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RESEARCH NOTE / NOTA CIENTÍFICA

SEROLOGICAL PROFILE OF CYSTIC ECHINOCOCCOSIS IN SERA FROM INDIVIDUALS IN SOUTHERN BRAZIL BY IMMUNOBLOT

PERFIL SEROLÓGICO DE EQUINOCOCOSIS QUÍSTICA EN LOS SUEROS DE INDIVIDUOS DEL SUR DE BRASIL POR *IMMUNOBLOT*

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Abstract

The cystic echinococcosis, caused by *Echinococcus granulosus*, is endemic in southern Brazil, especially in the state of Rio Grande do Sul (RS). Recent advances in imaging technology and in immunological tests provide powerful and non-invasive tools for its diagnosis based on the identification of cysts and detection of antibodies. We analyzed the serological profile of the genus *Echinococcus* by immunoblot in sera from patients from southern of Brazil who had been referred to the National reference service in hydatid disease between January 2010 and July 2013. Among 160 sera samples, 16 were reactive for at least one of the proteins (10, 18, 28, and 40 kDa), indicating that the pattern of reactivity can vary among the patients.

Keywords: cistic echinococcosis - *Echinococcus granulosus* - immunoblot - laboratorial diagnosis.

Resumen

La equinococosis quística es causada por *Echinococcus granulosus*, es endémica en el sur de Brasil, especialmente en Rio Grande do Sul (RS). Los recientes avances tecnológicos de imágenes y en pruebas inmunológicas proporcionan herramientas potentes y no invasivas para el diagnóstico con base en la identificación de los quistes y la detección de anticuerpos. En este trabajo se analizó el perfil serológico para el género *Echinococcus* por immunoblot en sueros de pacientes del sur de Brasil, que fueron enviados para el Servicio Nacional de Referencia en Hidatidosis entre enero de 2010 y julio de 2013. De las 160 muestras de suero, 16 fueron reactivas durante al menos una de las proteínas (10, 18, 28, y 40 kDa), lo que indica que el patrón de reactividad puede variar entre los pacientes.

Palabras claves: equinococosis quística - *Echinococcus granulosus* - immunoblot - diagnosis de laboratorio.

INTRODUCTION

The genus *Echinococcus*, Rudolphi 1801, comprises tapeworms belonging to the Taeniidae family. Based on light microscopy studies, four species that affect human are regarded as valid taxa within this genus (Eckert & Deplazes, 2004). All species present heteroxenous life-cycle, in which carnivores have a role as the definitive host by possessing adult worms in the small intestine, while herbivores are the intermediate hosts, in which the larval stage (metacestode) develops, mainly in the liver. *Echinococcus* transmission is principally maintained in a carnivore-herbivore-carnivore cycle in rural areas (Joshi *et al*., 1997).

Echinococcosis or hydatid disease is a zoonosis considered by the World Health Organization (WHO) as an emerging or re-emerging disease of increasing concern (Moro & Schantz, 2009). In South America, the disease is endemic in the southern cone of the continent, including the south of Brazil, and in the Andean region (Gavidia *et al*., 2008), where sheep farming is the main economic activity (De La Rue, 2008).

Because humans and carnivores share the same environment, people can become infected with *Echinococcus* eggs eliminated with carnivore (Lamberti *et al*., 2014). Humans are aberrant hosts and infection can result in life-threatening illness (Eckert & Deplazes, 2004). Herbivores become infected by a similar mechanism. The disease caused in humans and herbivores is named echinococcosis. Animal infection causes economic losses through the unsuitability of infected livers for marketing (Valiyeva *et al*., 2013; Cardona & Carmena, 2013).

The larval stage, commonly called aqueous vesicle or blister, consists of few or numerous spherical to sub-spherical isolated or contiguous vesicles macroscopically characterized as clear fluid-filled spheres. Histological examination reveals that these cysts contain a thicker outer laminated non-cellular layer (adventitious layer) composed of inflammatory cells and collagen deposited by host cells (Lewall & McCorkell, 1986). This layer is composed of carbohydrates and exhibits physiological and immunological activities, delivering antigens to the host and acting as a barrier against the immunological response (Gottstein & Hemphill, 1997). In general, it is treated with anthelmintics and, in many cases, surgery becomes necessary to remove the cyst (Eckert *et al*., 2000).

Humans produce a significant immune response during chronic cystic echinococcosis infection (Siracusano *et al.*, 2012). Serological tests use whole-crude fluid isolated from metacestodes from *Echinococcus granulosus* infection (Moro & Schantz, 2009). The serological profile in individuals living in an endemic area of Uruguay analyzed by the enzyme linked immunosorbent assay (ELISA) has been reported (Hernández *et al*., 2005). However, the situation in other endemic regions of South America is poorly understood. In Brazil there are few data on the occurrence of the disease. Early detection and use of an accurate method is fundamental (Pawlowski *et al*., 2001).

Echinococcus species share common antigens that can be used to diagnose all types of echinococcosis infections (Moro & Schantz, 2009). In this study, we applied an immunoblot technique using the total crude antigen extracted from *E. granulosus* hydatid fluid obtained from sheep to detect infection by *E. granulosus* in human serum samples from southern Brazil received by the National reference service in hydatid disease between January 2010 and July 2013.

MATERIAL AND METHODS

Serum samples collected during January 2010 and July 2013 from patients with suspected cystic echinococcosis by Central Public Health Laboratory were submitted to National Reference Service in Hydatid Disease (Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Brazil). This work was approved by the IPEC-Fiocruz institutional review board (nº 0002.1.000.009-04).

A total of 160 human serum samples were tested

for the presence of echinococcosis IgG antibodies using an immunoblot assay. In brief, four reactive bands were considered markers of a positive reaction (Pereira, 2014). These bands corresponded to molecular weights of 10, 18, 28, and 40 kDa and were present in sera from patients with surgically confirmed disease.

Of the total of 160 samples, 153 were from patients living in the state of Rio Grande do Sul (RS) and 7 from Santa Catarina (SC). Sera were considered reactive when they had IgG antibodies recognizing at least one of the four antigenic proteins used as reference.

RESULTS

Of the 160 samples, all were processed and 16 were considered positive by presenting an immune response to at least one of the proteins of interest (10, 18, 28, and 40 kDa). Of these 16 patients, eight were female and eight were male and the age range was wide, from 2 to 74 years. All the reactive patients were from municipalities in Rio Grande do Sul (Fig. 1).

Among reactive samples, nine were positive for all four proteins, one sample showed reactivity to three proteins (10, 18 and 40kDa) and six showed reactivity to only two proteins, five of which reacted with proteins 28 and 40 kDa, and only one reacting with proteins 10 and 18 kDa (Fig.2).

DISCUSSION

Cystic echinococcosis is a zoonosis of increasing concern worldwide. In the human host, accidental ingestion of eggs is followed by long-term growth of metacestode, resulting in chronic cyst-forming disease. Although the initial phase of the primary infection is always asymptomatic (Eckert & Deplazes, 2004), a significant immune response is produced (Siracusano *et al.*, 2012). During this phase, clinical signs are not pathognomonic and vary with the rate of parasite growth.

Like other tissue tapeworms, laboratory diagnosis based on direct parasite visualization is not available. Therefore, imaging techniques such as computed tomography (CT), ultrasound examination (US) and magnetic resonance imaging (MRI) play a role in diagnosing and staging the larval parasite (Stojkovic *et al.*, 2012). Serological tests are useful both for

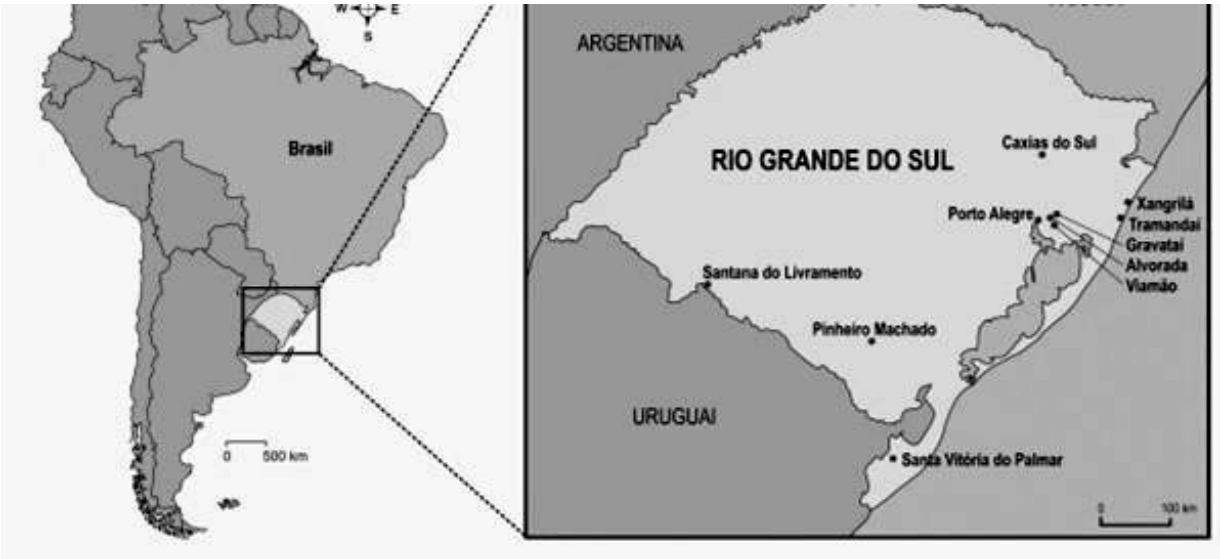

Figure 1. Origin of samples reactive in the immunoblot test received from southern Brazil. Illustration by Heloisa Diniz, Serviço de produção e tratamento de imagem, IOC, FIOCRUZ.

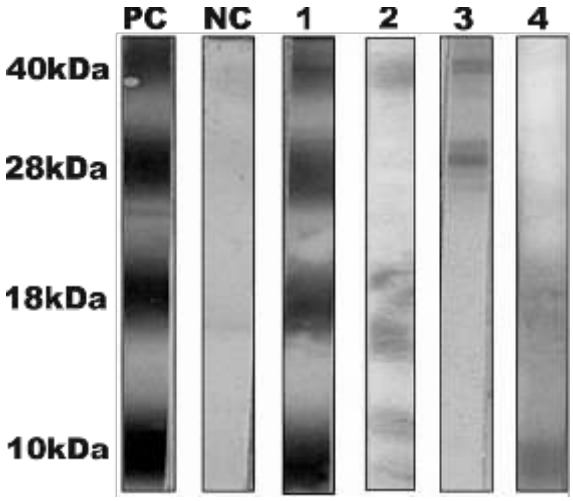

Figure 2. Reactive samples in immunoblot. PC – Positive control, NC – Negative control, 1- Reactive sample for 4 bands, 2 - Reactive sample for 3 bands, 3/4 - Reactive sample for 2 bands.

confirmation of suspected cases and epidemiological investigation (Zhang *et al.*, 2012).

Here, we utilized an immunoblot assay using crude antigens from bovine hydatid cyst fluid for the diagnosis of cystic echinococcosis. This approach deserves comments.

First, this tool was successfully used in Peru (Moro *et al.*, 2005) and Uruguay (Barbieri *et al.*, 1998).

Second, although our aim was not to answer epidemiological questions, the results confirm that human infection is not limited to Santana do Livramento (Larrieu & Zanini, 2012) or the border with Argentina (Monteiro *et al.*, 2014).

Third, it is known that species of *Echinococcus* share antigens. Thus, in those areas where more than one species is present, the cross-reactivity between them could be a problem for the correct diagnosis using immunological tests (Al-Yaman & Knobloch, 1989). However, the geographical distribution of *E. granulosus* and *E. vogeli* (species with occurrence in Brazil) are quite different (D'Alessandro, 1997). The latter occurs in the northeast region and causes polycystic echinococcosis, while the former is distributed in the southeast and cause cystic echinococcosis. Fourth, some researchers have made efforts to purify and identify specific antigens from *Echinococcus* sp., as well as to construct recombinant antigens, for use in ELISA and immunoblot assays (Liance *et al.*, 2000; Virginio *et al.*, 2003) to diagnose echinococcosis infections. However, these tests are not yet routinely used and have not been tested for all species of the genus.

Fifth, in other helminth infections heterologous antigens have been useful for immunodiagnosis. Due to the similarity shared between *Taenia crassiceps* (Zeder, 1800) and *Taenia solium* (Linnaeus, 1758) molecules, obtaining antigen extracts from *T. crassiceps* has been a good alternative source of antigens for immunodiagnosis of neurocysticercosis (Peralta *et al.*, 2010). Obtaining pigs naturally infected with *T. solium* larvae is often difficult, but the larval form of the species *T. crassiceps* reproduces asexually by intraperitoneal passage through Balb / c mice (Gomes *et al.*, 2007; Peralta *et al.*, 2002, 2010).

Another successful use of heterologous antigens occurs in the diagnosis of strongyloidiasis (Machado *et al.*, 2001). The most important limitation of immunodiagnosis is the difficulty

of obtaining infective larvae of *Strongyloides stercoralis* (Bavay, 1876), for use in the preparation of antigens. For this reason, studies have been performed using antigens of infective larvae of *Strongyloides ratti* (Sandground, 1925) and *S. venezuelensis* (Brumpt, 1934) to develop serological methods (Rodrigues *et al.*, 2004).

This study confirms that crude antigens of *E. granulosus* can be a useful source of antigens for immunological reactions to detect specific antibodies in patients with hydatid disease, as well as to show that the pattern of the proteins can be different from patient to patient.

It is important to remember that although being an important approach to help diagnosis of echinococcosis, the reactivity observed in immunoblot analysis using crude antigens of *E. granulosus* cannot serve as a definitive diagnosis, since it does not differentiate simple contact with the parasite (Liance *et al*., 2000), as well as past infections from current infections.

However, evaluation of all the tools that have the potential to improve identification of this zoonotic disease is important. Because of cross-reactivity, epidemiological information, working practices of the patient, as well as complementary image examination are important to determine a specific diagnosis (Li *et al*., 2010). The characterization of antigenic proteins that are reactive against reportedly positive *Echinococcus* sera is needed to improve the quality of diagnosis. Also, the use of recombinant proteins is promising. The possibility of this approach has been proven by previous studies (Virginio *et al*., 2003; Li *et al*., 2004), but further research is necessary to evaluate their sensitivity and specificity within the valid taxa of *Echinococcus*.

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