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K24 *T. GONDII* ISOLATE IS A HYBRID AND HAS THE VIRULENCE OF LINEAGE I ISOLATES

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Summary:

A permanently high virulence was found in tachyzoites of *T. gondii* K24 after serial passage in mice (90 passages during 324 days). Virulence tests revealed that a single tachyzoite of the 50th passage represented LD_{100} for mice. Analysis of genotype of K24 isolate was done by PCR/RFLP with ROP1/*Dde*I, SAG1/*Dde*I, 850/*Rsa*I and IGS/*Rsa*I and by RFLP/DNA with TGR1E sequence and *Pst*I enzyme. K24 isolate had an atypical genotype, with an association of type II (for ROP1, SAG1 genes and TGR1E sequence) and type I (for 850 gene) alleles, and a new pattern observed for IGS. All tested PCR/RFLP did not change through 2, 10, 20, 28, 40, 50, 60, 70, 81 and 90 tested passages. In RFLP/DNA with *Pst*I enzyme and TGR1E probe, K24 isolate produced a pattern with seven fragments of the size ranging from one to 23 kb and did not change through 7, 56, 70 and 83 tested passages. K24 *T. gondii* isolate is a hybrid and has the virulence of lineage I isolates.

KEY WORDS : *Toxoplasma gondii*, virulence, genotype, PCR-RFLP, RFLP/DNA.

Résumé : LA SOUCHE K24 DE *T. GONDII* EST UN HYBRIDE PRÉSENTANT LA VIRULENCE DES SOUCHES DE LA LIGNÉE I

Après passages successifs sur souris (90 passages en 324 jours), nous avons pu observer une augmentation de la virulence des tachyzoites de T. gondii. *Les tests de virulence ont en effet montré qu'un seul tachyzoite prélevé au 50ème passage induit une DL_{100} chez la souris. L'analyse du génotype de la souche K24, effectuée par (i) PCR/RPLF sur ROP1/*DdeI, *ROP1/*DdeI, *SAG1/*DdeI, *850/*RsaI *et IGS/*RsaI, *(ii) RFLP/DNA sur TGR1E, et (iii) l'enzyme* PstI, *a révélé l'existence d'une structure atypique, associant les allèles du type II (pour les gènes ROP1, SAG1 et la séquence TGR1E) et du type I (gène 850). Par contre, une nouvelle combinaison spécifique a été mise en évidence pour IGS. Les analyses génotypiques par PCR/RFLP effectuées au cours des passages (2, 10, 20, 28, 40, 50, 60, 70, 81 et 90), n'ont révélé aucune différence. L'analyse RFLP (*PstI *et TGR1E) de K24 a montré l'existence d'un patron de sept fragments de restriction (1 à 23 kb) qui ne varie pas au cours des 7, 56, 70 et 83ème passages. La souche K24 de* T. gondii *est donc un hybride présentant la virulence des souches de la lignée I.*

MOTS CLÉS : Toxoplasma gondii, *virulence, génotype, PCR-RFLP, RFLP/DNA.*

INTRODUCTION

T. gondii population has a clonal structure with three main lineages: I, II and III (Dardé *et al.*, 1992; Sibley & Boothroyd, 1992; Howe & Sibley, 1995). The strains of lineage type I are virulent in mice, while those of types II and III are avirulent (Howe & Sibley, 1995). All three lineages occur in different animal species and in humans from different geographical areas. The strains of lineage type II cause human toxoplasmosis and chronic infections in farm animals (Howe & Sibley, 1995). The strains of lineage type I are relatively rare in animals, while in humans they occur more frequently in patients with congenital (Fuentes *et al.*, 2001) and ocular toxoplasmosis (Grigg & Boothroyd, 2001). The isolates and strains isolated from domestic and wild animals usually consist of lineages type II and type III (Mondragon *et al.*, 1998; Cole *et al.*, 2000), but lineage type I was reported as well (Dubey *et al.*, 2002).

There are various strains with different virulence levels within *T. gondii* population. The virulence of the strains is usually determined on the basis of lethal dose of tachyzoites (Cruz, 1989). On the basis of lethal doses and tachyzoite virulence for mice different strains of *T. gondii* were divided into two groups: virulent strains ($LD_{100} < 10$ tachyzoites), which are able to cause acute lethal infection with ascites in mice, and avirulent strains ($LD_{100} > 10^3$), which cause chronic infection typical by the formation of tissue cysts in the brain of mice (Sibley & Boothroyd, 1992). Ferreira *et al.* (2001) divided *T. gondii* isolates into three groups according to mortality and period of survival in mice infected with the dose of ten tachyzoites. Group 1 included the isolates with 100 % mortality within 5-10 days post infection (p.i.). In group 2, the mortality of 100 % was recorded within 7-19 days p.i. The isolates from the third group showed 0 % mortality by 30-day p.i.

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Most of the strains of *T. gondii* were passaged in laboratory mice. Pathogenicity of some strains increased after passage with parenteral inoculation in mice (Jacobs & Melton, 1954). A loss of ability to produce oocysts after the infection in cats was observed in the cases of increased pathogenicity (Frenkel *et al.*, 1976). Frenkel & Ambroise-Thomas (1997) reported this phenomenon already after 30-35 passages of *T. gondii* in the form of tachyzoites. It is assumed that different phenotype features of the strains (different virulence in mice, ability or lack of ability to produce oocysts after the infection in cats) express different genotypes in the respective strains of *T. gondii*. Frenkel & Ambroise-Thomas (1997) concluded from their experiments that a long serial passage may lead to genomic changes and thus to the loss of ability to produce oocysts.

Genetic polymorphism of different *T. gondii* strains and isolates with regard to their virulence was studied by PCR-RFLP with the following markers: SAG1 with *Dde*I and *Sau96*I endonucleases (Sibley & Boothroyd, 1992; Howe & Sibley, 1994; Howe & Sibley, 1995; Mondragon *et al.*, 1998), ROP1 with *Dde*I, *Hba*I and *Hae*III endonucleases (Howe & Sibley, 1994; Howe & Sibley, 1995; Literák & Rychlík, 1999), 850 with *Rsa*I and *Sau*96I endonucleases (Sibley & Boothroyd, 1992; Howe & Sibley, 1995), the intergenic spacer (IGS) with *Spe*I, *Rsa*I and *Alu*I endonucleases (Fazaeli *et al.*, 2000b). A more detailed differentiation of the strains (fingerprint analysis) can be done by RFLP of total volume of chromosomal DNA using various enzymes, e.g. *Sal*I, *Pst*I, and various probes such as TGR1 E, TGR 6 and BS (Cristina *et al.*, 1991; Cristina *et al.*, 1995; Sibley & Boothroyd, 1992; Literák *et al.*, 1998).

The objective of the present study was to characterize virulence of the *T. gondii* K24 isolate from the Czech Republic by experimental inoculation in mice and to determine its genotype by the methods of molecular biology.

MATERIALS AND METHODS

T. GONDII ISOLATES

T. gondii K24 isolate was obtained from faeces of an infected domestic cat in 1995, Czech Republic (Literák *et al.*, 1998). The oocysts after sporulation were administered in drinking water to ten *Toxoplasma*-negative mice. Three months later microscopic examination of compressed specimens of mice brains was carried out to confirm the presence of tissue cysts of *T. gondii*. Brain tissue suspension from the specimens with confirmed presence of tissue cysts of *T. gondii* was used for intraperitoneal (i.p.) infection of two other mice. The procedure was repeated in the intervals of 4-6 months. Tachyzoites from K24 isolate were obtained by a modified procedure according to Dardé *et al.* (1992). The tissue cysts from the 15th passage were used for i.p. inoculation in the mice previously treated by subcutaneous (SC) injections of hydrocortisone sodium succinate in the dose of 40 mg/kg every other day for the total period of 20 days. Hydrocortisone injections were continued after the inoculation of mice with tissue cysts of *T. gondii* K24 isolate. Mice were killed after 8-10 days p.i. Peritoneal exudate of the mice contained tachyzoites, which DNA was isolated. Moreover, the tachyzoites were used for the preparation of infection dose for two other mice, which were inoculated i.p. injection. Subsequently in total 90 serial passages took place. The isolate in the form of tachyzoites was inoculated to mice in the intervals of 3-4 days. The mice were killed and tachyzoites obtained from peritoneal exudates were used for the preparation of next infectious dose and for DNA isolation. The procedure was repeated till the 90th passage when the experiment was ended after 324 days.

P-CZ is a strain isolated from a man in Prague, Czech Republic, in 1963 (Kouba *et al.*, 1974). The strain was classified into the category with genotype observed in mouse-virulent isolates (Literák *et al.*, 1998). Tachyzoites of the isolate were obtained from the National Institute of Public Health in Prague, where it has been maintained in the collection of isolates by continual passage in mice in the interval of 3-4 days. This isolate was used as virulent control. DNA was isolated from tachyzoites of this isolate.

K25 was isolated in 1995 in the form of oocysts from faeces of a domestic cat in Brno, Czech Republic. The isolate was classified into the category with genotype observed in mouse-avirulent isolates (Literák *et al.*, 1998). Continual passages of tissue cysts were carried out in mice. The isolate was used as avirulent control. DNA was isolated from tachyzoites of this isolate.

EXPERIMENTAL ANIMALS

Toxoplasma-negative mice of CD-1 strain (Anlab, Charles River, Prague) were used for the passage of the isolate and for the tests of virulence of K24 isolate tachyzoites. The weight of the mice was 20-22 g. The mice were housed in plastic boxes for laboratory rodents. Feed compound for laboratory mice and drinking water were available *ad libitum*.

TESTS OF VIRULENCE IN K24 ISOLATE TACHYZOITES

Eight groups of ten mice each were infected by i.p. inoculation of 10^6 to 10^1 tachyzoites (from 50th passage) as determined by dilution method. The period of survival of infected mice was recorded.

Isolation of DNA from tachyzoites

QIAamp Tissue Kit (Qiagen, Germany) was used for the isolation of DNA from tachyzoites of K25 and P-CZ isolates and from passages (2, 10, 20, 28, 40, 50, 60, 70, 81 and 90) of K24 isolate. Isolated DNA was resuspended in 200 µl of AE buffer and stored at -20° C.

RFLP analyses

Correlation of *Toxoplasma gondii* virulence with genotypes was assessed by PCR-RFLP and RFLP/DNA.

The genetic loci ROP1, SAG1, 850 and IGS were amplified by PCR; products were digested with appropriate endonucleases *Dde*I, *Dde*I, *Rsa*I, *Rsa*I, respectively, and electrophoresed in 2 % agarose gels containing ethidium bromide. For ROP1 the two primers were: 5´-CGTGACATATACTGCACTGAC-3´ and 5´-CATCTG-CAAACTCGATCAC-3´, each of the 40 reaction cycles consisted of 94° C for 30 s, 64° C for 1 min and 72° C for 2 min (Ossorio *et al.*, 1992). For SAG1, primers were: 5´-CAACGGTAATCACTCACGCG-3´ and 5´-CAATGTGCACCTGTAGGAAGC-3´. The conditions of PCR referred to by Sibley & Boothroyd (1992), were optimised to: 40 cycles of 94° C for 1 min, 52.5° C for 1.5 min, 72° C for 1.5 min. For 850, primers were: 5´-AAGGACCTGGTAACAGTCC-3´ and 5´-TCAAG-GCTTGGATGTTTCG-3´. The conditions of PCR referred to by Sibley & Boothroyd (1992), were optimised to: 40 cycles of 94° C for 1 min, 50° C for 2 min, and 72° C for 2 min. For IGS, primers were: 5´-TTCGCTTCATGCTTTTGGGC-3´ and 5´-TGAGC-CATTCGCAGTTTAGC-3´. The conditions of PCR referred to by Fazaeli *et al.* (2000b), were optimised to: ten cycles of 94° C for 30 s, 38° C for 1 min, 72° C for 1.5 min, 35 cycles of 94° C for 1 min, 48° C for 1.5 min, 72° C for 2 min.

Toxoplasmic DNA for RFLP/DNA was digested overnight at 37° C with endonuclease *Pst*I. The *Pst*I digests were separated on the 0.8 % agarose gel by electrophoresis and then transferred onto a nylon membrane (Hybond™-N, Amersham, UK) by Southern blotting. The membranes were hybridized with the probe TGR1E, labelled with horseradish peroxidase. The Southern blotting and hybridization conditions have been descried elsewhere (Cristina *et al.*, 1991; Cristina *et al.*, 1995). The probe was prepared by PCR with two primers: 5´-GGAGATGGTCGGGCGTATTG-3´ and 5´-CACCTGTGCCGCAAATGAAA-3´, and amplification conditions of 94° C for 5 min, 65° C for 2 min, 72° C for 40 s, followed by 35 cycles of 94° C for 30 s, 65° C for 40 s, 72° C for 40 s and stabilization of 94° C for 30 s, 65° C for 40 s, 72° C for 5 min. PCR product of 194 bp was purified from 1.5 % agarose gel by Qiagen Gel Extraction kit (Amersham). After hybridization, hyperfilm ECL (Amersham) was placed onto the nylon membrane and then exposed in dark for 1-2 h and developed.

RESULTS

Experimental inoculation in mice

Virulence tests in tachyzoites of K24 isolate from the 50th passage showed high virulence. The infectious dose corresponding to LD_{100} for mice was equal to a single tachyzoite. All infected mice died within the period of four to 11 days, depending on the infectious dose (Table I).

Infectious dose (tachyzoites)	10^6	10^5	10^4	10^3	10^2	10	1
Number of infected mice	10	10	10	10	10	10	10
Period till death (days)	**Number of dead mice**						
4	4	2					
5	5	8	1				
6	1		4				
7			5	2			
8				4	4	1	1
9				3	2	5	4
10				1	4	3	5
11						1	

Table I. – Virulence of tachyzoites from the 50th passage in mice (K24 isolate).

RFLP analysis

Despite the fact that they have a virulent phenotype, K24 isolate had an atypical genotype, with an association of type II (for ROP1, SAG1 genes and TGR1E sequence) and type I (for 850 gene) alleles, and a new pattern observed for IGS.

In PCR/RFLP (ROP1/*Dde*I) two large fragments of approximately 900 and 750 bp were seen in virulent control, one large and one small fragment of approximately 900 and 300 bp were seen in avirulent control and K24 isolate. In PCR/RFLP (SAG1/*Dde*I) single fragment was seen in virulent control, single but larger fragment was seen in avirulent control and K24 isolate. In PCR/RFLP (850/*Rsa*I) single fragment of approximately 700 bp was seen in virulent control and K24 isolate, avirulent control showed also single but smaller fragment of approximately 350 bp. In PCR/RFLP (IGS/*Rsa*I) two fragments of 1,630 and 260 bp were seen in virulent control, three fragments of 1,120, 500 and 260 bp in avirulent control, while K24 isolate showed different fragments of approximately 700 and 350 bp (Fig. 1). Patterns of all tested PCR/RFLP did not change through 2, 10, 20, 28, 40, 50, 60, 70, 81 and 90 tested passages.

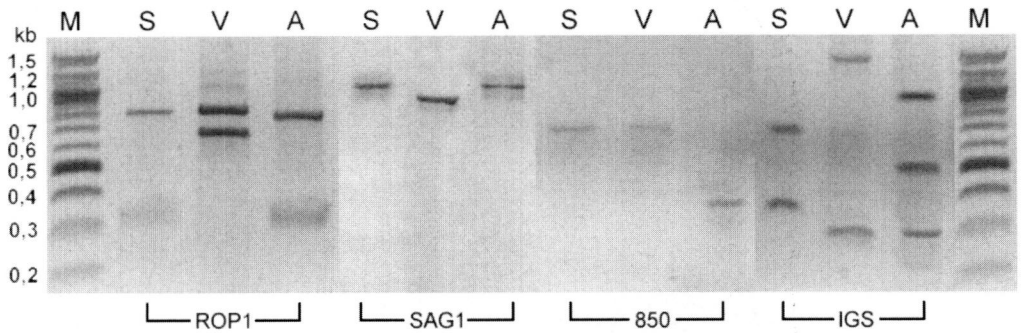

Fig. 1. – PCR-RFLP patterns of *T. gondii* strains of ROP1 cut with *Dde*I, SAG1 cut with *Dde*I, 850 cut with *Rsa*I and IGS cut with *Rsa*I. Lanes M are DNA size marker between 1.5 and 0.2 kb; lanes S are K24 *T. gondii* isolate; lanes V are virulent control (P-CZ strain); lanes A are avirulent control (K25 isolate).

In RFLP/DNA with *Pst*I enzyme and TGR1E probe, K24 isolate produced a pattern with seven fragments of the size ranging from i to 23 kb and did not change through 7, 56, 70 and 83 tested passages. This pattern of K24 isolate was the same with the pattern obtained by the same methods in 1998, when this isolate was first genotyped (Literák *et al.*, 1998).

DISCUSSION

After long-term continual passages (90 passages) of K24 isolate tachyzoites that were obtained from 15th passage in form of tissue cysts in mice, high virulence of tachyzoites from the 50th passage was found. A single tachyzoite represented LD_{100} for mice. Isolate K24 has been characterized yet by Literák *et al.* (1998), but the only tested infectious dose was 10^3 tachyzoites that were obtained after 6th passage of tissue cysts in mice. Out of 11 infected mice 2 died.

After a certain number of passages of *T. gondii* isolates in the form of tachyzoites the virulence may be changed. The phenotypic expression will show a failure to form oocysts and increased virulence for mice. Increased virulence was demonstrated for instance by Ruiz & Frenkel (1980) in seven *T. gondii* isolates during 5-10 passages, or by Literák & Rychlík (1999) in three isolates after five to 45 passages of tachyzoites in mice. After 35-61 passages of tachyzoites *T. gondii* isolates lost the ability to form oocysts (Dubey & Frenkel, 1973; Frenkel *et al.*, 1976).

Frenkel & Ambroise-Thomas (1997) concluded that the increased virulence and failure to form oocysts, which occurred after approximately 30-35 passages in the form of tachyzoites (approximately after 105-122 days), might be a consequence of genomic changes. Literák & Rychlík (1999) recorded increased virulence accompanied with current genomic changes in three Czech isolates by PCR-RFLP (ROP1/*Dde*I) and RFLP/DNA with TGR1E probe. In two of them the virulent genotype was recorded already after six passages of tachyzoites, in the third isolate this happened after the 38th passage of tachyzoites. Sibley & Boothroyd (1992) also reported increased virulence in some cases after fast and short passages. However, genomic changes were never observed.

On the basis of extensive genotype characterization of 22 Czech isolates of *T. gondii* in 1998, K24 isolate exhibited a genotype usually observed in mouse-avirulent isolates (Literák *et al.*, 1998).

During the long-term passages of K24 isolate in form of tachyzoites in mice, the virulent phenotype was observed when tested tachyzoites from 50th passage in mice. Despite of its virulent phenotype, K24 isolate had an atypical genotype, with an association of type II (for ROP1, SAG1 genes and TGR1E sequence) and type I (for 850 gene) alleles, and a new pattern observed for IGS.

Using different methods and different markers (genes and sequences) resulted in a different degree of correlation between genotype and biological characteristics of K24 strain. The situation wherein K24 isolate showed an atypical genotype despite its high virulence confirmed in mice has to be considered rare. There is only a report on few strains, which showed similar behaviour. Virulent MAS strain had a genotype usually observed in mouse-avirulent isolates detected by PCR/RFLP (GRA6/*Msa*I) (Fazaeli *et al.*, 2000a). When sequencing 213-1587 bp PCR/RFLP product (IGS/*Spe*I, *Rsa*I and *Alu*I) was done, the sequence of MAS and three other strains (RUB, TONT and CASTLE) differed from both virulent and avirulent controls (Fazaeli *et al.*, 2000b). After sequencing 0.3 kb part of SAG1 gene, Rinder *et al.* (1995) found three polymorphic sites that correlated with virulence in mice. Strain MAS showed genotype usually observed in mouse-virulent strains. Based on RFLP analysis with six genetic markers, MAS

strain was included among the strains with genotype type I, although there was a difference from genotype type I in two markers, L328 and 62 (Howe & Sibley, 1995). MAS probably originated from a recombination of virulent and avirulent genotypes, and therefore either virulent or avirulent genotypes are produced, or a completely different genotype occurs. Howe & Sibley (1995) characterized 106 *T. gondii* strains by RFLP with six genetic markers. All strains were classified into three groups with genotypes type I, II and III. Four strains with different genotypes were not included in any of four groups. Strains HART, SOU and B73 most probably originated by recombination of genotypes type II and III. Another strain P89 supposedly originated by recombination of genotypes types I and III (Howe & Sibley, 1995).

Although *T. gondii* undergoes predominant clonal evolution, at least in the cycles studied until now, some of its genotypes are hybrid. Ten of 18 analysed strains (TONT, SSI, P80, P89, ELG, RUB, MAS, CASTELLS, VAND and COUGAR) seem to derive from intermixing between the proposed two ancestral lineages (Grig *et al.*, 2001). They results showed that recombination can generate progeny with changed biological qualities (higher virulence), that may be result of combination of alleles that cooperate to confer increased pathogenicity.

We propose that K24 isolate is also hybrid with virulence of lineage I isolates.

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REFERENCES

Cole R.A., Lindsay D.S., Howe D.K., Roderick C.L., Dubey J.P., Thomas N.J. & Baeten L.A. Biological and molecular characterizations of *Toxoplasma gondii* strains obtained from Southern sea otters (*Enhydra lutris nereis*). *Journal of Parasitology*, 2000, *86*, 526-530.

Cristina N., Dardé M.L., Boudin G.T., Pestre-Alexandre M. & Ambroise-Thomas P. A DNA fingerprinting method for individual characterization of *Toxoplasma gondii* strains: combination with isoenzymatic characters for determination of linkage groups. *Parasitology Research*, 1995, *81*, 32-37.

Cristina N., Oury B., Ambroise-Thomas P. & Santoro F. Restriction-fragment-length polymorphisms among *Toxoplasma gondii* strains. *Parasitology Research*, 1991, *77*, 266-268.

Cruz A.A., Dreseen D.W. & Evans D.L. Western blot analyses and LD_{50} determination of *Toxoplasma gondii* isolates. *Veterinary Immunology Immunopathology*, 1989, *23*, 355-364.

Dardé M.L., Bouteille B. & Pestre-Alexandre M. Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. *Journal of Parasitology*, 1992, *78*, 786-794.

Dubey J.F. & Frenkel J.K. Experimental *Toxoplasma* infection in mice with strains producing oocysts. *Journal of Parasitology*, 1973, *59*, 505-512.

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

Fazaeli A., Carter P.E., Darde M.L. & Pennington T.H. Molecular typing of *Toxoplasma gondii* strains by GRA6 gene sequence analysis. *International Journal for Parasitology*, 2000a, *30*, 637-642.

Fazaeli A., Carter P.E. & Pennington T.H. Intergenic spacer (IGS) polymorphism: a new genetic marker for differentiation of *Toxoplasma gondii* strains and *Neospora caninum*. *Journal of Parasitology*, 2000b, *86*, 716-723.

Ferreira A.M., Martins M.S. & Vitor R.W.A. Virulence for BALB/c mice and antigenic diversity of eight *Toxoplasma gondii* strains isolated from animals and humans in Brazil. *Parasite*, 2001, *8*, 99-105.

Frenkel J.K. & Ambroise-Thomas P. Genomic drift of *Toxoplasma gondii*. *Parasitology Research*, 1997, *83*, 1-5.

Frenkel J.K., Dubey J.P. & Hoff R.L. Loss of stages after continuous passage of *Toxoplasma gondii* and *Besoitia jellisoni*. *Journal of Protozoology*, 1976, *23*, 421-424.

Fuentes I., Rubio J.M., Ramirez C. & Alvar J. Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: direct analysis from clinical samples. *Journal of Clinical Microbiology*, 2001, *39*, 1566-1570.

Grigg M.E., Bonnefoy S., Hehl A.B, Suzuki Y. & Boothroyd J.C. Success and virulence in *Toxoplasma* as the result of sexual recombination between two distinct ancestries. *Science*, 20001, *294*, 161-165.

Grigg M.E. & Boothroyd J.C. Rapid identification of virulent type I strains of the protozoan pathogen *Toxoplasma gondii* by PCR-restriction fragment length polymorphism analysis at the B1 gene. *Journal of Clinical Microbiology*, 2001, *39*, 398-400.

Howe D.K. & Sibley L.D. *Toxoplasma gondii*: analysis of different laboratory stocks of the RH strain reveals genetic heterogenity. *Experimental Parasitology*, 1994, *78*, 242-245.

Howe D.K. & Sibley L.D. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *Journal of Infectious Diseases*, 1995, *172*, 1561-1566.

Jacobs L. & Melton M.L. Modification in pathogenicity of a strain of *Toxoplasma gondii* by passage in various hosts. *American Journal of Tropical Medicine and Hygiene*, 1954, *3*, 447-457.

KOUBA K., JIRA J. & HÜBNER J. Toxoplasmosis. Avicenum, Prague, 1974, (in Czech).

LITERÁK I. & RYCHLÍK I. Genome changes in the *Toxoplasma gondii* strains during laboratory passages in mice. *Acta Veterinaria* (Brno), 1999, *68*, 203-208.

LITERÁK I., RYCHLÍK I., SVOBODOVÁ V. & POSPÍŠIL Z. Restriction fragment length polymorphism and virulence of Czech *Toxoplasma gondii* strains. *International Journal for Parasitology*, 1998, *28*, 1367-1374.

MONDRAGON R., HOWE D., DUBEY J.P. & SIBLEY L.D. Genotypic analysis of *Toxoplasma gondii* isolates from pigs. *Journal of Parasitology*, 1998, *84*, 639-641.

OSSORIO P.N., SCHWARTZMAN J.D. & BOOTHROYD J.C.A. *Toxoplasma gondii* rhoptry protein associated with host cell penetration has unusual charge asymetry. *Molecular and Biochemical Parasitology*, 1992, *50*, 1-16.

RINDER H., THOMSCHKE A., DARDÉ M.L. & LÖSCHER T. Specific DNA polymorphisms discriminate between virulence and non-virulence to mice in nine *Toxoplasma gondii* strains. *Molecular and Biochemical Parasitology*, 1995, *69*, 123-126.

RUIZ A. & FRENKEL J.K. Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. *American Journal of Tropical Medicine and Hygiene*, 1980, *29*, 1161-1166.

SIBLEY L.D. & BOOTHROYD J.C. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature*, 1992, *359*, 82-85.

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