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Short communication

Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France

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ABSTRACT

Toxoplasma gondii isolates have been classified into 3 genetic types. Little is known about genotypes of *T. gondii* isolates in wild animals in Europe. In this report, genotypes of *T. gondii* isolates from wildlife in France are described. Sera from wildlife were tested for antibodies to *T. gondii* with the modified agglutination test, and the hearts from animals with titers superior or equal to 1:6 were bioassayed individually in mice. *T.gondii* was isolated from 9 of 14 seropositive red foxes (*Vulpes vulpes*), 12 of 33 roe deer (*Capreolus capreolus*), 1 of 4 deer (*Cervus elaphus*), 1 of 7 mouflons (*Ovis gmelini musimon*) and 1 of 2 common mallards (*Anas platyrhynchos*). No isolate was obtained by bioassay in mice of 1 fallow deer (*Dama dama*) and of 3 European brown hares (*Lepus europaeus*). Genotyping of the 24 isolates using PCR-RFLP and microsatellite markers indicated that all were type II and none of these *Toxoplasma* isolates was virulent for mice.

1. Introduction

The protozoan *Toxoplasma gondii* is a zoonotic obligate intracellular parasite that infects humans and a wide variety of birds and mammals including felidae, which serve as hosts to the sexual phase of replication. *T. gondii* infections are widely prevalent in humans and animals worldwide (Tenter et al., 2000). The population structure of *T. gondii* in Europe and North America consists of three distinct clonal lineages known as types I, II and III (Sibley and Boothroyd, 1992). Recent studies have reported that the isolates of *T. gondii* from Brazil and South America are biologically and genetically different from those in North America and Europe (Dubey et al., 2002). In France, type II largely predominates in human toxoplasmosis (Ajzenberg et al., 2002) as well as in domestic animals (Dumètre et al., 2006),

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Table 1
T. gondii isolates from wildlife animals in France.

Host	Number of samples	Positive at 1:6 (%)[a]	Seroprevalence cut-off at 1:25 (%)	Number of samples bioassayed	Number of isolates	Isolation prevalence	Génotype
Roe deer (*Capreolus capreolus*)	60	36 (60)	24 (40)	33[b]	12/32	38%	Type II (12)
Mouflon (*Ovis gmelini*)	31	7 (23)	5 (16)	7[c]	1/4	25%	TypeII (1)
Red deer (*Cervus elaphus*)	24	4 (17)	1 (4)	4[b]	1/3	33%	Type II (1)
Fallow deer (*Dama dama*)	4	1 (25)	1 (25)	1	0/1	0	
Hare (*Lepus europaeus*)	23	3 (13)	2 (9)	3[b]	0/2	0	
Fox (*Vulpes vulpes*)	19	14 (74)	14 (74)	14[b]	9/13	69%	Type II (9)
Mallard (*Anas platyrhynchos*)	4	2 (50)	2 (50)	2	1/2	50%	Type II (1)

[a] Samples bioassayed in mice when MAT titer ≥1:6, when possible
[b] One bioassay not analysed because all mice died between days 1 and 3.
[c] Three bioassays not analysed because all mice died between days 1 and 3.

though little is known about *T. gondii* prevalence and genotype distribution in French wildlife species.

In this paper, parasite isolation was attempted from several wildlife species and isolates were genetically characterized using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and microsatellite markers.

2. Materials and methods

Roe deer (*Capreolus capreolus*, $n = 60$), mouflon (*Ovis gmelini musimon*, $n = 31$), red deer (*Cervus elaphus*, $n = 24$), fallow deer (*Dama dama*, $n = 4$), red fox (*Vulpes vulpes*, $n = 19$), European brown hares (*Lepus europaeus*, $n = 23$) and common mallard (*Anas platyrhynchos*, $n = 4$) were hunted in France during the hunting seasons 2003–2008. The blood samples from hunted animals were collected from the thoracic cavity with a syringe. Fluid samples were tested for antibodies to *T. gondii* with the modified agglutination test (MAT) as previously described (Dubey and Desmonts, 1987). Sera were diluted two-fold, starting at a 1:6 dilution. In accordance with previous studies, sera with an agglutination titer of 1:25 or higher were considered positive.

Hearts were collected and placed in sterile plastic collectors containing a suspension of 0.9% (w:v) saline with antibiotics added (120,000 U/L penicillin-G and 120 mg/L streptomycin). Hearts of individuals with a positive agglutination reaction (starting at a 1:6 dilution) were bioassayed in outbreed female Swiss Webster mice (Charles River Laboratory, France) (Villena et al., 2004). The whole heart, or maximum of 200 g of heart, was mixed and incubated at 37 °C for 2.5 h with trypsin (final concentration 0.25%). The suspension was then filtered, centrifuged, washed and suspended in saline solution containing penicillin-G and streptomycin. The homogenate was inoculated intraperitonealy in 3–6 mice, depending on the volume of the centrifugation pellet. Mice were tested for seroconversion with the MAT 4 weeks postinoculation (pi) and finally sacrificed 60 days pi. Tissue cysts in brains of seropositive mice were detected by microscopic examination.

Brain cysts from seropositive mice were isolated by percoll gradient centrifugation and DNA was then extracted using QIAamp DNA minikit (Qiagen, Courtaboeuf, France). Strain typing was performed using three PCR–RFLP markers (*SAG1*, *SAG2*, and *GRA7*). Genetic characterization was also performed at six microsatellite loci in a multiplex PCR assay (*TUB2*, *TgM-A*, *W35*, *B17*, *B18* and *M33*) (Ajzenberg et al., 2005).

3. Results and discussion

Recently, attention has been focused on the genetic and the biological variability among *T. gondii* isolates. Little is known about the genetic type and virulence in mice exposed to *T. gondii* isolates in wildlife in Europe. In this study, parasite isolation was attempted in several wildlife species from France and isolates were characterized using RFLP-PCR and microsatellite analysis.

T. gondii antibodies were found in 14 of 19 (73.7%) red foxes, with titers between 1:25 and 1:6400 (Table 1) and parasite isolation was successful in 9/13 seropositive animals (69.2%). In a large survey on red foxes in the USA, with the same test and the same cut-off, Dubey et al. (1999) reported a seroprevalence of 85.9% (243/283). A high seroprevalence was also found in Belgium (98%, Buxton et al., 1997) and in Hungary (68%, Jakubek et al., 2007). Seroprevalence in North Europe was lower: 38% in Sweden (Jakubek et al., 2001), 31% in Norwegian and Swedish foxes (Kapperud, 1978), 20% in the United Kingdom and 47% in Ireland (Hamilton et al., 2005; Wolfe et al., 2001). As for parasitological prevalence, De Lalla et al. (1967) isolated *T. gondii* from 5 of 17 (29%) Italian red foxes whereas seroprevalence was 43.3%. *T. gondii* was isolated from one seropositive fox (Dubey et al., 2004) and was type II. All the 9 strains isolated from foxes were type II and avirulent for mice.

Thirty-six of the 60 roe deer (60%) showed antibodies with titers between 1:6 and 1:6400. Thirty-three bioassays were performed, 12 isolates were obtained from animals with antibodies titers between 1:25 and 1:6400. *T. gondii* antibodies were found in 4 of 24 red deer (17%) with titers of 1:6 (2), 1:10 (1) and 1:25 (1) and a viable parasite was isolated from the heart of one red deer with a titer of 1:6. No parasite was isolated from fallow deer with only one positive with a titer of 1:25. In the Czech Republic, 15% and 14% of the tested red deer and roe deer, respectively, had antibodies against *T. gondii*, but tissue cysts were not isolated from these two species (Hejlicek et al., 1997). Kapperud (1978) reported seroprevalence of 12% and 63% and Vikoren et al. (2004) 7.7% and 33.9% in Norwegian red and roe deer respectively. Recently, using the MAT and a cut-off titer of 1:25, Gauss et al. (2006) showed that antibodies were detected in 15.6% of red deer ($n = 441$) and

21.9% of roe deer ($n = 33$) and Gamarra et al. (2008) found *T. gondii* antibodies in 39.2% of roe deer ($n = 238$) in Spain. Using the same cut-off, we found a prevalence of 4% in 24 red deer, and 40% in 60 roe deer. No tissue cyst was found in 309 examined red deer, in 117 roe deer and in 8 fallow deer from the Czech Republic (Hejlicek et al., 1997). The present study demonstrates that *T. gondii* can be isolated from naturally infected roe and red deer. Little data is available from fallow deer. In the Czech Republic, Hejlicek et al. (1997) showed the presence of antibodies against *T. gondii* in all the 3 animals analyzed but failed to isolate *T. gondii* from fallow deer as in the present study. More recently, Bartova et al. (2007) found a lower prevalence (16.8%, $n = 143$). With MAT at 1:25, 24% of the 79 fallow deer had antibodies to *T. gondii* (Gauss et al., 2006) in Spain.

T. gondii antibodies were found in 7 of 31 mouflons (23%) with titers between 1:6 and 1:6400. We observed similar seroprevalence of *T. gondii* infection in mouflons (16%, $n = 31$) compared to Gauss et al. (2006) in Spain (14.8%, $n = 27$) and Hejlicek et al. (1997) in the Czech Republic (10%, $n = 20$). Moreover, viable parasites were recovered from 1 of the 4 seropositive mouflons analyzed (for the 3 other samples, all the mice died from bacterial infection).

Consumption of infected meat from cervids was identified as a source of *T. gondii* infection in humans (Ross et al., 2001) as well as evisceration and handling of game may represent risks for human infection (Dubey, 1994).

The brown hare (*L. europaeus*) is a common species of wild mammals in Europe where they are extensively hunted. Hejlicek et al. report a parasitological prevalence of 4% in *L. europaeus* (6 of 164). We detected *T. gondii* antibodies in only 9% of sera from brown hares but failed to isolate viable parasites.

Two of the four mallards sampled had *T. gondii* antibodies with titer of 1:50 and the parasite isolation was successful in 1 case out of 2. A previous report of isolation of *T. gondii* from common mallards by Literak et al. (1992) recovered the parasite from 22 of the 184 mallards analyzed (12%).

The prevalence of antibodies against *T. gondii* varied considerably between the different species examined (from 4 to 74%). This may be due to species difference in diet or behavior and susceptibility to infection. Genotyping of the 24 *T. gondii* isolates using the 3 PCR–RFLP markers and the 6 microsatellite markers revealed a type II genotype for all isolates (Table 1) and none of the *Toxoplasma* isolates was virulent for mice. These results are in agreement with the observed genotype of animal strains in France. All the strains isolated from domestic (sheep, $n = 8$; Dumètre et al., 2006) or wildlife species (tawny owl, $n = 1$, Aubert et al., 2008 and wild boars, $n = 21$, Richomme et al., 2009) were avirulent for mice and were from type II.

These results underline that wildlife species may serve as an important reservoir for transmission of *T. gondii*.

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References

Ajzenberg, D., Cogné, N., Paris, L., Bessières, M.H., Thulliez, P., Filisetti, D., Pelloux, H., Marty, P., Dardé, M.L., 2002. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J. Infect. Dis. 186, 684–689.

Ajzenberg, D., Dumètre, A., Dardé, M.L., 2005. Multiplex PCR for typing strains of *Toxoplasma gondii*. J. Clin. Microbiol. 43, 1940–1943.

Aubert, D., Terrier, M.E., Dumetre, A., Barrat, J., Villena, I., 2008. Prevalence of *Toxoplasma gondii* in some raptors from France. J. Wildl. Dis. 44, 172–173.

Bartova, E., Sedlak, K., Pavlik, I., Literak, I., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in wild ruminants from the countryside or captivity in the Czech Republic. J. Parasitol. 93, 1216–1218.

Buxton, D., Maley, S.W., Pastoret, P.P., Brochier, B., Innes, E.A., 1997. Examination of red foxes (*Vulpes vulpes*) from Belgium for antibody to *Neospora caninum* and *Toxoplasma gondii*. Vet. Rec. 141, 308–309.

De Lalla, F., Bechelli, G., Cavallini-sampieri, L., 1967. Osservazioni sierologiche e parassitologiche sulla diffusione della toxoplasmosi nelle volpi dell'area di Siena. La clinica Veterinaria 90, 393–400.

Dubey, J.P., 1994. Toxoplasmosis. J. Am. Vet. Med. Assoc. 205, 1593–1598.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.

Dubey, J.P., Graham, D.H., De Young, R.W., Dahl, E., Eberhard, M.L., Nace, E.K., Won, K., Bishop, H., Punkosdy, G., Sreekumar, C., Vianna, M.C., Shen, S.K., Kwok, O.C., Sumners, J.A., Demarais, S., Humphreys, J.G., Lehmann, T., 2004. Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. J. Parasitol. 90, 67–71.

Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., Ragozo, A.M., Nishi, S.M., Shen, S.K., Kwok, O.C., Hill, D.E., Thulliez, P., 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sao Paulo, Brazil: unexpected findings. Int. J. Parasitol. 32, 99–105.

Dubey, J.P., Storandt, S.T., Kwok, O.C., Thulliez, P., Kazacos, K.R., 1999. *Toxoplasma gondii* antibodies in naturally exposed wild coyotes, red foxes, and gray foxes and serologic diagnosis of Toxoplasmosis in red foxes fed *T. gondii* oocysts and tissue cysts. J. Parasitol. 85, 240–243.

Dumètre, A., Ajzenberg, D., Rozette, L., Mercier, A., Dardé, M.L., 2006. *Toxoplasma gondii* infection in sheep from Haute-Vienne, France: Seroprevalence and isolate genotyping by microsatellite analysis. Vet. Parasitol. 142, 376–379.

Gamarra, J.A., Cabezón, O., Pabón, M., Arnal, M.C., Luco, D.F., Dubey, J.P., Gortázar, C., Almeria, S., 2008. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. Vet. Parasitol. 153, 152–156.

Gauss, C.B., Dubey, J.P., Vidal, D., Cabezon, O., Ruiz-Fons, F., Vicente, J., Marco, I., Lavin, S., Gortazar, C., Almeria, S., 2006. Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain. Vet. Parasitol. 136, 193–200.

Hamilton, C.M., Gray, R., Wright, S.E., Gangadharan, B., Laurenson, K., Innes, E.A., 2005. Prevalence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in red foxes (*Vulpes vulpes*) from around the UK. Vet. Parasitol. 130, 169–173.

Hejlicek, K., Literak, I., Nezval, J., 1997. Toxoplasmosis in wild mammals from the Czech Republic. J. Wildl. Dis. 33, 480–485.

Jakubek, E.B., Brojer, C., Regnersen, C., Uggla, A., Schares, G., Bjorkman, C., 2001. Seroprevalences of *Toxoplasma gondii* and *Neospora caninum* in Swedish red foxes (*Vulpes vulpes*). Vet. Parasitol. 102, 167–172.

Jakubek, E.B., Farkas, R., Pálfi, V., Mattsson, J.G., 2007. Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in Hungarian red foxes (*Vulpes vulpes*). Vet. Parasitol. 144, 39–44.

Kapperud, G., 1978. Survey for toxoplasmosis in wild and domestic animals from Norway and Sweden. J. Wildl. Dis. 14, 157–162.

Literak, I., Hejlicek, K., Nezvaal, J., Folk, C., 1992. Incidence of Toxoplasma gondii in populations of wild birds in the Czech Republic. Avian pathol. 21, 659–665.

Richomme, C., Aubert, D., Gilot, E., Ajzenberg, D., Mercier, A., Ducrot, C., Ferté, H., Delorme, D., Villena, I., 2009. Genetic characterization of *Toxoplasma gondii* from wild boar (*Sus scrofa*) in France. Vet. Parasitol. 164, 296–300.

Ross, R.D., Stec, L.A., Werner, J.C., Blumenkranz, M.S., Glazer, L., Williams, G.A., 2001. Presumed acquired ocular toxoplasmosis in deer hunters. Retina 21, 226–229.

Sibley, L.D., Boothroyd, J.C., 1992. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. Nature 359, 82–85.

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217–1258.

Vikoren, T., Tharaldsen, J., Fredriksen, B., Handeland, K., 2004. Prevalence of *Toxoplasma gondii* antibodies in wild red deer, roe deer, moose, and reindeer from Norway. Vet. Parasitol. 120, 159–169.

Villena, I., Aubert, D., Gomis, P., Ferte, H., Inglard, J.C., Denis-Bisiaux, H., Dondon, J.M., Pisano, E., Ortis, N., Pinon, J.M., 2004. Evaluation of a strategy for *Toxoplasma gondii* oocyst detection in water. Appl. Environ. Microbiol. 70, 4035–4039.

Wolfe, A., Hogan, S., Maguire, D., Fitzpatrick, C., Vaughan, L., Wall, D., Hayden, T.J., Mulcahy, G., 2001. Red foxes (*Vulpes vulpes*) in Ireland as hosts for parasites of potential zoonotic and veterinary significance. Vet. Rec. 149, 759–763.