

## CLASS SPECIFIC ANTIBODY RESPONSES TO NEWBORN LARVA ANTIGENS DURING *TRICHINELLA SPIRALIS* HUMAN INFECTION

MENDEZ-LOREDO B.\*, MARTÍNEZ y ZAMORA R.\*\*, CHAPA-RUIZ R.\* & SALINAS-TOBÓN R.\*

### Summary :

A follow-up study of the class antibody responses to newborn larva (NBL) antigens in individuals involved in an outbreak of human trichinellosis was carried out by ELISA assays. The data showed that similar kinetics of antibody responses of different magnitude developed in trichinellosis patients; it was low by week 3, a peak raised by week 5 and decreased from week 7 up to the end of the study. The IgA-ELISA assay was the most sensitive and specific while the IgM was the least sensitive and specific. IgA antibodies to NBL antigens were detected in 80 % of patients while IgE, IgG and IgM responses were observed in 44, 31 and 19 % of the patients by week 3, respectively. From weeks 5 to 7, IgA antibodies were found in 89 to 100 % of the patients while lower percentages (0-82 %) were found for the other isotypes. Reactivity of IgA, IgE, IgG and IgM to NBL antigens decreased from week 37 to 57 after infection (0-38 %). These results suggest that detection of IgA antibodies may be useful for early diagnosis and epidemiological studies in human trichinellosis.

**KEY WORDS :** human trichinellosis, humoral immune response, newborn larva.

Characterization of the immune responses elicited in the host infected with *T. spiralis* as well as parasite components recognized by effectors of these responses has relevance in diagnosis, control and understanding of all aspects of parasitic diseases, including pathology (Parkhouse & Harrison, 1989). Several studies have shown that many *T. spiralis* antigens in particular surface and secreted components, may be stage (or age-) specific (Philipp *et al.*, 1980; Jungery *et al.*, 1983). In addition, an independent variation of individual immunoglobulin classes to defined antigenic epitopes in every parasite compartment and stage has been observed (Philipp *et al.*, 1981; Almond & Parkhouse, 1986; Parkhouse & Harrison, 1989; Takashi *et al.*, 1990). This information have provided the basis for stage-specific diagnosis, protection and analysis of pathological reactions. Based on this,

\* Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, I.P.N. Apdo. Postal CON-238 C.P. 06400, México, D.F. CP 11340, México.

\*\* Hospital General de Zona, IMSS, Eje Central Lázaro Cárdenas 445, México, D.F. CP 06900, México.

Correspondence: R. Salinas Tobón.

Tel.: 57 29 60 00 ext 62486 – Fax: 57 29 60 00 ext 62489.

e-mail: msalinas@redipn.ipn.mx

a follow-up study in humans infected with *T. spiralis* was carried out to analyse the IgM, IgG, IgA and IgE responses produced to NBL total soluble extract (TSE) during infection by ELISA assays.

## MATERIALS AND METHODS

### DESCRIPTION OF THE OUTBREAK

The outbreak of trichinellosis took place in February 1989, in Toluca State of Mexico where 22 adults were infected by eating undercooked sausages prepared from pork meat. The description of the outbreak was previously described by Salinas *et al.* (1996).

### CLINICAL FEATURES AND TREATMENT

By week 3 postinfection, the most frequent signs and clinical manifestations observed were fever > 38° (100 %), myalgia (76 %), periorbital and/or facial edema (70.6 %), weakness (47 %) and headache (47 %). No complications were observed. Laboratory findings revealed that all patients showed an elevated eosinophil cell count (> 500/mm<sup>3</sup>), total IgE (> 100 µg/100 mL) and levels of plasma creatine phosphokinase (> 50 IU/L). All patients were treated by anthelmintic drug(s) and the initial response to treatment was assessed in clinical terms on the basis of fever and myalgias; 12 patients received 600 mg mebendazole per day for 15 days. Of the 12, three received 1,500 mg thiabendazole per day for further seven days and one received 40 mg prednisolone per day for seven days. The remaining five received 1,500 mg thiabendazole per day for 15 days and two of them also received 40 mg prednisolone. Based on other laboratory tests, clinical findings were found normal by week 6 or 7 after infection in most patients.

### SERUM SAMPLES

At least three serum samples were collected from each of the 22 individuals involved in the trichinellosis outbreak. Serum samples were taken at different intervals after infection by weeks 3, 4, 5, 7, 12, 15, 37 and 57.

Control serum samples were collected from 56 individuals. Among these, 26 were from healthy adults and the remaining 30 from adults infected with other parasites. Aliquots of sera were stored at  $-20^{\circ}\text{C}$  in an equal amount of glycerol to avoid freeze-thawing (Salinas *et al.*, 1996).

#### PARASITES

*T. spiralis* was maintained by several passages in outbred BALB/c mice. Recovery of muscle larvae, adult worms and newborn larvae (NBL) was performed as described by Dennis *et al.* (1970).

#### ANTIGENS

NBL TSE was prepared from 18-h-old NBL following the method described by Philipp *et al.* (1980).

#### ELISA ASSAYS

Detection of IgM and IgG antibodies was performed by indirect ELISA assays while IgA and IgE were quan-

tified by indirect amplified ELISA assays as described by Salinas *et al.* (1996) with some modifications. These included the use of peroxidase-conjugated goat antibody specific for IgM or IgG (heavy-chain reactive, Cappel Lab.) at 1/8000 and 1/4000 dilutions, for IgM or IgG detection, respectively. For IgA or IgE detection, goat IgG anti-human IgA at 1/4000 dilution or IgE at 1/1000 dilution (heavy-chain reactive, Cappel Lab.), and peroxidase-conjugated rabbit anti-goat IgG ( $\gamma$ -chain reactive, Cappel, Lab.) was diluted 1/8000 or 1/4000, respectively. In all assays  $5\ \mu\text{g ml}^{-1}$  NBL TSE were used. Absorbances were determined by ELISA plate reader (Dynatech Industries) at 492 nm.

## RESULTS

ELISA assays were standardized to measure antibody responses in humans involved in the outbreak of trichinellosis to analyse the kinetics of antibody production against NBL TSE throughout the

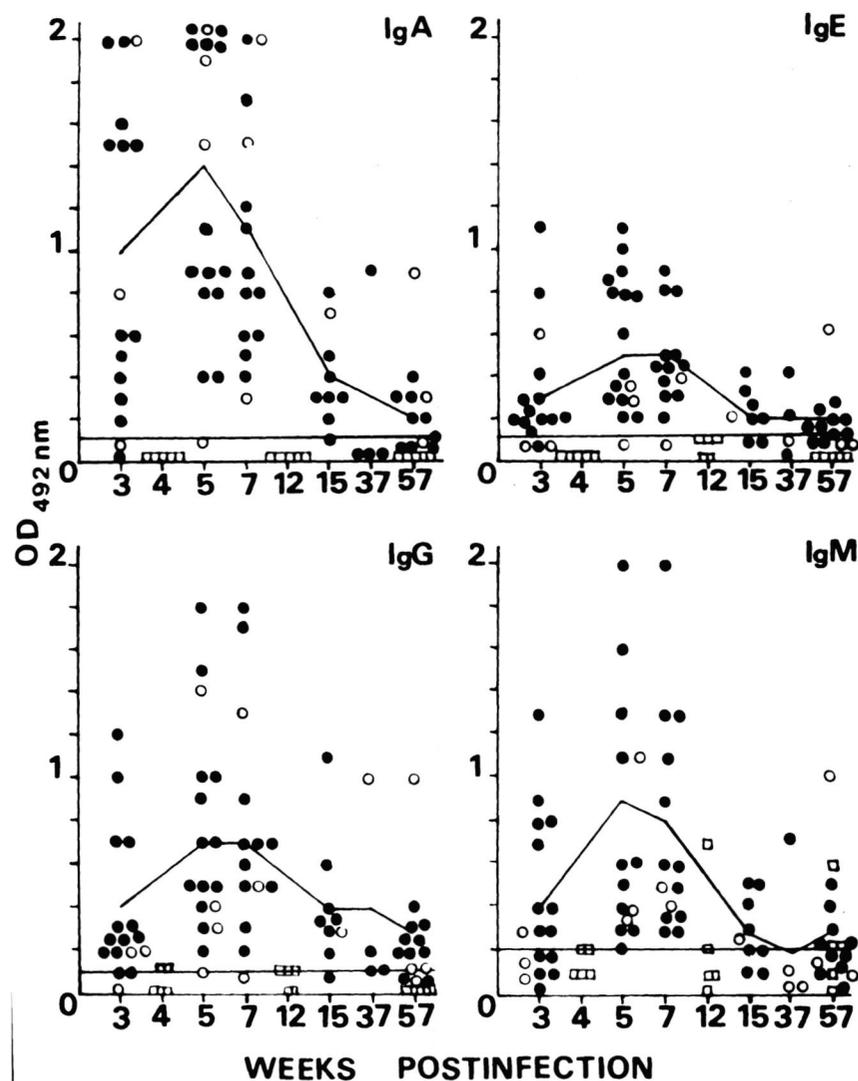


Fig. 1. – Human IgA, IgE, IgG and IgM antibody responses to NBL antigens of *Trichinella spiralis* during one year of infection. ELISA assays were carried out using  $5\ \mu\text{g/ml}$  NBL TSE of *T. spiralis* and serum samples collected at different time intervals after infection from symptomatic individuals with positive (●) or negative (○) biopsy and from asymptomatic individuals (□) to detect class antibody responses. Continued horizontal lines represent the mean plus a standard deviation of OD values from serum samples from healthy individuals.

infection. The data obtained in this study revealed that trichinellosis patients developed a class specific antibody response to NBL antigens. In general, similar kinetics of antibody responses were observed although some differences were detected.

The general trend in antibody production is depicted as the mean OD values of serum samples of trichinellosis patients and is shown in Figure 1. It was low by week 3, a peak risen by week 5 and decreased from week 7 up to the end of the study. Furthermore, the IgA antibody titers developed in trichinellosis patients were the highest while the IgE antibody levels were the lowest. In addition, the IgG and IgM antibody responses were slightly higher than the IgE and lower than the IgA responses during infection. To show if ELISA assays using NBL TSE were more sensitive than those performed in a former study

(Salinas *et al.*, 1996) employing TSL-1 antigens, negative serum samples of asymptomatic individuals for TSL-1 antigens were tested for early production of antibodies to NBL. However, asymptomatic individuals did not show reactivity to NBL antigens during infection.

Due to the wide distribution of parasitic infections in our country, a group of serum samples collected from individuals infected with other parasites were tested in each assay. Initially, a cut-off value was calculated considering the mean plus three standard deviations of the OD values of serum samples from healthy individuals (Fig. 2). The cut-off value of ELISA assays for IgM was higher than those observed for the other antibody classes. The ELISA results showed cross-reactivity in all immunoglobulin-ELISA assays except that of IgA. Reactivity of serum samples in IgM-ELISA assays

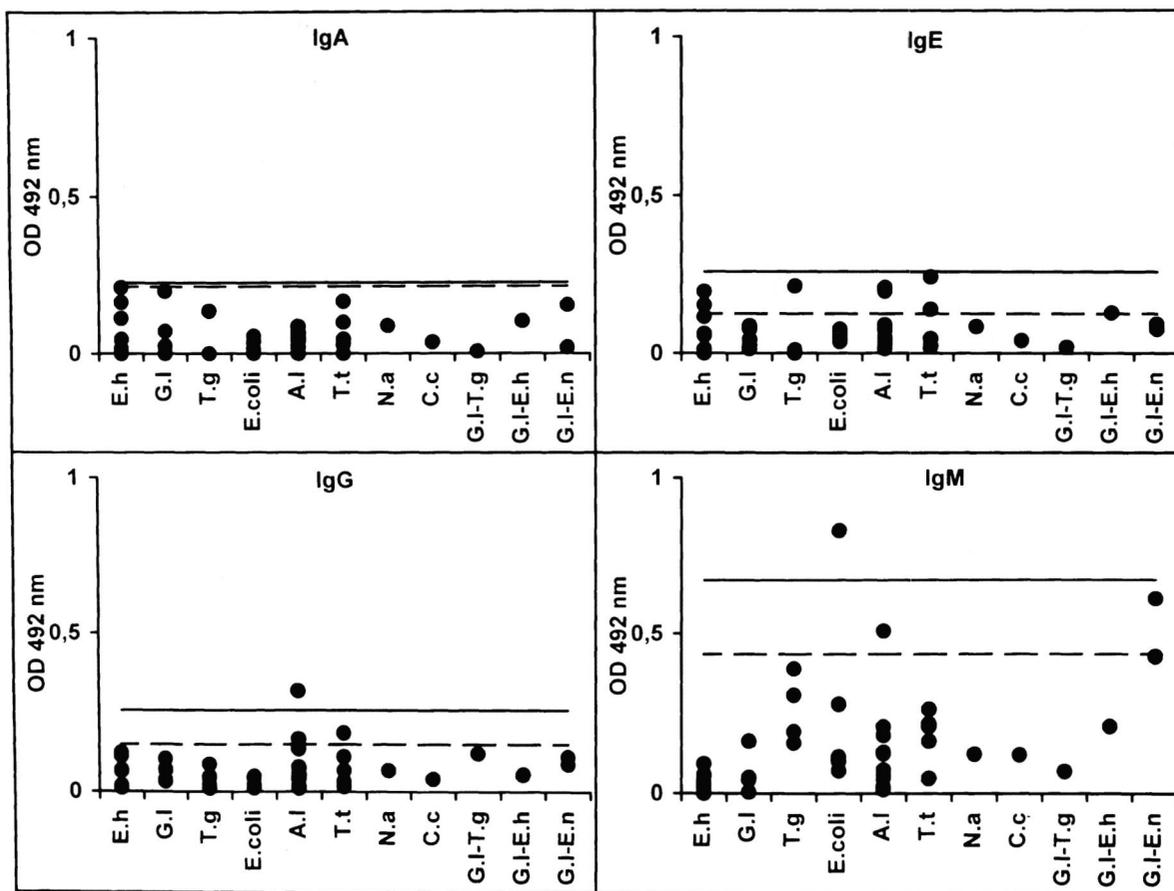


Fig. 2. – Cross reactivity of serum samples from individuals infected with other parasites to NBL antigens of *T. spiralis*. ELISA assays were carried out using NBL TSE of *T. spiralis* and serum samples collected from individuals infected with other parasites such as: *Entamoeba histolytica* (E.h.), *Giardia lamblia* (g.l.), *Toxoplasma gondii* (T.g.), *Entamoeba coli* (E.C.), *Ascaris lumbricoides* (A.l.), *Trichuris trichiura* (T.t.), *Necator americanus* (N.a.), *Cysticercus cellulosae* (C.c.), *Giardia lamblia* and *Toxoplasma gondii* (G.l.-T.g.), *Giardia lamblia* and *Entamoeba histolytica* (G.l.-E.h.) or *Giardia lamblia* and *Endolimax nana* (G.l.-E.n.) to detect cross-reactive IgA, IgE, IgG and IgM antibodies. Discontinued horizontal lines indicate  $X \pm 3 \sigma$  OD values from healthy individuals. Continued horizontal lines represents  $X \pm 3 \sigma$  OD values of serum samples from individuals infected with other parasites. The latter was taken as the threshold value of positivity.

Antigen	Antibody detection	Weeks post-infection					
		3	5	7	15	37	57
NBL	IgA	81.3	94.1	100	88.9	37	38.5
	IgE	43.8	82.4	78.6	33.3	25	7.8
	IgG	31.3	88.2	85.7	77.8	25	30.8
	IgM	18.8	47.1	35.7	0	0	23
TSL-1 <sup>a</sup>	IgA	75	100	100	100	100	92.3
	IgE	62.5	100	100	100	100	92.3
	IgG	43.8	100	100	100	100	100
	IgM	50	100	100	100	100	76.9

NBL: soluble whole extract of newborn larvae.

TSL-1: affinity-purified TSL-1 antigens of muscle larvae.

a: data from Salinas *et al.*, 1996.

Positivity rate was calculated on the basis of a threshold of positivity as the mean plus 3 standard deviations of OD values from serum samples of subjects with other parasitic infections.

Table I. – Positivity rate of specific isotypes by ELISA assays on serum samples taken from trichinellosis patients during one year of infection.

was the highest as compared with that observed in the IgE and IgG ELISA assays.

The IgA-ELISA assay was the most sensitive and specific while the IgM was the least sensitive and specific (Table I). IgA antibodies to NBL antigens were detected in 80 % of patients while IgE, IgG and IgM responses were observed in 44, 31 and 19 % of the patients by week 3, respectively. From weeks 5 to 7, IgA antibodies were found in 89 to 100 % of the patients while lower percentages (0-82 %) were found for the other isotypes. Reactivity of IgA, IgE, IgG and IgM to NBL antigens decreased from week 37 to 57 after infection (0-38 %). The fact that IgM reactivity was observed in none of the patients by weeks 15 and 37 might be due to the lesser number of serum samples analysed by these weeks. Serum samples taken from the same patients were also negative by week 57 in IgM ELISA assays. These percentages of positive values for trichinellosis patients were compared with those obtained in a previous study (Salinas *et al.*, 1996) in which TSL-1 glycoproteins were used.

It was observed that although the highest sensitivity was observed from weeks 5 to 37 in all the assays, by week 3 postinfection 75 % positivity was detected when IgA antibodies were determined (Table I).

## DISCUSSION

The specificity and heavy-chain class of antibodies produced during infection are important factors in the host-parasite interplay; antibodies involved in protective immunity and pathology, antibodies lacking of effectors properties that may act as blocking antibodies or as markers of acute or chronic

phase of infection have been demonstrated in different host-parasite interactions (Parkhouse & Harrison, 1989). Thus, the present study is the first report that analyses the kinetics of human class antibody production to NBL TSE using ELISA assays.

The data obtained reveal similar kinetics of IgM, IgG, IgA and IgE responses but of different magnitude. The fact that the quantity and quality of antibodies elicited by NBL antigens vary, may be due to genetic differences between human hosts (Negrao-Correa *et al.*, 1999), chemical nature and density of the antigenic epitopes on NBL components (Jungery *et al.*, 1983; De Vos *et al.*, 1992), and the total number of NBL produced by the female adult worm in the host (Pozio *et al.*, 1993).

Unlike kinetics of class antibody responses to TSL-1 antigens reported in a previous study (Salinas *et al.*, 1996), the kinetics of class antibody responses to NBL suggested a transient exposure of the patients to the migratory larva as a result of natural infection. In addition, the antibody response may also be affected by the anthelmintic treatment. Thus, this issue should be further studied (Fourestié *et al.*, 1988; Kurniawan *et al.*, 1995).

Similar times of appearance of antibodies against NBL, measured by different techniques, have been observed in rodents (Philipp *et al.*, 1981; Almond & Parkhouse, 1986), humans (Kazura, 1981; Venturiello *et al.*, 1995) and pigs (Marti *et al.*, 1986). In the latter, it has been clearly demonstrated that antibodies to NBL have a critical role in protective immunity (Marti *et al.*, 1986). In contrast, although very little is known regarding the immune elimination of *T. spiralis* from the human host (Capo & Despommier, 1996), data obtained from clinical observations of human cases suggest that the survival of adult worm is considerably prolonged as that seen in pigs (Behnke *et al.*, 1994). Thus, it might be speculated that both hosts may show similar immune responses.

In this respect, the IgG (Kazura, 1981; Venturiello *et al.*, 1995) and possibly the highest level of IgA antibodies produced in patients analysed in this study may have an effector role either to kill NBL *in vivo* as suggested by *in vitro* antibody-dependent cell mediated destruction of parasite larvae tests or in intestinal immunity (Almond & Parkhouse, 1986; De Vos *et al.*, 1992). In fact, the specific class antibody responses to NBL antigens, elevated levels of total IgE and eosinophiles observed in trichinellosis patients suggest that a Th2 response is involved (Finkelman *et al.*, 1991). However, in contrast to the high IgA response, low specific IgE responses to NBL accompanied by elevated levels of total and TSL-1-specific IgE (Salinas *et al.*, 1996) were also observed. This might represent a mechanism for the evasion of the immune system by

the parasite already described in helminth infections (Pritchard, 1993). Thus, there is still controversial whether a Th2 response contributes to protective immunity or is an immunoregulatory consequence in helminth infection (Finkelman *et al.*, 1991).

On the other hand, a knowledge of the kinetics of antibody responses has allowed to identify antibodies indicative of early versus chronic infections (Parkhouse & Harrison, 1989). In our study it was striking to find IgA antibodies which were detected in a high percentage of the serum samples tested early in the infection by an amplified method. This offers an advantage for early diagnosis of human trichinellosis since pathology could be prevented. Furthermore, the IgA response reflects the length of acute stage of the infection, which offers advantages to determine the recovery of patients from the infection.

In all ELISA assays carried out in this study, except that for IgA, cross-reactive antibodies were detected suggesting that antibodies produced in humans may cross react with non-specific epitopes on NBL antigens such as phosphorilcoline which has been found as a cross-reacting epitope between *T. spiralis*, and other helminths and protozoan (Lal & Ottesen, 1989; Choy *et al.*, 1991). Therefore, it is likely that some of the NBL antigens bear such an epitope and other chemically-different epitopes, which could be cross-reactive. The findings mentioned above emphasize the importance to further characterize the relevant epitopes bearing NBL antigens among which are those recognized by human IgA antibodies.

This analysis will lead to the identification of suitable epitopes for specific diagnosis in early stage of the infection.

## ACKNOWLEDGEMENTS

This work was supported by a grant of DEPI. RCR and RST are grateful to COFFA for financial support. The authors wish to thank Dr. Guadalupe Ortega-Pierres and Dr. Javier Hernández-Sánchez for critical review of the manuscript.

## REFERENCES

- ALMOND N.M. & PARKHOUSE R.M.E. Immunoglobulin class specific responses to biochemically defined antigens of *Trichinella spiralis*. *Parasite Immunology*, 1986, 8, 391-406.
- BEHNKE J.M., DEHLAWI M.S., ROSE R., SPYROPOULUS P.N. & WAKELIN D. The response of hamsters to primary and secondary infection with *Trichinella spiralis* and to vaccination with parasite antigens. *Journal of Helminthology*, 1994, 68, 287-294.
- CAPO V. & DESPOMMIER D.D. Clinical aspects of infection with *Trichinella* spp. *Clinical Microbiology Reviews*, 1996, 9, 47-54.
- CHOY W.F., NG M.H. & LIM P.L. *Trichinella spiralis*: light microscope monoclonal antibody localization and immunochemical characterization of phosphorylcholine and other antigens in the muscle larva. *Experimental Parasitology*, 1991, 73, 172-183.
- DENNIS D.T., DESPOMMIER D.D. & DAVIS N.J. Infectivity of the newborn larva of *Trichinella spiralis* in the rat. *Journal of Parasitology*, 1970, 56, 974-977.
- DE VOS T., DANELL G. & DICK T.A. *Trichinella spiralis*: Dose dependence and kinetics of the mucosal immune response in mice. *Experimental Parasitology*, 1992, 75, 99-111.
- FINKELMAN F.D., PEARCE E.J., URBAN J.F. JR. & SHER A. Regulation and biological function of helminth-induced cytokine responses. *Immunology Today*, 1991, 12, 62-66.
- FOURESTIÉ V., BOUGNOUX M.E., ANCELLE T., LIANCE M., ROUDOT-THARAVEL F., NAGA H., PAIRON-PENNACHIANI M., RAUSS A. & LEJONG J.L. Randomized trial of albendazole versus thiabendazole plus fluobendazole during an outbreak of human trichinellosis. *Parasitology Research*, 1988, 75, 36-41.
- JUNGERY M., CLARK N.W.T. & PARKHOUSE R.M.E. A major change in surface antigens during the maturation of newborn larvae of *Trichinella spiralis*. *Molecular and Biochemical Parasitology*, 1983, 7, 101-109.
- KAZURA J.W. Host defense mechanisms against nematode parasite: destruction of newborn *Trichinella spiralis* larvae by human antibodies and granulocytes. *The Journal of Infectious Diseases*, 1981, 143, 712-718.
- KURNIWAN A., ATKINSON R., SARTONO F., YAZDANBAKHSH M. & MAIZELS R.M. Differential decline in filaria-specific IgG1, IgG4 and IgE antibodies in *Brugia malayi*-infected patients after diethylcarbamazine chemotherapy. *The Journal of Infectious Diseases*, 1995, 172, 1567-1572.
- LAL R.B. & OTTESEN E.A. Phosphocholine epitopes on helminth and protozoal parasites and their presence in the circulation of infected human patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1989, 83, 652-655.
- MARTI H.P., MURRELL K.D. & GAMBLE H.R. *Trichinella spiralis*: antifecundity and antinewborn larvae immunity in swine. *Experimental Parasitology*, 1986, 62, 370-375.
- NEGRAO-CORREA D., ADAMS L.S. & BELL R.G. Variability of the intestinal immunoglobulin E response of rats to infection with *Trichinella spiralis*, *Heligmosomoides polygyrus* or *Nippostrongylus brasiliensis*. *Parasite Immunology*, 1999, 21, 287-297.
- PARKHOUSE R.M.E. & HARRISON L.J.S. Antigens of parasitic helminths in diagnosis, protection and pathology. *Parasitology*, 1989, 99, S5-S19.
- PHILIPP M., PARKHOUSE R.M.E. & OGILVIE B.M. Changing proteins on the surface of a parasitic nematode. *Nature*, 1980, 287, 538-540.
- PHILIPP M., TAYLOR P.M., PARKHOUSE R.M.E. & OGILVIE R.M.E. Immune response to stage-specific surface antigens of the parasitic nematode *Trichinella spiralis*. *Journal of Experimental Medicine*, 1981, 154, 210-215.

- POZIO E., VARESE P., GOMEZ MORALES M.A., CROPPA P., PELICIA D. & BRUSCHI F.: Comparaison of human trichinellosis causes by *Trichinella spiralis* and *Trichinella britovi*. *American Journal of Tropical Medicine and Hygiene*, 1993, 48, 568-575.
- PRITCHARD D.I. Immunity to helminths: is too much IgE parasite-rather than host-protective? *Parasite Immunology*, 1993, 15, 5-9.
- SALINAS-TOBÓN M.R., MÉNDEZ LOREDO B., ALCÁNTARA-GONZÁLEZ N., VALDÉZ CRUZ C., MARTÍNEZ Y ZAMORA R., ORTEGA-PIERRES M.G. AND CHAPA-RUIZ M.R. Class and subclass specific antibody responses to TSL-1 antigens during *Trichinella spiralis* human infection, in: Proceedings of the 9th International Conference on Trichinellosis. Ortega-Pierres G., Gamble R., Van Knapen F. & Wakelin D. (eds), CINVESTAV, México, 1996, 453-462.
- TAKAHASHI Y., MIZUNO N., UNO T., AISAKA A. & ARAKI T. A spectrum of antibody response with time after *Trichinella spiralis* infection in rats. *Journal of Parasitology*, 1990, 76, 230-239.
- VENTURIELLO S.M., GIAMBARTOLOMEI G.H. & CONSTANTINO S.N. Immune cytotoxic activity of human eosinophils against *Trichinella spiralis* newborn larvae. *Parasite Immunology*, 1995, 17, 555-559.