Genetic diversity among isolates of *Trichinella spiralis* from the Province of Buenos Aires, Argentina

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Abstract

Random Amplified Polymorphic DNAs, (RAPDs) are used to study the occurrence of *Trichinella britovi* and T5 among domestic animals in the Province of Buenos Aires, Argentina and to assess the genetic diversity among isolates of *T. spiralis* from this area in a number of infected hosts. All the local isolates proved to be *T. spiralis*. Six of the eight primers used indicate that the Buenos Aires isolates are distinct from each other as they produce a considerable number of polymorphic bands. Our overall estimates are relatively higher than other intraspecific distances previously estimated within species of this genus and among *T. spiralis* isolates. Such high degrees of variability observed among local isolates and between isolates from Buenos Aires and Spain should be taken into account when defining isolates within this species, and considering differences in the epidemiology of *T. spiralis*.

Introduction

At present, the incidence and prevalence of infection of humans due to *Trichinella spiralis* are low in Western Europe and the United States (Capó & Despommier, 1996; Gottstein, 1997), although common source outbreaks occur in southern South America (Chile and Argentina). The incidence of infection in the most densely populated province of Argentina (Province of Buenos Aires; 307,430 km^2, 15,560,377 hab., National census 1991) has dramatically increased in the last decade, from four affected localities in 1992 with 149 human cases to 32 localities affecting 477 individuals in 1997 (Montali, 1997).

According to the current nomenclature of the genus, *Trichinella* contains five species (*T. spiralis, T. nativa, T. pseudospiralis, T. nelsoni* and *T. britovi*) plus three phenotypes of uncertain taxonomic level (T5, T6 and T8) (Pozio *et al*., 1992a, 1992b). Several of these have produced infections in humans (MacLean *et al*., 1989; Gari-Toussaint *et al*., 1993, Murrell & Bruschi, 1994); the most pathogenic being *T. spiralis* followed by *T. nativa* and *T. britovi* (Pozio *et al*., 1992a; Murrell & Bruschi, 1994). The infecting species will also determine, among other factors, the duration of the incubation period (Murrell & Bruschi, 1994).

Even though recent investigations show that *T. britovi* and *T. nativa* are more commonly found in a sylvatic cycle (Pozio, 1996; Gottstein *et al*., 1997), *T. britovi* has also been found in the domestic pig (Rodriguez *et al*., 1996) and has invaded the domestic habitat under certain conditions (Pozio, 1998). The swine producing environment in the Province of Buenos Aires can present conditions that favour the transference of *T. britovi* into the domestic cycle. These include the family rearing of pigs on household, restaurant or slaughterhouse remains which attract rodents. More importantly, pigs are allowed to pasture in areas where carcasses of wild animals can be used as food.

DNA based studies (RFPLs: restriction fragment length polymorphism, and PCR) have been successfully employed to compare isolates of species within the genus *Trichinella* (Klassen *et al*., 1986; Dupouy-Camet *et al*., 1991).

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Random amplified polymorphic DNAs (RAPDs) have been used to assess variation within this genus where one of the genotypes, T5, showed 87% genetic similarity to *T. britovi* (Dupouy-Camet *et al*., 1993, 1994) and 40% similarity in a more recent study (Rodriguez *et al*., 1996), displaying a very low swine infectivity index (Dame *et al*., 1987; Minchella *et al*., 1989). PCR-single strand conformation polymorphism (SSCP) has recently been used to identify *Trichinella* isolates, and the RAPDs technique has been suggested as a useful tool that provided concordant results with other molecular based studies (Gasser *et al*., 1998). Moreover, a PCR based detection system has recently been designed to identify all *Trichinella* species, the PCR primers used being constructed using RAPD sequences (Wu *et al*., 1998).

With regard to intra-specific studies, previous analyses of five strains of *T. spiralis* showed differences in RFLP patterns between isolates obtained from hosts in different geographical regions (Wang *et al*., 1995). However RAPDs have not yet been widely used to evaluate genetic variability within *T. spiralis* nor to find primers that would produce isolate specific banding patterns. Parasite genetic variability is crucial when considering host susceptibility and thus there is a need to obtain more precise knowledge of parasite diversity before developing therapeutics or vaccines (Cox, 1991).

DNA techniques are used in this paper to study the occurrence of *T. britovi* and T5 (due to its genetic similarity with *T. britovi*) among pigs in the Province of Buenos Aires and to assess the genetic diversity of *T. spiralis* from this provincial area and with Chilean and Spanish isolates.

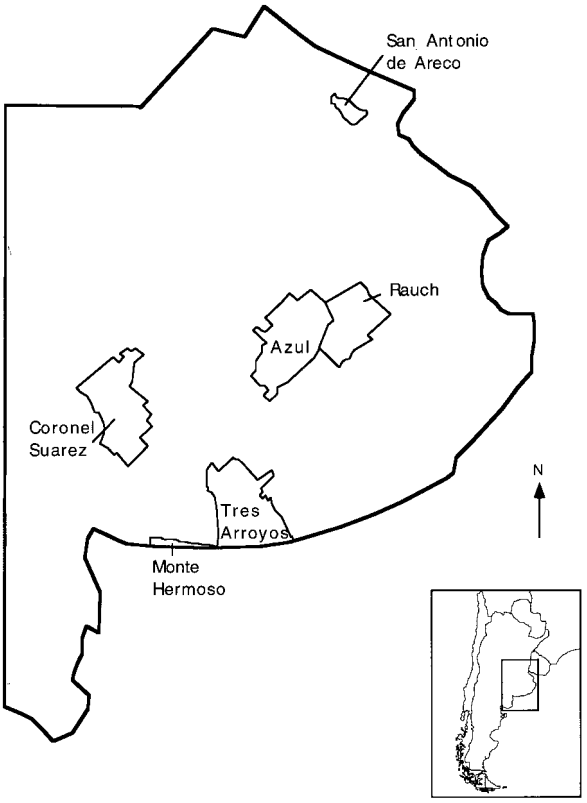

Fig. 1. The localities in the Province of Buenos Aires (Argentina) from which isolates of *Trichinella* larvae were obtained.

Materials and methods

Parasite and DNA isolation

Domestic pig muscle and cold meat samples were received from different localities in the Province of Buenos Aires, Argentina (fig. 1, table 1): San Antonio de Areco (AR), Azul (AZ), Coronel Suarez (CS), Rauch (RA), Monte Hermoso (MH), Tres Arroyos (TA), some of these localities are more than 1000 km apart, and one from Santiago de Chile, Chile (SC). Two samples were kindly provided by Dr Martinez-Fernandez from Spain already in mouse muscle tissue: *T. spiralis* Spain (SP) and *T. britovi* (Tb). *Trichinella* larvae were liberated from ground muscle through pepsin digestion (1:10.000) in acid conditions (1% HCl) and 500 larvae were inoculated into CF1 mice for maintenance and reproduction and, after 30 days, a new pepsin digestion was performed from two mouse specimens and the larvae were obtained. Reproductive capacity indexes (RCI) were calculated for each isolate as the number of larvae recovered from a given amount of tissue for a given amount of inoculated parasites (number of larvae recovered/number of larvae inoculated). Total DNA was extracted from larval pools (per location) as described in Dame *et al*. (1987).

Table 1. Isolates of *Trichinella* assayed by RAPD.

Isolate	Locality	Region	RCI	Recurrence in swine (years)	Outbreak occurrence
AR	San Antonio de Areco	N	41.7	–	1996/1997
AZ	Azul	C	136	1	1996
CS	Coronel Suarez	C	46	1	–
RA	Rauch	C	0	2	–
MH	Monte Hermoso	S	83.03	2	–
TA	Tres Arroyos	S	81.5	3	1996/1997
SC	Santiago de Chile	–	55.9	n.a.	n.a.
SP	Spain (GM-1)	–	15.2	n.a.	n.a.
Tb	*T. britovi* (Monegrillo)	–	24.1	n.a.	n.a.

Reproductive capacity index (RCI): number of larvae recovered/ number of larvae given. N, northern, C, central and S, southern regions in the province of Buenos Aires; n.a., not analysed.

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Table 2. RAPD primers used in a survey of *Trichinella spiralis* isolates.

Primer	Sequence (5′–3′)	N_t	N_p	N_{sp}	N_{br}
A01	CCC AAG GTC C	9	7	6	1
A02	GGT GCG GGA A	6	2	3	3
A03	AAG ACC CCT C	11	7	5	3
A05	CAC CAG GTG A	11	9	7	2
A06	GAG TCT CAG G	5	0	4	1
A08	ACG CAC AAC C	8	0	2	3
A09	CTA ATG CCG T	7	4	5	2
A10	ACG GCG TAT T	11	2	7	4

N_t, total number of bands for each primer; N_p, number of bands polymorphic within *T. spiralis;* N_{sp}, and N_{br}, number of bands species specific for *T. spiralis* and *T. britovi* respectively.

RAPD PCR procedure

Reaction mixtures (50 μl) typically contained 0.5 U Taq polymerase (Promega), 5 μl 10× thermal reaction buffer (Promega), 150/200 ng primer (Biodynamics), 100 μM of each deoxynucleoside triphosphate (Promega), and 50/100 ng of genomic DNA. Amplifications were carried out in a Sontec thermal cycler. The reactions were cycled 45 times through the following temperature regimes: 94°C for 1 min, 36°C for 1 min and 72°C for 2 min. Prior to the first cycle and after the final cycle longer denaturation and extension steps were applied. To test PCR products, controls were routinely used. After amplifying with ten different primers, eight were selected to perform the analyses provided reproducible and clear banding patterns were produced (table 2, fig. 2).

Analysis of PCR products

Samples were resolved electrophoretically on 1.4% agarose gels, visualized through ethidium bromide fluorescence and photographed. Sizes of amplified fragments were estimated by comparison with various standard DNA markers: λ*Eco*R1-*Hin*dIII double digest (Promega) and ϕ-*Hae*III digests (Promega). A presence/absence matrix was constructed for each band amplified with the eight primers for each of the isolates analysed. The assumption for the construction of this matrix was that bands of the same size were considered to represent the same sequence and were therefore scored as present for the isolates that displayed that band.

Different genetic distance measurements were calculated (Nei, 1972; Nei & Li, 1985) using this matrix to display the genetic relationships among the local isolates and with isolates from elsewhere (SP and Tb) (table 3). Overall genetic distance measurements were calculated amongst the Buenos Aires isolates (CS, RA, TA, AR, AZ and MH), between Buenos Aires isolates and the Chilean isolate (SC), between the Argentinean isolates and the Spanish isolate (BA vs. SP) and between the Buenos Aires isolates and *T. britovi*. Pairwise calculations were performed with the Rapds program (Black, 1997). Cluster analyses were carried out by the unweighted pair group method using arithmetical means (UPGMA) (Sneath & Sokal, 1973) through the Neighbour program in Phylip 3.5 (Felsenstein, 1993).

Results and Discussion

The isolates analysed produced a wide range in values for the reproductive capacity index (RCI) which has

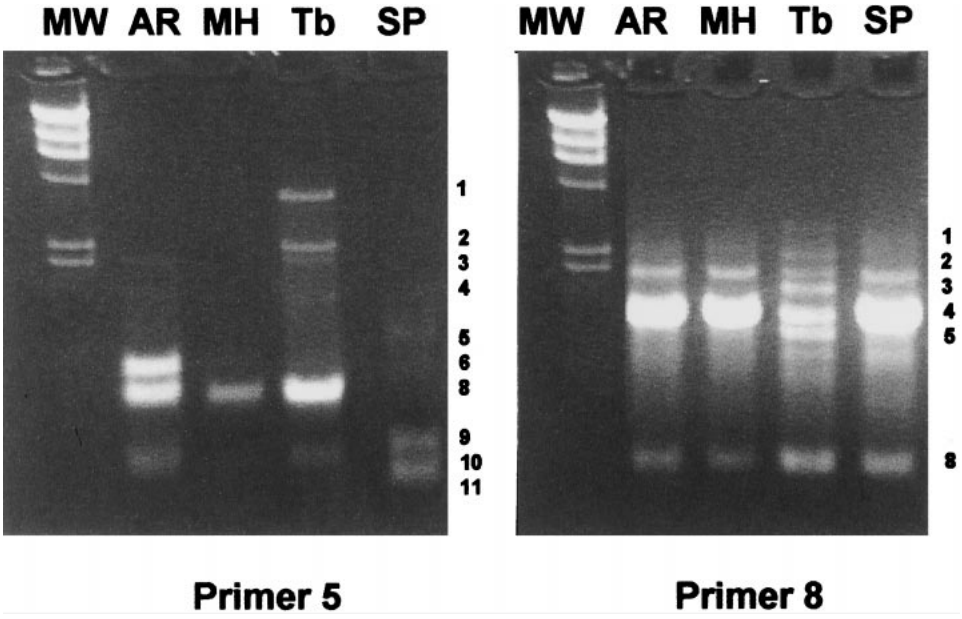

Fig. 2. Banding patterns produced by RAPDs with some local isolates of *Trichinella* (AR and MH) and reference strains (SP and Tb) showing primers which are polymorphic (Primer 5) and monomorphic within *T. spiralis* (Primer 8). (MW: Molecular weight marker λ *Hin*dIII) (Numbers correspond to the identification of bands for the construction of the presence/absence matrix).

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Table 3. Nei (1972) distance values between pairs of populations of *Trichinella* calculated from RAPD markers (above the diagonal) and Nei & Li's (1985) similarity index (1-%S) (below the diagonal).

	CS	RA	AR	TA	SC	AZ	MH	SP	Tb
CS	–	0.3430	0.5286	0.5423	0.4851	0.4851	0.4537	0.6417	0.8660
RA	0.1212	–	0.5816	0.5145	0.4851	0.5145	0.5145	0.5688	0.8489
AR	0.3016	0.3898	–	0.4697	0.5286	0.5000	0.5557	0.4697	0.7859
TA	0.3333	0.3214	0.2830	–	0.5423	0.5145	0.5145	0.5688	0.7952
SC	0.2581	0.2759	0.3455	0.3846	–	0.5145	0.5423	0.5688	0.8135
AZ	0.2286	0.2727	0.2698	0.3000	0.2903	–	0.5145	0.6183	0.8489
MH	0.2121	0.2903	0.3559	0.3214	0.3448	0.2727	–	0.6417	0.8135
SP	0.4828	0.4074	0.2941	0.4583	0.4400	0.4483	0.5185	–	0.7376
Tb	0.8095	0.8305	0.7500	0.8113	0.8182	0.7778	0.7627	0.7255	–

See table 1 for key to isolates.

been widely discussed as a taxonomic feature (Bolas-Fernandez & Wakelin, 1989, 1990). In the present study, the numbers do not correspond to the recurrence of infection in swine in each locality nor to the occurrence of outbreaks over the last three years in each locality, nor do they correspond to the genetic distances amongst the isolates based on RAPDs (table 1).

The group of eight primers used for RAPDs analysis generated 68 bands ranging from five to 11 per primer (table 2). Nineteen amplification products were species specific (present only in *T. britovi*) allowing future identification with a single RAPD reaction. Some primers did not display intraspecific variability (primers 6 and 8), and this is similar to the results of Rodriguez *et al*. (1996) where two primers produced similar banding patterns for all isolates of *T. spiralis*. On the other hand, the profiles generated by primers 1, 2, 3, 5, 9 and 10 (for some of the profiles see fig. 2) indicate that the isolates in Buenos Aires are distinct from each other since they produce different RAPD profiles. This suggests that most isolates are to some extent genetically isolated, since they display significant average genetic distances between isolates (0.2849–0.5031) (Nei, 1972; Nei & Li, 1985). Intraspecific distances have been estimated previously within species of this genus; i.e. among isolates of *T. nativa* ($1\text{-}S_{ab}=0.30$), *T. britovi* ($1\text{-}S_{ab}=0.17$) (Dupouy-Camet *et al*., 1994) and (1-%S = 0.37) (Rodriguez *et al*., 1996) and *T. spiralis* (1-%S = 0.25) (Rodriguez *et al*., 1996). Our overall estimates are relatively higher, and this could be attributed to a higher number of 10-mer primers used and to the diverse origin of the isolates.

The cluster analysis of both genetic distance measurements (Nei, 1972; Nei & Li, 1985) does not reflect the geographical distances separating locations from which the isolates originated (fig. 3). Similarly, a lack of correlation was found for isolates of *T. spiralis* and *T. britovi* by Rodriguez *et al*. (1996). Central populations (AZ, RA and CS) are clustered with a clearly southern population (MH) and with the Chilean isolate (SC). Two distant populations (TA and AR, northern and southern respectively) are grouped with the Spanish isolate (SP), these two Argentine locations have suffered outbreaks of human *T. spiralis* infections in the last two years despite the isolates displaying variable RCI values (high and medium) (table 1). No clear pattern for the geographical origin of *T. spiralis* in the Province of Buenos Aires can be derived from this phenogram. However, one possiblility for this grouping, despite the vast distance separating the locations, suggests some common source of *T. spiralis* for both Argentina and Spain and a later introduction to Chile, although more samples are needed to confirm this. Due to the geographical isolation of Spain and Argentina and the strict swine importation/exportation standards in both countries it is unlikely that introduction would have occurred through swine in the past five decades, but rodents might have been present in the stockfeed of trading ships.

Trichinella britovi remains separated from all the other isolates, and displayed high distance value with the Buenos Aires isolates (0.8264) and the Spanish isolate (0.7376). This supports the RFLP result, with no evidence of other species or genotype being found among the Buenos Aires isolates. Southern blot analysis of genomic DNA from all Buenos Aires isolates showed the same banding patterns using five restriction enzymes (*Sau*3A, *Hae*III, *Cla*I, *Hind*III and *Eco*RI) after hybridization with the T1 (*T. spiralis*) specific probe. However, the T5 specific

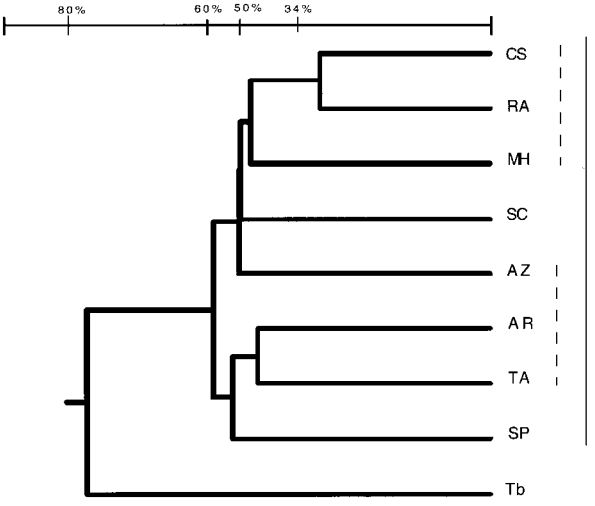

Fig. 3. Phenogram constructed with Nei & Li's (1985) genetic distances obtained from RAPD data through UPGMA linking isolates of *Trichinella* (% of genetic difference among isolates are displayed within the phenogram). ____ , *T. spiralis* _ _ _ _, isolates from the Province of Buenos Aires; SC, isolate from Chile; SP, isolate from Spain; Tb, *T. britovi*.

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probe did not hybridize with any of the local isolates indicating the absence of T5 among the Buenos Aires or Chilean isolates. Despite the high (Dupouy-Camet *et al*., 1994) or medium (Rodriguez *et al*., 1996) genetic similarity of this phenotype with *T. britovi,* pUPB-2 is still specific for T5.

Parasitism provides ample opportunities for sympatric speciation and the most relevant evidence for ongoing sympatric divergences comes from the parasitological literature (de Meeus *et al*., 1998) as parasites constitute a large proportion of known species diversity (Renault *et al*., 1996). Substantial work has been undertaken since the 1960s to biologically characterize geographical isolates of the genus *Trichinella* and in those cases where biological differences were inconclusive and controversial, it was necessary to develop more sensitive methods to precisely define the isolates (Mydynski & Dick, 1985; Wakelin & Goyal, 1996). The present contribution has shown the extent of the variability among isolates of *T. spiralis* in the Province of Buenos Aires and that the RAPDs technique can be useful to differentiate isolates. Bearing in mind the potential of *T. spiralis* for diversification, the high degree of variability encountered among local isolates and between the Buenos Aires and Spanish isolates should be taken into account when defining isolates within this species, as polymorphism may reflect epidemiologically relevant characters (McManus & Bowles, 1996) and influence disease diagnosis (Guo *et al*., 1997). Therefore, RAPDs have provided new and useful information as an initial screen of the genetic makeup of *T. spiralis* isolates.

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References

Black, W. (1997) *Rapds, Rapddist 1.0*. Department of Microbiology, Colorado State University, Fort Collins, Colorado.

Bolas-Fernandez, F. & Wakelin, D. (1989) Infectivity of *Trichinella* isolates in mice is determined by host inmune responsiveness. *Parasitology* **99**, 83–88.

Bolas-Fernandez, F. & Wakelin D. (1990) Infectivity, antigenicity and host responses to isolates of the genus *Trichinella*. *Parasitology* **100**, 491–497.

Capó, V. & Despommier, D. (1996) Clinical aspects of infection with *Trichinella* spp. *Clinical Microbiological Reviews* **96**, 47–54.

Cox, F.E.G. (1991) Variation and vaccination. *Nature* **349**, 193.

Dame, J.B. & McCutchan T.F. (1995) Cloning and characterization of ribosomal DNA from *Plasmodium berghei*. *Molecular and Biochemical Parasitology* **8**, 263–279.

Dame, J.B., Murrell, K.D., Worley, D.E. & Schad, G.A. (1987) *Trichinella spiralis*: genetic evidence for synanthropic subspecies in sylvatic hosts. *Experimental Parasitology* **64**, 195–203.

de Meeûs, T., Michalakis, Y. & Renaud, F. (1998) Santa Rosalia revisted: or Why are there so many kinds of parasites in "The Garden of earthly delights"? *Parasitology Today* **14**, 10–13.

Despommier, D.D. (1983) Biology. pp. 75–142 *in* Campbell, W.C. (*Ed*.) *Trichinella and trichinellosis*. New York, Plenum Press.

Dupouy-Camet, J., Robert, F., Guillou, J.P., Vallet, C., Perret, C. & Soule, C. (1993) Genetic analysis of *Trichinella* isolates with random amplified polymorphic DNA markers. *in* Campbell, W.C., Pozio, E. & Bruschi, F. (*Eds*) *Trichinellosis*. Proceedings of the 8th International Conference on Trichinellosis 1993. Rome, Instituto Superiore di Sanitá Press.

Dupouy-Camet, J., Robert, F., Guillou, J.P., Vallet, C., Perret, C. & Soule, C. (1994) Identification of *Trichinella* isolates with random amplified polymorphic DNA markers. *Parasitology Research* **80**, 358–360.

Dupouy-Camet, J., Soule, C., Guillou, J.P., Rouer, E., Lavareda de Souza, S., Ancelle, T. & Benarous, R. (1991) Detection of repetitive sequences of *Trichinella spiralis* by the polymerase chain reaction in experimentally infected mice. *Parasitology Research* **77**, 180–182.

Felsestein, J. (1993) *PHYLIP (Phylogeny inference package) v. 3.5c*. Department of Genetics, The University of Washington, Seattle, Washington.

Gari-Toussaint, M., Bernard, E., Quaranta, J.F., Marty, P., Soler, C., Ozouf, N. & Caux, C. (1993) First report in France of an outbreak of human trichinellosis due to *Trichinella britovi*. pp. 465–468 *in* Campbell, W.C., Pozio, E. & Bruschi, F. (*Eds*) *Trichinellosis*. Proceedings of the 8th International Conference on Trichinellosis 1993. Rome, Instituto Superiore di Sanitá Press.

Gasser, R.B., Zhu, X.Q., Monti, J.R., Dou, L., Cai, X. & Pozio, E. (1998) PCR-SSCP of rDNA for the identification of trichinella isolated from mainland China. *Molecular and Cellular Probes* **12**, 27–34.

Gottstein, B., Pozio, E., Connolly, B., Gamble, H.R., Eckert, J. & Jakob, H.P. (1997) Epidemiological investigation of trichinellosis in Switzerland. *Veterinary Parasitology* **72**, 201–207.

Guo, Z.G., Gross, U. & Johnson, A.M. (1997) *Toxoplasma gondii* virulence markers identified by random amplified polymorphic DNA polymerase chain reaction. *Parasitology Research* **83**, 458–463.

Klassen, G.R., Thiessen, J.L. & Dick, T.A. (1986) Strain specific 1.7 kilobase repetitive deoxyribonucleic acid sequence family in *Trichinella spiralis*. *Molecular and Biochemical Parasitology* **21**, 227–233.

MacLean, J.D., Viallet, J., Law, C. & Staudt, M. (1989) Trichinosis in the Canadian Arctic: report of five outbreaks and a new clinical syndrome. *Journal of Infectious Diseases* **160**, 513–520.

McManus, D.P. & Bowles, J. (1996) Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. *International Journal for Parasitology* **26**, 687–704.

Minchella, D.J., Barnstetter, B.A. & Kazacos, K.R. (1989) Molecular characterization of sylvatic isolates of *Trichinella spiralis*. *Journal of Parasitology* **75**, 388–392.

Montali, J.G. (1997) Dirección Provincial de Ganadería. Ministerio de Asuntos Agrarios. Informe de la Primera Reunión Nacional de Triquinosis, Buenos Aires, Argentina.

Murrell, K.D. & Bruschi, F. (1994) Clinical trichinellosis. pp. 117–150 *in* Sun, T. (*Ed*.) *Progress in clinical parasitology.* Boca Ratón, Florida, CRC Press.

Mydynski, L.J. & Dick , T.A. (1985) The use of enzyme polymorphisms to identify genetic differences in the genus *Trichinella*. *Journal of Parasitology* **71**, 671–677.

Nei, M. (1972) Genetic distance between populations. *American Naturalist* **106**, 283–292.

Nei, M. & Li, W.H. (1985) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA* **76**, 5296–5273.

Pozio, E. (1996) Experimental and human influence on the ecology of *Trichinella spiralis* and *Trichinella britovi* in Western Europe. *Parasitology* **113**, 527–533.

Pozio, E. (1998) Trichinellosis in the European Union. Epidemiology, ecology and economic impact. *Parasitology Today* **14**, 35–38.

Pozio, E., La Rosa, G., Murrell, K.D. & Lichtenfels, J.R. (1992a) Taxonomic revisions of the genus *Trichinella*. *Journal of Parasitology* **78**, 654–659.

Pozio, E., La Rosa, G., Rossi, P.V. & Murrell, K.D. (1992b) Biological characterization of *Trichinella* isolates from various host species and geographical regions. *Journal of Parasitology* **78**, 647–653.

Pozio, E., Varese, P., Morales, M.A., Croppoi, G.P., Pellucia, D. & Bruschi, F. (1993) Comparison of human trichinellosis caused by *T. spiralis* and *T. britovi*. *American Journal of Tropical Medicine and Hygiene* **48**, 568–575.

Renault, F., Clayton, D. & de Meeûs, T. (1996) Biodiversity and evolution in host-parasite associations. *Biodiversity and Conservation* **5**, 963–974.

Rodriguez, E., Nieto, J., Castillo, J.A. & Garate, T. (1996) Characterization of Spanish *Trichinella* isolates by random amplified polymorphic DNA (RAPD). *Journal of Helminthology* **70**, 335–343.

Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical taxonomy: the principles and practice of numerical classification.* San Francisco, Freeman.

Timm, R.M & Clauson, B.L. (1987) *Coevolution*. McGraw-Hill.

Wakelin, D. & Denham, D.A. (1983) The inmune response. pp. 265–308 *in* Campbell, W.C. (*Ed*.) *Trichinella and trichinellosis*. New York, Plenum Press.

Wakelin, D. & Goyal, P.K. (1996) *Trichinella* isolates: parasite variability and host responses. *International Journal for Parasitology* **26**, 471–481.

Wang, H., Zhang, Y., Lao, W. & Wu, Z. (1995) Restriction fragment length polymorphism (RFLP) analysis of genomic DNA of five strains of *Trichinella spiralis* in China. *Chinese Medical Sciences Journal* **10**, 131–135.

Wu, Z., Nagano, I. & Takahashi, Y. (1998) The detection of *Trichinella* with polymerase chain reaction (PCR) primers constructed using sequences of random amplified polymorphic DNA (RAPD) or sequences of complementary DNA encoding excretory-secretory (E-S) glycoproteins. *Parasitology* **117**, 173–183.

Zarlenga, D.S, Al-Yaman, F., Minchella, D. & La Rosa, S. (1991) A repetitive DNA probe specific for a North American sylvatic genotype in *Trichinella*. *Molecular and Biochemical Parasitology* **48**, 131–138.

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