COMPARISON OF IGM, IGG1 AND IGG2 RESPONSES TO *TRICHINELLA SPIRALIS* AND *TRICHINELLA BRITOVI* IN SWINE

SERRANO F.J.*, PÉREZ-MARTIN J.E.*, CARRÓN A.* & NAVARRETE I.*

Summary :

Pigs infected with *T. spiralis* and *T. britovi* were followed by double (IgG) and triple antibody ELISA (IgG1, IgG2 and IgM) during a 12-week-period. Specific IgG and IgG1 responses were similar and showed a significant relation with the infecting doses and intensity of infection. Response to *T. britovi* was slightly lower than in groups infected with the same dose of *T. spiralis*. IgG2 response was weak and almost undetectable in the lowest infected pigs, but relationship with the intensity of infection was unclear. IgM antibodies showed rapid but transient increases, generally simultaneous to peaks of IgG response.

KEY WORDS : *Trichinella*, pig, IgG1, IgG2, IgM.

In contrast to extensive studies on humoral response to *Trichinella* infections in humans and rodents, studies in swine have been mainly limited to detection of IgG response. As far as we know, the role of IgG isotypes in swine trichinellosis has not yet been investigated. We present herein a preliminary study of the IgG1, IgG2 and IgM response by using a triple antibody ELISA method.

MATERIAL AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN

Thirty Iberian pigs, about 25 kg of body weight, were used in this study. Experimental design and number of larvae per gram (lpg) obtained in pillars of diaphragm at the end of the experiment are shown in Table II. Sera were obtained at 0, 7, 17, 21, 27, 34, 44, 54, 68 and 84 days post-infection (dpi). Group 1 and 2 were established *a posteriori* for analysis of ELISA results, in basis to the level of infection.

ANTIGENS

Muscle larvae of *T. spiralis* and *T. britovi* were obtained by pepsin digestion as described by Serrano *et al.* (1992) and allowed to migrate one hour by a gradient of 0.5-2 % of carboxymethylcellulose in RPMI 1640. After washing, larvae were used as source of crude antigens (CA) or incubated 24 hours in RPMI 1640 to obtain ES antigens as described by Serrano *et al.* (1993). *Trichinella* isolates were kindly identified by Dr. E. Pozio (Istituto Superiore di Sanità, Rome, Italy).

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

An indirect double antibody ELISA was used for IgG as described by Serrano *et al.* (1992) with minor modifications. Triple antibody ELISA methods were used for IgG1, IgG2 and IgM. Second antibodies and peroxidase conjugates used in each method are shown in Table I. For triple antibody ELISA, after incubation of sera, each sample was incubated simultaneously with anti-porcine IgG1, IgG2 or IgM antibodies and washing buffer (individual controls). Optical density was read at 450 nm and corrected by substraction of individual control in triple antibody ELISA, and expressed as percentage of reactivity of a positive control.

ELISA	Anti-porcine antibody	Peroxidase conjugate
IgG	(Not applicable)	Rabbit anti-swine IgG (Sigma A-5670)
IgG1	Mouse anti-IgG1 (Labgen CTS6135)	Rabbit anti-mouse IgG (Sigma A-9044)
IgG2	Mouse anti-IgG2 (Labgen CTS6135)	Rabbit anti-mouse IgG (Sigma A-9044)
IgM	Rabbit anti-IgM (ICN 643901)	Goat anti-rabbit IgG (Sigma A-9169)

Table I. – Secondary antibodies and conjugates of double and triple antibody ELISA methods.

RESULTS AND DISCUSSION

Muscle infections were detected in all infected pigs. Accordingly to Kapel and Gamble (2000), we found that *T. britovi* produced moderate levels of infection, in spite of high infective

* Unidad de Parasitología y Enfermedades Parasitarias, Departamento de Medicina y Sanidad Animal, Facultad de Veterinaria, Universidad de Extremadura, 10071 Cáceres, Spain.
Fax: 003492725710 – Tel.: 0034927257132 – e-mail: fserrano@unex.es

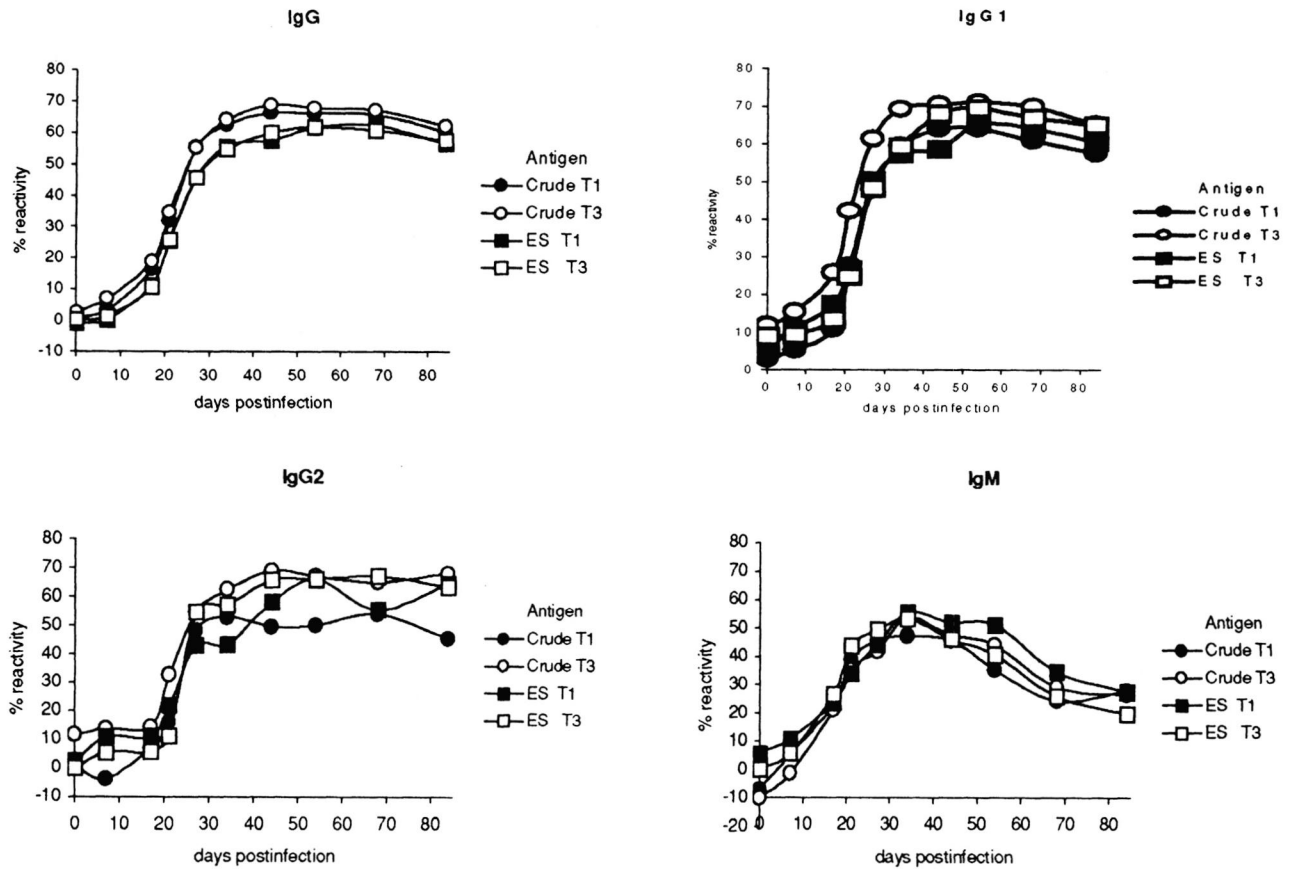

Fig. 1. – Recognition of IgG, IgG1, IgG2 and IgM antibodies in all the infected pigs (groups 1-5) by crude larvae extracts and excretory-secretory (ES) antigens of *T. spiralis* (T1) and *T. britovi* (T3).

Exp. Group	n	Infecting dose		Larvae per gram
		Larvae/kg	Species	Mean ± S.D.
1	5	150	*T. spiralis*	2,668 ± 1,531
2	5	150	*T. spiralis*	878 ± 133
3	5	150	*T. britovi*	436 ± 131
4	5	20	*T. spiralis*	31 ± 24
5	5	2	*T. spiralis*	0.5 ± 0.8
6	5	0	–	0.0 ± 0

Table II. – Experimental design of experimental groups and level of infection obtained by artificial digestion.

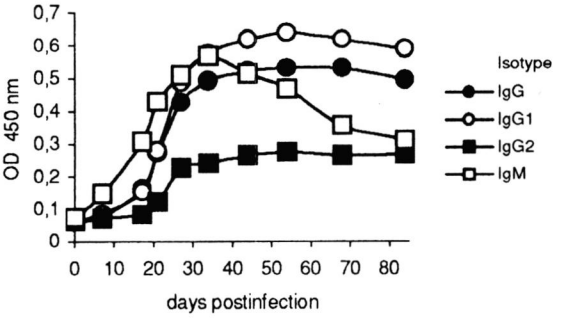

Fig. 2. – Comparison of IgG, IgG1, IgG2 and IgM responses against *Trichinella* antigens in all the infected swine (groups 1 to 5).

doses (Table II). Comparison of ELISA results with respect of the antigen, showed no significant differences with any Ig isotype (Fig. 1). Other studies have found also a close antigenic relationship of *Trichinella* species, although the use of heterologous antigens may decrease slightly the sensitivity (Serrano *et al.* 1992; Kapel & Gamble, 2000). Antibody profiles obtained with all the antigens in infected pigs showed similar time course of IgG and IgG1 levels (Fig. 2). Detection of IgG2 was weak but parallel to IgG1. Levels of IgM antibodies increased during the 4-5 weeks but a clear reduction was seen thereafter. All isotype responses correlated significantly with the infecting doses from 17 to 84 dpi, specially at 27 for IgG1 (r = 0.82), and the number of lpg, but this correlated only weakly with the IgG2 at 27 dpi (r = 0.19). IgG levels differed significantly among the experimental groups, starting at 17-27 dpi, excepting between groups 3 and 4, and finally group 2-4 at 54-68 dpi (Fig. 3). Groups 1-2 were very similar in the IgG1 responses and were indistinguishable from groups 3-4 at the end of experiment. The

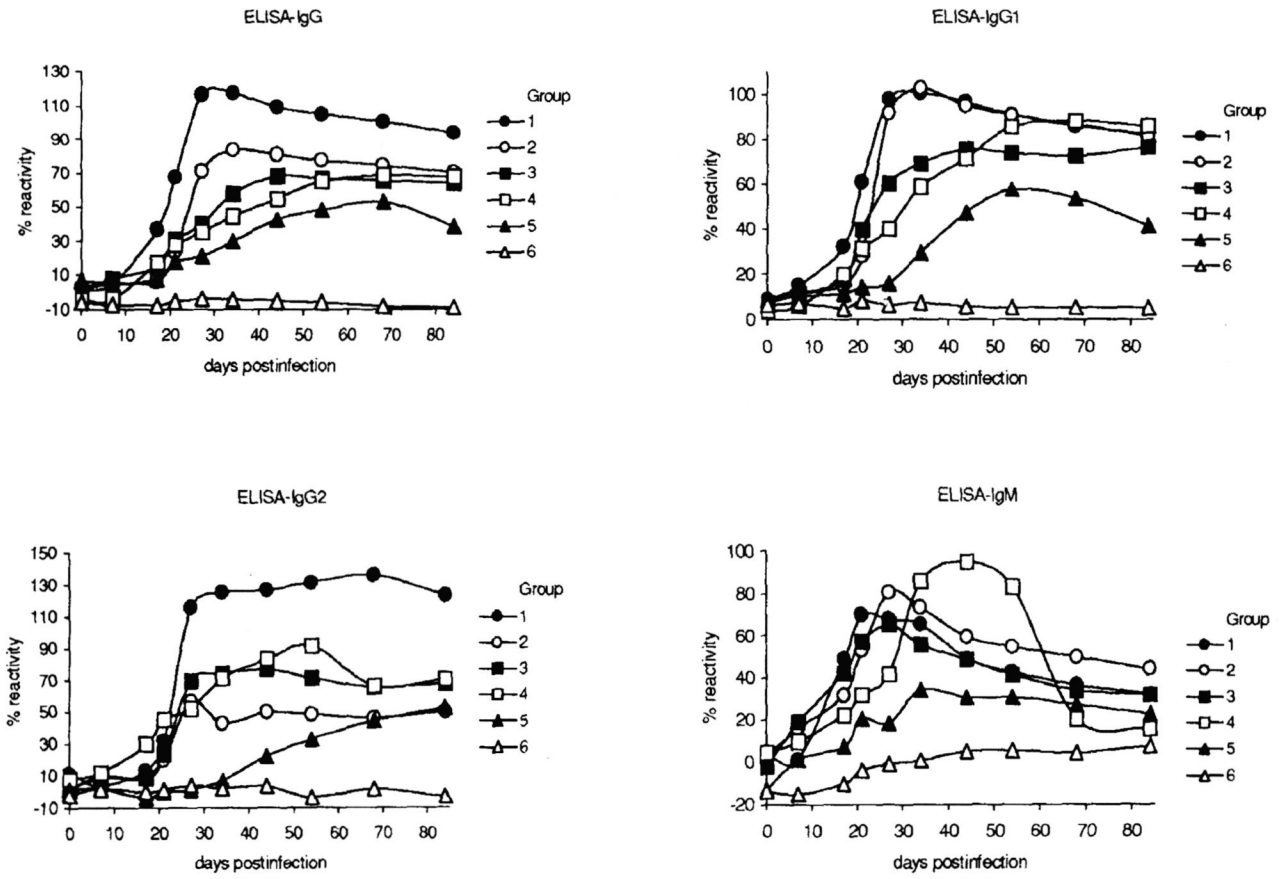

Fig. 3. – Kinetics of specific IgG, IgG1, IgG2 and IgM antibodies by ELISA in pigs infected with different levels of infection of *T. spiralis* (groups 1, 2, 4 and 5), *T. britovi* (group 3) and non infected pigs (group 6) against crude and ES antigens of both species.

IgG2 response was variable and only the group 1 showed significant differences with remaining groups after the 27 dpi and between groups 4 and 5 at 17-21 dpi. IgM response of groups 1, 2 and 3 was very similar. Group 4 showed a delayed peak of response, whereas only a moderate response was found in group 5.

These results showed that IgG response was mainly supported by the IgG1 isotype. Molecular genetic studies reveal that cDNA of IgG1 is the most frequently encountered IgG subclass (Kacskovics *et al.*, 1994) but its function is unknown. Although protective response against *Actinobacillus pleuropneumoniae* in pigs has been related with IgG1 isotype (Furesz *et al.*, 1998), and IgG2 seems related with resistance to parasites in calves (Gasbarre *et al.*, 1993), further works need to be accomplished in this area.

REFERENCES

GASBARRE L.C., LEIGHTON E.A. & DAVIES C.J. Influence of host genetics upon antibody responses against gastrointestinal nematode infections in cattle. *Veterinary Parasitology*, 1993, *46*, 81-91.

KACSKOVICS I., SUN J. & BUTLER J.E. Five putative subclasses of swine IgG identified from the cDNA sequences of a single animal. *Journal of Immunology*, 1994, *153*, 3565-3573.

KAPEL C.M. & GAMBLE H.R. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. *International Journal for Parasitology*, 2000, *30*, 215-221.

FURESZ S.E., WILKIE B.N., MALLARD B.A., ROSENDAL S. & MACINNES J.I. Anti-haemolysin IgG1 to IgG2 ratios correlate with haemolysin neutralization titres and lung lesion scores in *Actinobacillus pleuropneumoniae* infected pigs. *Vaccine*, 1998, *16*, 1971-1975.

SERRANO F., PÉREZ E., REINA D. & NAVARRETE I. *Trichinella* strain, pig race and other parasitic infections as factors in the reliability of ELISA for the detection of swine trichinellosis. *Parasitology*, 1992, *105*, 111-115.

SERRANO F., PÉREZ E., REINA D. & NAVARRETE I. Recognition of antibodies against *Trichinella spiralis* antigens in sera of stray dogs using ELISA and western blot methods. *In*: Trichinellosis. Campbell W.C., Pozio E. & Bruschi F. (eds), Istituto Superiore di Sanità Press, Rome, 1993, 381-386.