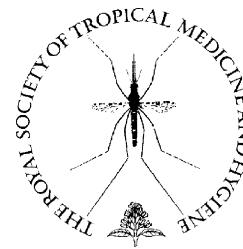




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Immunoparasitological evaluation of *Trichinella spiralis* infection during human pregnancy: a small case series

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Summary The clinical background, vertical transmission of *Trichinella spiralis* and helminthotoxic activity of sera were evaluated in four pregnant women infected at different gestation times. Parasitological and immunological parameters were analyzed. Patients presented clinical symptoms and a seroconversion pattern typical of *Trichinella* infection, independently of the gestation trimester. All patients, including one patient treated with mebendazole, gave birth to healthy infants at full term. Studies performed in placentas and umbilical cords by artificial digestion and/or immunofluorescence did not reveal the presence of parasites or parasite antigens. Specific antibodies were found in placental extraction and maternal sera by immunofluorescence, ELISA and/or immunoelectrotransfer blot assay. Specific IgG, IgE and IgA were found in the umbilical cord sera. One umbilical cord demonstrated immune-complexed specific IgM and circulating parasite antigens were found in the corresponding infant up to 10 months after birth. Patients' sera were able to kill newborn larvae in cytotoxicity assays, even in the absence of specific antibodies; this effect was abrogated by mifepristone. The results suggest that in human trichinellosis during pregnancy there is an enhanced helminthotoxicity to newborn larvae, dependent in part on progesterone, leading to a mild or moderate course of the infection. The results also indicate that the transplacental passage of migrating larvae is possible.

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1. Introduction

Trichinellosis is a food-borne parasitic disease endemic in many countries worldwide. It is acquired by the ingestion of

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either raw or undercooked pork, meat from wild animals and even meat from herbivorous animals bearing larvae of the nematode *Trichinella* spp. (Ancelle et al., 1998; Campbell, 1988; Murrell and Bruschi, 1994). The life cycle of the nematode consists of three phases: intestinal, migrant and muscular.

The presence of pregnant women during outbreaks of trichinellosis represents an important challenge for physicians since the treatment and clinical course of the infection in pregnant woman is still unclear. Knowledge about the influences that this parasitic disease may have on pregnancy, fetal development and wellness of the newborn is still scarce and controversial. Few cases have been reported, documenting abortions, premature births and only a few normal deliveries. In these cases, the mechanisms which led to abortions or premature births remain unknown (Aldridge, 1931; Blondheim et al., 1984; Fetchko et al., 1952; Klein, 1978; Kociecka, 1981; Schwartz, 1949).

Transplacental passage of migrant larvae to the offspring has been described in rats, rabbits, ferrets, guinea pigs and mice (Boulos et al., 2005; Cosoroabă and Orjanu, 1998; Hartmanova and Chroust, 1968; Nuñez et al., 2002; Wang et al., 2005; Webster and Kapel, 2005). There are few reports of the transplacental passage of migrant larvae to the fetus in human trichinellosis (Dubinský et al., 2001; Kuitunen-Ekbaum, 1941) and many aspects of this parasite infection during pregnancy remain to be elucidated.

In previous studies we demonstrated a synergism between the Th2-type responses generated by both the pregnant status and the infection with the nematode. This synergism was reflected in an enhanced parasitocidal activity against *T. spiralis* newborn larvae (NBL) and not against the adult worm, resulting in a lower degree of infection in pregnant rats. This increased helminthotoxic activity was demonstrated to be driven by progesterone (Nuñez et al., 2002, 2005).

The purpose of this study was to report the clinical and serological features of trichinellosis in four pregnant women. The immunoserological features of their newborn and/or infants, a comparative study of the isotypes of specific immunoglobulins detected in the newborn and their mothers and parasitological analysis of the placenta and umbilical cord were carried out to assess the possibility of transplacental passage of larvae. Furthermore, the ability of mothers' sera to mediate the killing of the NBL in cytotoxicity assays and the role of progesterone were studied.

2. Materials and methods

2.1. Patients

The clinical background and laboratory test results of four pregnant women involved in different trichinellosis outbreaks in Argentina are described:

2.1.1. Patient one

The patient was a 22-year-old in the third trimester of pregnancy who acquired the infection through the ingestion of pork products from a commercial source and was seen in June 2003. By day 11 post-infection (p.i.) myalgia

and eosinophilia of 17% were observed. No anthelmintic treatment was administered and hospitalisation was not needed. The woman gave birth to a healthy newborn at the 40th week of gestation (1 month p.i.) by caesarean surgery.

2.1.2. Patient two

The patient was a 30-year-old in the first trimester of gestation who acquired the infection through the ingestion of pork salami and was seen in May 2005. Clinical manifestations of the disease included fever (38.3–39.7 °C), diarrhoea during the first week p.i., facial oedema, myalgia and headache. On day 21 p.i. she had to be hospitalised and was medicated with ibuprofen, dipyrone, acetaminophen and erythromycin eye drops. Anthelmintic treatment with mebendazole (400 mg/day) for 9 days was administered. No secondary manifestations (endocarditis, pneumonitis, encephalitis) were observed. She also had an episode of metrorrhagia not related to an abortion process. Ultrasound studies at different times of gestation and amniocentesis for karyotyping found no fetal abnormalities. Serum activities of lactate dehydrogenase (LD) and creatine phosphokinase (CPK) were elevated up to three and eight times the normal values on days 24 and 27 p.i., respectively. CPK levels fell to normal values by day 34 p.i., whereas the LD fell to normal values by day 42 p.i. Total leukocyte count reached a maximum by day 27 p.i., reaching values of 11 800 cells/mm³. Eosinophil counts increased up to 18% at the end of the analysed period (day 41 p.i.). In the 38th week of gestation (8 months p.i.) the woman gave birth to a healthy newborn by normal delivery.

2.1.3. Patients three and four

Patients three and four were 26-year-olds who were infected during the first trimester of pregnancy and seen in June 2003 and April 2004, respectively. They were infected by the consumption of a pork product (ham). At the time of the serological testing (18 days and 1 month p.i., respectively) they presented with myalgia, headache and fever. Eosinophil percentage in patient three reached only 9% of the white blood cell count. Unfortunately, additional laboratory data were not available. No anthelmintic treatment was administered to the patients and hospitalisation was not needed. Both women gave birth to one healthy newborn each at full term.

2.2. Sera

Serum samples from infected mothers and their infants were obtained by venous puncture. Cord blood samples were obtained at delivery. Placental extraction serum, which represents mostly the maternal serum, was obtained according to the method of Malan Borel et al. (1991). In cytotoxicity assays, serum samples from patients in the acute phase of the infection (anti-*T. spiralis* newborn larvae cytotoxic serum) and sera from non-pregnant women, not infected with *Trichinella* spp. were used as positive and negative controls, respectively. In all cases serum samples were aliquoted and kept at –20 °C until used.

2.3. Placentas and umbilical cords: parasitological and histochemical analysis

Placentas and umbilical cords from patients one and two were obtained at delivery. One-third and one-quarter of each placenta, respectively (>50 g each) and umbilical cord samples (27.5 g and 47.7 g each) were subjected to the artificial digestion method according to Larsh and Kent (1949). Analysis of the histological structure of the placenta obtained from patient two was made by fixation in 10% formaldehyde and inclusion in paraffin for subsequent staining with hematoxylin and eosin.

Trichinella spp. antigens were investigated in placental tissue by immunofluorescence (IF). Paraffin from tissue sections was removed (Venturiello et al., 2007) and Fc receptors present in the trophoblast blocked by incubation with undiluted normal human serum. Sections were then incubated with an anti-*T. spiralis* rat serum suitably diluted in PBS plus 0.1% Tween 20 (PBST-0.1%). After washing, an anti-rat serum conjugated to fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA, USA) diluted in Evans blue plus 20% v/v normal human serum was added and incubated as above. All steps were carried out at 37 °C with three washes with PBST-0.1% in between. Slides were then air-dried and mounted using buffered glycerine. Slides were observed under a fluorescence microscope (Zeiss, Oberkochen, Germany). A negative control (where the anti-*T. spiralis* serum was replaced by normal rat serum) was also included.

2.4. Detection of specific total gammaglobulins by ELISA and immunofluorescence

Total gammaglobulins were detected by IF according to Costantino et al. (2001), employing cryostat sections of free *T. spiralis* muscle larva (ML) as antigen. Serum samples were considered positive when fluorescence of the parasite cuticle was observed under a fluorescence microscope (Zeiss). Positive and negative controls were run in each assay.

Total gammaglobulins against *T. spiralis* were detected by indirect ELISA employing as antigen the ML excretory-secretory products (ML-ESP) obtained as previously described (Nuñez et al., 2000). Briefly, flat-bottomed PolySorp polyvinyl microtitre plates (Nunc, Roskilde, Denmark) were coated with ML-ESP at 5 µg/ml. After coating and between each step, plates were washed with PBST-0.05%. Plates were blocked using non-fat dried milk in PBS. Serum samples were then added diluted 1/200. A horseradish peroxidase-conjugated anti-human gammaglobulin serum (Biosys, Compiègne, France) suitably diluted in PBST-0.05% was added. Colour reaction was developed using *o*-phenyldiamine (Sigma, St Louis, MO, USA) and H₂O₂. The reaction was stopped by addition of 4N H₂SO₄ and the optical density (OD) read at 490 nm in an ELISA reader (Metertech Σ960). Positive and negative serum samples were run as controls. All samples were run in duplicate. The cutoff OD value previously determined was 0.4, equivalent to the mean plus 3 SD of 50 *Trichinella*-free human sera.

2.5. Analysis of serum samples by immunoelectrotransfer blot assay

Serum samples from mothers and children were analysed by immunoelectrotransfer blot assay (IETB) according to Nuñez et al. (2000). For detection of total specific gammaglobulins, a biotinylated goat anti-human IgG (H+L) serum (Vector Laboratories) followed by a macromolecular complex of avidin and biotinylated peroxidase (Vector Laboratories) were added according to the manufacturer's directions. For detection of IgA a rabbit anti-human α chain (Dako Corporation, Carpinteria, CA, USA) was used and followed by a peroxidase-conjugated goat anti-rabbit serum (Sigma) suitably diluted. For detection of IgG, a goat anti-human γ chain (Sigma) was used followed by a peroxidase-conjugated rabbit anti-goat serum (Bethyl Laboratories, Montgomery, TX, USA) adequately diluted. For detection of specific IgM a rabbit anti-human μ chain (Dako Corporation) was used, followed by a peroxidase-conjugated goat anti-rabbit serum (Sigma) suitably diluted. IgE detection was carried out employing a goat anti-human ε chain (Vector Laboratories) followed by a peroxidase-conjugated rabbit anti-goat serum (Bethyl Laboratories) suitably diluted. Blots were developed using a solution containing α-chloronafthol and H₂O₂. Reactions were stopped by the addition of tap water. Samples were considered positive when the *Trichinella*-specific bands of ~45 and ~55 kDa were developed.

2.6. Detection of circulating antigens by ELISA

The method employed by Attallah et al. (2003) was used with some modifications. Briefly, plates were coated with a 1/100 dilution of each serum sample in carbonate buffer at pH 9.6 and incubated for 1 h at 37 °C and overnight at 4 °C. After washing with PBST-0.05% the mAb PG6B1, with reactivity against the ~45–55 kDa components of the ML-ESP, kindly provided by Dr Bruschi, at 15 µg/ml was employed followed by a horseradish peroxidase-conjugated anti-mouse IgG (H+L) (Diagnostics Pasteur, Marnes-La-Coquette, France). Colour reaction was developed and stopped as above. A standard curve of ML-ESP diluted in normal serum ranging from 25 ng/ml to 5000 ng/ml was run in each plate. All samples were run in duplicate.

2.7. Detection of circulating specific IgM-bearing immune complexes by ELISA

IgM-bearing immune complexes were detected in serum samples by capture ELISA. Briefly, flat-bottomed PolySorp polyvinyl microtitre plates (Nunc) were coated with a rabbit anti-human μ chain (Dako Corporation) at 10 µg/ml in carbonate buffer, pH 9.6, for 1 h at 37 °C and overnight at 4 °C. After coating and between each step plates were washed three times with PBST-0.05%. Plates were blocked using 0.3% non-fat dried milk in PBST-0.05% for 1 h at 37 °C. Serum samples were then added diluted 1/100 in PBST and incubated for 1 h at 37 °C and overnight at 4 °C. The reaction was continued by the addition of mAb PG6B1 at 15 µg/ml followed by a horseradish peroxidase-conjugated anti-mouse

IgG (H+L) (Diagnostics Pasteur). Colour reaction was developed and stopped as above. Positive and negative controls were run. Results were expressed as positivity ratio (PR) calculated as OD sample/OD normal sera. Samples were run in duplicate.

2.8. Cytotoxicity assay

The cytotoxicity assay was carried out according to a protocol employed routinely in our laboratory (Nuñez et al., 2005; Venturiello et al., 1993). Briefly, the test was performed in flat-bottomed microwell modules (Nunc). Thirty microlitres of a larval suspension in Eagle's minimum essential medium with Earle's salts (MEM, Gibco, NY, USA), obtained by in vitro culture of adult female worms and containing approximately 50 NBL, was placed in each well and the exact number of larvae was counted. Ten microlitres of each serum from non-*Trichinella*-infected pregnant, *Trichinella*-infected pregnant (patients), non-infected non-pregnant women or anti-*T. spiralis* newborn larvae cytotoxic serum and 100 µl of a leukocyte suspension from a non-infected non-pregnant woman obtained by dextran sedimentation and containing 5×10^6 cells/ml were added to each well. Sera were used undiluted. Reactions were kept for 20 h at 37°C in an atmosphere of 5% CO₂ in air. In order to evaluate the role of progesterone in the effector mechanism of NBL killing, larvae were also incubated with sera from pregnant women and leukocytes which had been previously resuspended in MEM with the progesterone receptor antagonist mifepristone (RU486, 200 ng/ml; Sigma). NBL death was measured by direct microscopy by two independent observers and the mortality percentage calculated according to the following formula: % Mortality = $[(NBL_i - NBL_f)/NBL_i] \times 100$ where NBL_i and NBL_f are the numbers of NBL counted at the beginning and at the end of the reaction, respectively. Experiments were done in duplicate.

2.9. Statistical methods

Percentages of NBL mortality were analysed by two-way ANOVA with repeated measures of one factor, using SPSS 12.0 (SPSS Inc. Chicago, IL, USA).

3. Results

3.1. Placental and umbilical cord samples: parasitological and histochemical studies

No *Trichinella* spp. larvae were found by the artificial digestion method in either the placental tissues or the umbilical cords of either patient one or two. No parasite antigens were detected by IF in sections of the placenta belonging to patient two. The histopathological study of the placenta showed basal mononuclear villitis, mild villous oedema, foci with villous immaturity and mild lymphocytic deciduitis. Nevertheless, it was considered to be a normal third trimester placenta.

3.2. Detection of specific total gammaglobulins and isotypes anti-muscle larvae excretory-secretory products

In patient one, by day 11 p.i. anti-*T. spiralis* ML-ESP total gammaglobulins were detected by two serological techniques (ELISA and IETB; Table 1). At delivery (1 month p.i.), the IF, ELISA and IETB showed high levels of specific antibodies compared to the sample obtained on day 11 p.i. in both serum and placental extraction serum. Anti-ML-ESP immunoglobulins of all the isotypes were present as shown by IETB analysis. In the umbilical cord blood belonging to infant one, specific immunoglobulins to the parasite were detected in the IgG, IgA and IgE isotypes (Table 2). Two months after birth the infant was immunoserologically tested for total gammaglobulins, being positive by IF and ELISA and also displaying in IETB the typical specific bands of *T. spiralis* ML-ESP of ~45 and ~55 kDa. Both the patient and her son were serologically tested 2 years later. At this time, the mother was immunoserologically positive for total gammaglobulins whereas her son was negative by IF, ELISA and IETB.

After 1 month p.i., patient two had specific total gammaglobulins detected by IF, ELISA and IETB. Moreover, specific total gammaglobulins were also detected in the placental extraction serum (Table 1). In umbilical cord blood of infant two, specific total gammaglobulins to the parasite were detected by IF and IETB. By IETB, anti-ML-ESP activity was detected in the IgG, IgA and IgE isotypes. The latter specific isotypes were also found in the serum sample of the infant 10 months after birth. No specific free IgM was found by IETB in any of the samples (Table 2).

Specific total gammaglobulins were detected by IF, ELISA and IETB at the first sampling time for patient three (18 days p.i.), and patient four, (1 month p.i., Table 1). Immunoserological follow up of infant four was possible only at 16 months of age and found no specific gammaglobulins (Table 1).

3.3. Detection of circulating antigens and IgM immune-complexes by ELISA

Neither circulating antigens nor IgM-bearing immune complexes were detected in the placental extraction serum and the cord blood belonging to infant one. Both the serum sample from the acute phase (1 month p.i.) and the placental extraction serum of patient two presented a positive ELISA for circulating antigens, rendering values of antigenaemia of 20 and 21 ng/ml, respectively. Serum samples from infant two taken from the umbilical cord and a sample taken at 10 months after birth also gave positive results, with antigenaemia values of 74 ng/ml and 110 ng/ml, respectively. This mother was negative for IgM-bearing immune complexes, whereas her infant's serum samples (umbilical cord and peripheral blood taken at 10 months after birth) gave positive reactions (PRs of 4.2 and 3.2 respectively).

3.4. Cytotoxicity assay

Sera from the four pregnant women infected with *Trichinella* were able to induce NBL death despite the lack of anti-NBL

Table 1 Immunoserological study (detection of specific total gammaglobulins) of sera from different samples obtained from mothers and their infants at different times

Serum sample	Disease stage	Sampling time	Source	ELISA ^a	IF	IETB
Patient 1	Acute	Pregnancy	Peripheral	+(0.470)	—	+
		11 days p.i.				
	Convalescence	Delivery	Peripheral	+(1.230)	+	+
		1 month p.i.	Placental extraction	+(0.921)	+	+
		7 months p.i.	Peripheral	+(1.126)	+	+
Infant 1	Chronic	2 years p.i.	Peripheral	−(0.360)	+	+
	Birth	Umbilical cord	−(0.245)	+	+	
	2 months	Peripheral	+(0.427)	+	+	
	2 years	Peripheral	−(0.294)	—	—	
Patient 2	Acute	Pregnancy	Peripheral	+(0.922)	+	+
		1 month p.i.				
	Convalescence	Delivery	Placental	+(0.498)	+	+
		8 months p.i.	extrac-			
Infant 2	Chronic	1.5 years	Peripheral	+(0.806)	+	+
	Birth	Umbilical cord	−(0.361)	+	+	
	10 months	Peripheral	−(0.018)	—	+	
Patient 3	Acute	Pregnancy	Peripheral	+(1.042)	+	+
		18 days p.i.				
Patient 4	Acute	3.5 months p.i.	Peripheral	+(1.682)	+	+
Patient 4	Acute	Pregnancy	Peripheral	+(0.431)	+	+
		1 month p.i.				
Infant 4		16 months	Peripheral	−(0.094)	—	—

IF: immunofluorescence; IETB: immunoelectrotransfer blot assay; p.i.: post-infection.

^a ELISA cutoff optical density: 0.400.

antibodies in their sera and adherence of the leukocytes to the parasite. When leukocytes were preincubated with the progesterone antagonist mifepristone (RU486) this mortality was abrogated ($31.0 \pm 8.7\%$ vs. $9.7 \pm 8.2\%$; $P < 0.0001$). On the other hand, similar results were obtained with sera from non-*Trichinella* infected pregnant women, which also induced NBL death. This mortality was also abrogated in the presence of RU486 ($18.7 \pm 2.4\%$ vs. $3.3 \pm 1.2\%$; $P < 0.001$). Positive and negative controls carried out with anti-*T. spiralis* newborn larvae cytotoxic serum and sera from non-pregnant, non-infected women had values of $64.9 \pm 8.3\%$ and $6.3 \pm 2.4\%$ respectively. Similar results were obtained when these controls were carried out with leukocytes preincubated with RU486.

Table 2 Comparative study by immunoelectrotransfer blot assay of anti-*Trichinella spiralis* isotypes in placental extraction serum from infected mothers and umbilical cord serum from their infants

Serum sample	IgG	IgM	IgA	IgE
Patient 1	+	+	+	+
Infant 1	+	—	+	+
Patient 2	+	+	+	+
Infant 2	+	— ^a	+	+

^a Positive for specific IgM-bearing immune complexes.

4. Discussion

In this study we report four cases of trichinellosis during pregnancy. The clinical symptoms (fever, myalgia, diarrhoea and/or facial oedema) presented by the patients corresponded to the typical trichinellosis syndrome (Murrell and Bruschi, 1994). The signs and symptoms of the disease were mild in patients one, three and four and moderate in patient two, according to the classification of Kassur et al. (1981). Although the intensity of the acute phase of the infection is related to the parasite load of the pork ingested (Dupouy-Camet et al., 2002), we suggest that the lack of severe manifestations as well as the absence of secondary complications of the disease are due to the helminthotoxic properties of the pregnant women's sera. This would contribute to the decrease in the quantity of parasites infecting the host as demonstrated by our previous work in the murine model (Nuñez et al., 2002, 2005).

We also suggest that the pronounced facial oedema and high temperature reached by patient two could be due not only to the hypersensitivity reaction produced by the migration of larvae through the bloodstream and tissues but also to the shedding of antigens following parasite destruction due to the anthelmintic treatment received by the patient.

The administration of benzimidazole derivatives during pregnancy has long been a matter of discussion, especially the prescription of mebendazole, which has been demonstrated to be a potent teratogenic drug in rats (Yoshimura,

2003). No embryofetotoxic effects, e.g. structural abnormalities leading to serious medical, surgical or cosmetic consequences of mebendazole were observed in the baby born to patient two. This observation is in line with reports suggesting that mebendazole would not represent a major teratogenic risk in humans (Acs et al., 2005; Diav-Citrin et al., 2003).

Regarding the immunological diagnosis during the acute phase of the infection all mothers were positive by the three conventional serological tests, that is the detection of total gammaglobulins anti-ML-ESP by ELISA, IF and IETB, at around 20 days, the expected time according to our experience (Costantino et al., 2001). Subsequent testing of patients (one, two and three) during the convalescence and/or chronic phases gave positive results even after two years p.i.. Serology tests at this time p.i. have only epidemiological value, since by this time muscle larvae are already encapsulated in striated muscle and antiparasitic drugs are ineffective (Dupouy-Camet et al., 2002; Soulé and Dupouy-Camet, 1991). Serum samples obtained from peripheral and/or placental (placental extraction) blood was available for analysis at the time of delivery in patients one and two, and these samples also had a high titre of antibodies. This finding would indicate that despite the pregnant status, which has been considered a state of immunosuppression by various authors (Menendez, 1995; Sacks et al., 1999; Szekeres-Bartho et al., 1989), the patients' immune systems could respond strongly to parasite antigen stimulation.

Serum samples from the umbilical cords of the infants born to patients one and two were tested in parallel with placental extraction sera from their mothers obtained at delivery. Both infants had specific anti-ML-ESP IgG, IgA and IgE (Table 2). The finding of specific IgG could be explained by the ability of this immunoglobulin to cross the placental barrier employing the Fc γ receptor (transcytosis) (Yoshida et al., 2006). The presence of specific IgA and IgE in these samples is most surprising as the phenomenon of transcytosis has not previously been reported for these isotypes. However, Malek et al. (1997, 1998) have demonstrated that IgA would be able to cross the placental barrier by a passive diffusion phenomenon governed by a maternal-fetal concentration gradient. Even though additional studies are needed, our results also suggest the transplacental passage of IgE.

The possibility that samples from infants one and two were contaminated with maternal blood may be ruled out for if that had been the case, these samples would have presented specific free IgM, which was detected in the maternal placental extraction serum samples.

It is noteworthy that infant two presented the same immunoglobulin isotypes as his mother even 10 months after birth, a time by which all maternally derived immunoglobulins are already catabolised. Although no specific free IgM could be detected as a marker of congenital infection, this isotype was detected in the umbilical cord and peripheral serum samples as an IgM-ML-ESP immune complex. On the other hand, these complexes were not present in infant one.

Unlike in infant one, the umbilical cord and peripheral serum samples from infant two obtained at 10 months of age and the placental extraction serum contained parasite circulating antigens. These results indicate either the

transplacental passage of parasite antigens, as has been demonstrated in other parasitic diseases (Carlier et al., 1980; Druilhe et al., 1976) or the secretion of parasite antigens into the bloodstream due to a congenital infection with *Trichinella* spp.

This report is an attempt to elucidate the clinical course of human trichinellosis during pregnancy and may be summarized by the following points: (a) all the patients gave birth to healthy infants without any pathological processes in the placenta, (b) no teratogenic effects were found in the infant born to the mother treated with mebendazole, (c) the clinical picture presented by the patients was that of a typical *Trichinella* spp infection, which varied from mild to moderate independently of the gestation trimester at which the infection occurred, (d) no secondary manifestations or sequelae were found, most probably due to progesterone-dependent immunoactivation, leading to a decrease in the parasite burden due to an helminthotoxic effect towards the NBL, (e) on the basis of the immunoserological studies performed in one of the infants (presence of all the immunoglobulin isotypes and circulating antigen) it was speculated that the transplacental passage of larvae to the fetus occurred, and (f) the differences found in the immunoserological parameters between the infants would seem to be independent of the trimester of gestation when the infection of the mothers occurred.

Although a greater number of patients should be analysed, the results presented herein contribute to a better understanding of the complex host-parasite relationship in trichinellosis during pregnancy.

Authors' contributions: SMV and GGN designed the study protocol; GGN and SNC carried out the assays; all authors carried out the clinical assessment and analysis and interpretation of the data. All authors contributed to the preparation and revision of the manuscript and read and approved the final version. SMV and GGN are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: Not specifically required; the work was carried out in accordance with the code of ethics of the Declaration of Helsinki for experiments involving humans.

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