ten loci (SAG1 and the nine markers used in [7]) also using the polymerase chain reaction-restriction fragment length polymorphism method [8]. Three of the samples from the fatal cases had type I allele at Apico, whereas one had type II allele at all markers, and all four were interpreted as type II.

The strain that killed the cat in Switzerland was isolated by inoculation in mice and later maintained in cell cultures, but the genotyping was also performed directly from frozen tissues of the cat [7]. The genotyping of the strains that caused the deaths of the arctic foxes was done directly from the brain tissue of the foxes [8].

Species	Country of origin	Number of cases	Genotyping method used	Reference
Arctic fox (<i>Vulpes lagopus</i>)	Norway	4	PCR-RFLP	[8]
Cat (<i>Felis catus</i>)	Finland	6	MS	[4]
	Switzerland	1	PCR-RFLP	[7]
Eurasian red squirrel (<i>Sciurus vulgaris</i>)	Finland	3	MS	[3]
European brown hare (<i>Lepus europaeus</i>)	Finland	14	MS	[2]
Mountain hare (<i>Lepus timidus</i>)	Finland	4	MS	[2]

Table 1. Recent genotyping results of cases of fatal toxoplasmosis in different animal host species in Europe.

Taken together, *T. gondii* parasites belonging to the endemic genotype II that are typically nonvirulent in mice caused these altogether 32 fatal infections in altogether five different animal host species (Table 1). Genotype II was the only genotype detected from these fatal cases. Surprisingly, none of these animals had appeared to have any clear immunodeficiency or other predisposing factor. By contrast, the hares that had died from toxoplasmosis were actually heavier, in better bodily condition, than the hares that had died of other causes [2]. Interestingly, some host species, such as the European brown hares [2] and possibly also Eurasian red squirrels [3], appear extremely susceptible to the acquired *T. gondii* infection. They have relatively high proportional mortality rates from toxoplasmosis: the proportion of animals examined post-mortem that had died from toxoplasmosis is substantial.

3. Conclusions

Two conclusions and one question arise from these results summarized above:

1. These infections were naturally acquired, which supports the endemic status and dominance of *T. gondii* genotype II in Europe. These results also show its spread north, and that the parasite appears unstoppable by the harsh winters and remote locations. Not on-

ly animals, but undoubtedly also humans can encounter *T. gondii* even in the northernmost parts of Europe.

2. These results further affirm that no especially virulent *T. gondii* strain is required for the infection to kill a host. Moreover, these were naturally acquired infections, implying the infection doses have been within limits of what may be encountered in the nature and the infection routes probably the ones these hosts should be most adapted to.

The question remaining unanswered is the prevalence, role, and importance of *T. gondii* strains belonging to other genotypes than type II in Europe. More investigations in this field are ongoing, and needed. Strains belonging to other genotypes, possibly more virulent ones, could be found by characterizing more strains that cause severe or fatal infections.

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