Journal of Saudi Chemical Society (2012) **16**, 203–207

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Diagnostic value of different antigenic fractions of hydatid cyst fluid from camel and sheep in Kingdom of Saudi Arabia

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Received 20 December 2010; accepted 2 January 2011
Available online 5 January 2011

KEYWORDS

Serodiagnosis;
Enzyme-linked immuno-electrotransfer blot (EITB);
Hydatidosis;
Echinococcus granulosus;
KSA

Abstract Hydatid cyst fluids (HCF) crude extracts from camels and sheep slaughtered in Riyadh region, KSA were subjected to Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE) and Western blot analysis. Sera from 17 confirmed human cases of hydatidosis, 25 patients with other parasitic infections and 10 clinically healthy subjects were used to evaluate the diagnostic value of the different antigenic fractions of these extracts. Immunoblotting results revealed that, at least 11 major discrete protein fractions (110–8 kDa) were recognized by sera from hydatidosis patients, sera from patients with other parasitic diseases showed cross-reactivity with few of these bands. The cluster of bands (38–35 kDa) that may be a breakdown of "Arc 5" antigen (39–38 kDa) was detected by 100% and 94% of sera from hydatidosis cases with HCF extracts from camel and sheep, respectively. This cluster showed also some cross reactivity (20% and 8%) with control sera from patients with other parasitic infections with camel and sheep HCF extracts, respectively. Polypeptides at 24–22, 16 and 8 kDa which may probably correspond to antigen B subunits were also identified by all samples from hydatidosis patients with sheep HCF extracts and by 100%, 65% and 74% with camel HCF extracts respectively. Sera from control subjects did not react with any of

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Peer review under responsibility of King Saud University.
doi:10.1016/j.jscs.2011.01.001

Production and hosting by Elsevier

these polypeptides (24–22, 16 and 8 kDa). According to our results, the identified molecular weight bands (16 and 8 kDa using HCF crude extracts from sheep and 24–22 kDa using HCF crude extracts either from camel or sheep) represent good candidates for immunodiagnosis of hydatidosis.

1. Introduction

Hydatidosis is a disease caused by infection with the metacestode stage of the dog tapeworm, *Echinococcus granulosus*. The disease represents a significant public health problem in several North Africa and Middle East countries (Battelli et al., 2002; Kamhawi, 1995; Sadjjadi, 2006; Shambesh et al., 1997). Several studies indicated that hydatid disease is an endemic zoonosis in the Kingdom of Saudi Arabia (KSA) affecting both humans and their domestic animals (Abu-Eshy, 1998; Adewunmi and Basilingappa, 2004; Al Mofleh et al., 2000; Fahim and Al Salamah, 2007; Ibrahim, 2010; Rashed et al., 2004).

There is usually no direct parasitological evidence for the presence of cysts in organs or tissues and in most cases the early stages of infection are asymptomatic. Over the last decade diagnosis of hydatid disease was improved due to the use of imaging techniques including ultrasonography, computed tomography (CT scanning) and magnetic resonance imaging (MRI) supported by immunological assays for confirmation of clinical diagnosis (World Health Organization, 2003; Zhang et al., 2003).

Hydatid cysts of *E. granulosus* develop in internal organs (mainly the liver and lungs) of humans and intermediate hosts (herbivores including: sheep, horses, cattle, pigs, goats and camels) as unilocular fluid-filled bladders (Zhang et al., 2003). Most studies have focused on hydatid cyst fluid (HCF) antigens as an invaluable source of antigenic material for immunodiagnosis (Burgu et al., 2000; Kanwar et al., 1994; Musiani et al., 1978; Oriol et al., 1971; Piantelli et al., 1977; Pozzuoli et al., 1974). Detection of antibodies to the well characterized lipoproteins antigen B (AgB) and antigen 5 "Arc 5" (Lightowlers et al., 1989; Oriol and Oriol, 1975; Verastegui et al., 1992), the major antigenic components of HCF, have received most of the attention for such diagnostic approach. Although AgB and "Arc 5" have proved to be diagnostically valuable, there are difficulties related to their lack of sensitivity and specificity and problems with the standardization of their use (Babba et al., 1994). Nevertheless, several studies evaluated the immunogenicity of crude or semi-purified HCF antigens derived from sheep (Rogan et al., 1991) or camel hosts (Ramzy et al., 1999; Shambesh et al., 1995) with sera from patients with hydatidosis which resulted in the development of promising diagnostic systems. However, there are considerable differences among the various tests in both specificity and sensitivity. Enzyme-linked immunoelectrotransfer blot (EITB) was reported to be the most sensitive serological assay for confirmation of hydatidosis (Verastegui et al., 1992). It also showed high specificity due to the high resolution of HCF antigenic components (Kharebov et al., 1997).

It is generally accepted that parasites accommodated in different hosts or collected from a variety of geographical localities would be of distinguished strains and even considered as different species (Thompson and McManus, 2002), likely to produce diverse antigenic constituents. This may explain why different laboratories using antigens prepared from different host species obtain different anti-hydatid antibody relativities.

The present study was designed to evaluate and compare the diagnostic value of different antigenic fractions of HCF crude extracts from camel and sheep origin using EITB technique.

2. Materials and methods

2.1. Antigens preparation

Fertile hydatid cysts were obtained from six sheep and three camels within 24 h of slaughter in the main abattoir in Riyadh region, Kingdom of Saudi Arabia. A single lot of crude antigen extract was prepared from each animal source according to Ramzy et al. (1999) with slight modifications. Briefly, hydatid cyst fluid (HCF) was aspirated aseptically and 25× protease inhibitor cocktail (Roche, Basel, Switzerland) in 100 mM phosphate buffer, pH 7.0 was immediately added. HCF was centrifuged at 3000*g* for 30 min at 4 °C and the supernatant was collected and dialyzed, using cellulose membrane with molecular weight cut-off of 3.5 kDa, against three changes of deionized water per 24 h over three successive days. The dialyzate was centrifuged again at 3000*g* for 30 min at 4 °C and the supernatant collected. Protein content was estimated by the Bio-Rad Bradford protein assay kit (Bio-Rad AG, Glattbrugg, Switzerland) using bovine plasma gamma globulin as a standard. The two prepared antigen extracts were divided into aliquots and stored at −70 °C until used.

2.2. Serum samples

Serum samples for the present study were collected from 17 surgically confirmed human cases with hydatidosis (group A).

Control sera were obtained from 25 patients with parasitic infections other than *E. granulosus* (group B), and from 10 clinically healthy subjects with no history of living in endemic areas and free from parasitic infections (group C). Group B, consisted of 4 cases with amoebic liver abscesses, 3 cases with ascariasis, 3 cases with schistosoiasis, 5 cases with fascioliasis, 5 cases with cysticercosis and 5 cases of ancylostomiasis.

2.3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Enzyme-linked immunoelectrotransfer blot (EITB)

HCF extracted antigens from sheep and camel were analyzed basically as described by Laemmli (1970). Briefly, 100 mg from each source were separated by SDS–PAGE under denaturing conditions using 5–20% gradient gels. Fractionated proteins were electrophoretically transferred to nitrocellulose membranes as described by Verastegui et al. (1992). After blotting, nitrocellulose sheets were cut into 0.3 cm strips, blocked in 0.01 M phosphate buffered saline (pH 7.4) with 0.05% Tween