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Evaluation of immunodiagnostics for toxocarosis in experimental porcine cysticercosis

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Summary We assessed whether immunodiagnostic tests for cysticercosis can cross-react with the currently available immunodiagnostic tests for *Toxocara canis* in an established animal model for cysticercosis infection in pigs, known host for *Toxocara*. We examined by TES-enzyme-linked immunosorbent test and immunoblot assay for toxocarosis and cysticercosis the baseline and final follow-up sera of 10 pigs, before and after (3 months) infection with *Taenia solium*. After successful cysticercosis infection, the nine evaluable pigs became seropositive to *T. solium* (enzyme-linked immunoelectrotransfer blot assay), but did remain seronegative for *Toxocara* in both assays, documenting the lack of cross-reactivity with anti-*T. solium* antibodies in both *T. canis* assays. These findings should help clinicians better interpret serology for toxocariosis and cysticercosis in endemic areas for both helminth infections.

keywords toxocarosis, cysticercosis, immunodiagnosis, epilepsy, pig

Introduction

Toxocarosis is distributed worldwide with higher prevalence in low-resource tropical countries, where the humid climate favours the survival of parasite eggs in the soil and poor sanitary conditions increase the probability of infection (Schantz & Glickman 1978; Schantz 2000; Taylor & Holland 2001). Transmission does not only occur in rural areas, but also in urban areas because of contamination of the public gardens with dog and cat stools. Although most infections are asymptomatic, migrating larval stages of animal intestinal roundworms (gen. *Toxocara, Toxascaris, Ascaris*, etc.) can cause inflammatory tissue reactions in the human host leading to two main clinical syndromes, visceral and ocular/encephalic larva migrans (Taylor *et al.* 1988; Magnaval *et al.* 1997; Nicoletti *et al.* 2002).

Definitive diagnosis could only be provided by histological demonstration of the parasite in biopsy material. However, it is rarely clinically justified to obtain biopsy material and thus the diagnosis rests on non-specific imaging findings and confirmatory serology. Previous serological tests based on somatic or excretory/secretory (E/S) antigenic preparations of adult or larval *Toxocara canis* were unreliable because of low sensitivity and specificity. An enzyme-linked immunosorbent test (ELISA) using soluble *Toxocara* excretory-secretory antigens (TES-Ag) from second-stage larvae is now the most widely used assay (De Savigny *et al.* 1979). Although this TES-ELISA has reasonable sensitivity (73%) and specificity (92%) (Glickman *et al.* 1978), cross-reactivity with other helminth infections occurs (Speiser & Gottstein 1984; Lynch *et al.* 1988). Given that TES-Ag is a complex mixture, including specific and non-specific fractions (Nicholas *et al.* 1984; Kennedy *et al.* 1987), immunoblot is recommended as a confirmatory test (Magnaval *et al.* 1991). In this immunoblotting, TES-Ag is separated into two groups: low-molecular weight antigen bands (LMW; 24–35 kDa) and high-molecular weight bands (HMW bands) (132, 147 and 200 kDa) (Maizels *et al.* 1984; Magnaval *et al.* 1991). LMW bands (usually associated to the HMW complex) are indicative of toxocarosis, whereas isolated clusters of HMW are considered a possible cross-reaction to other helminth antigens. Recombinant antigens seem to have

increased specificity (Yamasaki *et al.* 2000), although they are not yet widely available.

One of the parasites most frequently involved in the differential diagnosis is the larval stage of *Taenia solium*, aetiological agent of cysticercosis, mainly in low-resource areas where the two helminths are often co-endemic and human neurocysticercosis (NCC) is the major cause of acquired epilepsy (White 1997; Garcia *et al.* 2003). NCC has a cosmopolitan distribution, with higher prevalence in Latin America, Asia, Africa and Oceania, but it is increasingly diagnosed in industrialized countries because of the immigration of *T. solium*-infected individuals, with over 1000 cases per year in the USA (Schantz *et al.* 1998). Considering that both *Toxocara* and *T. solium* larvae may generate a variety of neurologic disorders, epilepsy included, the availability of reliable serological tools is a crucial requisite for clinical diagnosis and research purposes.

We took advantage of an established animal model for cysticercosis infection in pigs (Gonzalez *et al.* 2005) (also a known host for *Toxocara*) to assess whether immunodiagnostics for cysticercosis can cross-react with the currently available immunodiagnostic tests for *T. canis*.

Methods

Firstly, as a proof of concept, we examined whether *Toxocara* immunoblot assays could detect specific bands in pigs. To maximize the chances of finding positive cases, we examined by immunoblot sera samples from 43 rural pigs 18 months or older, sampled in field conditions.

In phase 2, we examined by TES-ELISA and immunoblot assay for toxocarosis and cysticercosis the baseline and final follow-up sera of 10 pigs bought to a commercial farm, before and after oral infection with *T. solium* (one proglottid per month to each pig) (Gonzalez *et al.* 2005). These pigs were part of control groups of vaccine studies, and were humanely euthanized 3 months after infection. All laboratory personnel were blind to the pre- and post-infection status of sera.

Toxocara serology

An in-house assay was initially used to determine the presence of specific anti-*Toxocara* IgG in the 43 rural pigs sampled in the field. This assay uses E/S antigen obtained from L2 and L3 larvae of *T. canis* in protein-free media. The E/S product was processed by immunoelectrotransfer blot as described by Tsang *et al.* (1983), by using gradient 4–16% acrylamide gels. It detects seven bands of molecular weights 24, 28, 30, 35, 132, 147 and 200 kDa. A subgroup of the rural pig samples and all experimental samples were evaluated to detect an anti-*Toxocara*-specific IgG, by TES-ELISA and immunoblot by using commercial kits (Toxocara ELISA IgG, CYPRESS Diagnostics, Langdorp, Belgium; Toxocara WB IgG, LDBIO Diagnostics, Lyon, France) as described by the manufacturers. A polyclonal goat anti-porcine IgG conjugate was used instead of the anti-human IgG conjugate included in the kits prepared to test human sera. We considered a positive result only those samples reacting to two or more LMW bands.

T. solium serology

Taenia solium serologic status was determined by enzyme-linked immunoelectrotransfer blot as previously described (Tsang *et al.* 1989). Briefly, this assay uses seven purified *T. solium* glycoprotein antigens (diagnostic bands GP50, GP42–39, GP24, GP21, GP18, GP14 and GP13, the number indicating the respective molecular weight in kDa) in an immunoblot format to detect infection-specific antibodies. Reaction to at least one band is considered positive.

Results

Almost all pigs from the field (40/43) were positive to *Toxocara* antibodies with at least one band (of low or HMW) on the in-house immunoblot assay. Thirty-eight were positive to at least one LMW band, and 30 of them were positive to two or more LMW bands. Repeated serology using the commercial kit, on a sub-sample of 10 sera, showed the perfect concordance between the two assays. This pig population included similar proportions of animals seropositive and seronegative to *T. solium* antibodies. No differences in seroprevalence of anti-*Toxocara* antibodies was found with regard to *T. solium* antibody status.

Experimental animals

At baseline, all 10 pigs proved seronegative to *T. solium*. One of them was seropositive to *T. canis* on immunoblot (presence of two LMW bands, 24–35 kDa, and three HMW bands) but negative in TES-ELISA. This animal was excluded from the study as considered naturally infected with ascarid parasites.

After successful experimental cysticercosis infection, all nine evaluable pigs became seropositive to *T. solium* with three or more of the seven diagnostic antibody bands. All these pigs showed multiple viable cysticerci at necropsy (mean 141, median 93; range: 1–470 cysts) but no other tissue parasites. No records of intestinal nematodes were taken in this experiment. At the time of necropsy (3 months after *T. solium* infection), all nine samples tested

negative for *Toxocara* in both ELISA and immunoblot assay. However, HMW bands were observed in seven animals (two bands in six pigs, one band in one pig) in the immunoblot assay.

Discussion

Using this porcine cysticercosis infection model, we documented the lack of cross-reactivity with anti-*T. solium* antibodies in the *T. canis*-tested assays (negative results of TES-ELISA and absence of any specific LMW bands in the immunoblot assay), despite the success of the experimental porcine cysticercosis, which was proven at necropsy and by serology. These findings demonstrate that at least in the initial 3 months of infection, established cysticercosis does not cross react with toxocarosis. Our study design cannot rule out that cross reactions were established in later stages of cysticercosis infection. However, by month 3, the cysts are fully developed and in humans, probably less heavily infected by *T. solium* eggs than our pigs, the risk that a toxocarosis assay cross reacts with cysticercosis should be less important.

At the same time, the occurrence of HMW bands in the post-*T. solium* infection sera confirmed their low specificity, previously observed testing sera from human subjects with various helminthic diseases other than cysticercosis (Yamasaki *et al.* 2000). Albeit unlikely as the experiment was performed in controlled and clean conditions, it is, however, possible that our experimental pigs became infected with other nematodes during the course of the study and thus these HMW bands cannot be attributed with certainty to a cross reaction with anti-*T. solium* antibodies.

Although several authors claimed high specificity in the immunoblot assay for *Toxocara* serodiagnosis, to the best of our knowledge, this is the first evidence of absence of cross-reactivity of LMW fractions drawn out by testing known sera containing anti-*T. solium* antibodies. Concomitant *T. solium* and *T. canis* infection is a possible event in areas endemic for both helminths, and albeit cysticercosis is a much more known cause of neurological symptoms, toxocarosis should not be ruled out as a cross-reactive response/possible origin of neurological disorders.

Interpretation of serological results should also take into account the background seroprevalence level in the studied population. In endemic areas for both infections, up to 25% of general population may have specific antibodies to *T. solium* (many of them representing exposure only, without established infection), and up to 60–90% to *Toxocara* (Magnaval *et al.* 2001; Garcia *et al.* 2003). The results obtained in this study should help clinicians to better interpret concomitant-positive serology for toxocarosis and cysticercosis, which could occur with some frequency in endemic areas for both helminths. However, further efforts, mainly devoted to the development of tests based on the antigen detection, are needed to improve the immunological diagnosis of helminth infections.

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Evaluation des diagnostics immunologiques de la toxocarose dans la cysticercose porcine expérimentale

Nous avons évalué si les tests de diagnostic immunologique de la cysticercose pouvaient avoir des réactions croisées avec le test immunologique actuellement disponible de *Toxocara canis* dans un modèle animal établi pour l'infection de la cysticercose chez le porc, hôte connu de *Toxocara*. Nous avons examiné par test ELISA et par un test sur immunoblot pour toxocarose et cysticercose le sang de 10 porcs a la base et a la fin du suivi, avant et après (trois mois) d'infection avec *Taenia solium*. A la suite d'une infection efficace de cysticercose, les 9 porcs évaluables sont devenus séropositifs pour *T. solium* (test enzymatique par immunoelectrotransfert) mais sont restés séronégatifs pour *Toxocara* pour tous les tests. Cela révèle donc le manque de réaction croisée avec les anticorps anti-*T. solium* dans les deux tests pour *T. canis*. Ces observations devraient aider les cliniciens a mieux interpréter la sérologie de toxocarose et cysticercose dans les régions endémiques pour les deux helminthoses.

mots clés toxocarose, cysticercose, diagnostic immunologique, épilepsie, porc

Evaluación del inmunodiagnóstico de Toxocariasis en Cisticercosis Porcina Experimental

Hemos evaluado si las pruebas inmunodiagnósticas para cisticercosis pueden tener reacciones cruzadas con las pruebas inmunodiagnósticas que actualmente hay en el mercado para *Toxocara canis* en un modelo animal establecido para infección por cisticercosis en cerdos, los cuales se sabe son hospederos de *Toxocara*. Se ha realizado la prueba para toxocarosis y cisticercosis, mediante TES-ELISA e immunoblot, a los sueros de 10 cerdos, al inicio del estudio y después del seguimiento, antes y 3 meses después de infectarles con *Taenia solium*. Tras una infección exitosa, los 9 cerdos evaluables seroconvirtieron para *T. solium* (ensayo de inmunoelectrotransferencia ligado a enzima) pero se mantuvieron seronegativos para *Toxocara* con ambas pruebas, demostrando la falta de reactividad cruzada entre los anticuerpos anti-*T. solium* y ambas pruebas para *T. canis*. Estos hallazgos deberían ayudar a los clínicos en una mejor interpretación de la serología para toxocarosis y cisticercosis en áreas endémicas para ambas infecciones helminticas.

palabras clave toxocarosis, cisticercosis, inmunodiagnóstico, epilepsia, cerdo