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

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. Poly-peptides 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...
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; Sadjadi, 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 25x 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 dialy- yzate 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, Glattb- rugg, 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 schistosoriasis, 5 cases with fascioliasis, 5 cases with cysticercosis and 5 cases of encystostomiasis.

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...
20 (PBS/T) and 5% non fat dry milk for 30 min, and incubated with human sera (1:250 in PBS/T with 5% fetal calf serum [FCS]) for 2 h at 37 °C. After washing the strips were incubated in alkaline phosphatase conjugated antimouse IgG antibodies (Promega, Madison, WI, USA) in PBS/T with 5% FCS for 2 h at 37 °C. After washing the nitrocellulose strips were developed with the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT, Sigma Chemical Co., St. Louis, MO, USA) in 100 mM Tris–HCl with 100 mM NaCl and 5 mM MgCl₂ for 15 min at 37 °C.

Molecular weight estimates were made by comparing the mobility of the different antigen fractions with those of Pre-stained Protein Marker, Broad Range (7–175 kDa), (New England Biolabs, USA) which were separated in the same gel of a hydatid fluid sample and electrotransferred to the same sheet.

3. Results

Sera of the 17 confirmed hydatidosis cases (group A) recognized at least 11 major bands in HCF antigens originated from camel and sheep cysts (Fig. 1) with molecular weights of (110, 97, 80, 65–60, 55–50, 45–40, 38–35, 30, 24–22, 16.8 kDa). Some sera from patients with other parasitic diseases (group B) showed cross-reactivity with few numbers of these bands. Sera from clinically healthy individuals (group C) did not show any reactivity to these polypeptides. Fig. 2, represents the differences in the reactivity to these antigenic fractions from camel and sheep HCF extracts with the studied sera groups.

The cluster of bands at 45–40 kDa was detected by 94% and 77% of hydatidosis cases with HCF extracts from camel and sheep respectively. Though, this cluster of bands (45–40 kDa) was not detected by any of the controls with HCF camel extracts, it was detected by 16% of control sera from group B with HCF sheep extracts (2 cases of ancylostomiasis and 2 cases of fascioliasis).

While, the cluster of band at 38–35 kDa was detected by all hydatidosis sera samples with HCF camel extracts and by 94% with HCF sheep extracts. This cluster showed also some cross reactivity (20% and 8%) with control sera from group B with camel and sheep HCF extracts respectively (3 cases of cysticercosis and 2 cases of amoebic liver abscesses with HCF camel extracts and 2 cases of cysticercosis with HCF sheep extracts).

The cluster of bands at 24–22 kDa was found to be common (100%) in all hydatidosis cases using camel and sheep

![Image](im.png)
Another group (Kanwar et al., 1992); they reported that sera and sheep. Our data are in agreement with those obtained by genic fractions (110–8 kDa) in HCF preparations from camel individuals).

Other parasitic infections and clinically healthy sera samples (patients with other parasitic diseases and from normal controls (group C) did not react with any of these polypeptides. Though, the relatively low molecular weight of bands at 16 and 8 kDa were detected by 100% of sera from hydatidosis cases with sheep HCF extracts; they were detected by 65% and 74% of CE cases with camel HCF extracts, respectively. These bands (16 and 8 kDa) showed 100% specificity using both antigen extracts.

4. Discussion

The present work is one of the first studies in Saudi Arabia to analyze protein extracts of HCF collected from infected camels and sheep using SDS–PAGE and EITB techniques to identify their antigenic fractions and compare the diagnostic values of these fractions. Sera samples from surgically confirmed hydatidosis cases were used for identifying antigenic determinants in HCF which are unshared with control sera samples (patients with other parasitic infections and clinically healthy individuals).

Immunoblotting results identified 11 major discrete antigenic fractions (110–8 kDa) in HCF preparations from camel and sheep. Our data are in agreement with those obtained by another group (Kanwar et al., 1992); they reported that sera from surgically confirmed cases of hydatidosis reacted with 12 polypeptides with molecular weights of 8–116 kDa by Western blotting using hydatid antigens from sheep.

In the present study, sera of the studied groups showed some variations in recognizing the 11 detected protein fractions (110–8 kDa) between HCF preparations from camel and sheep. Though, the cluster of bands at 45–40 kDa was one of the most frequently recognized bands by CE cases (94%), this in addition to absolute specificity with HCF camel extracts. It was detected by only 77% sensitivity and 84% specificity with HCF sheep extracts.

It is interesting to note that, the cluster of bands (38–35 kDa) that may be a breakdown of “Arc 5” antigen (39–38 kDa) (Shepherd and McManus, 1987) was detected in the present study by 100% and 94% of sera from CE cases with HCF extracts from camel and sheep, respectively. Detection of antigen “Arc 5” is one of the most widely used immunodiagnostic procedures for hydatid disease (Zhang et al., 2003).

On the other hand, a relatively low specificity (80% and 92%) was recognized towards these polypeptides with camel and sheep HCF extracts respectively. This coincides with many studies that reported cross-reactivity of antigen “Arc 5” (39–38 kDa subunits) with sera from patients with other parasites (Lightowers and Gottstein, 1995; Liu et al., 1993; Ortona et al., 2000; Poretti et al., 1999; Rott et al., 2000) as a major problem. The cross reactivity of this antigen may be attributed to its phosphorylcholine epitope, first described by Shepherd and McManus (1987) and later confirmed by Lightowers et al. (1989).

Furthermore, the immunogenic polypeptides at 24–22, 16 and 8 kDa which may probably correspond to antigen B subunits were also seen in the current study by all sera samples from CE patients with sheep HCF extracts and by 100%, 65% and 74% with camel HCF extracts respectively. These polypeptides (24–22, 16 and 8 kDa) showed by 100% specificity, as sera from 25 patients with other parasitic diseases and from 10 clinically healthy individuals did not react with any of these polypeptides with antigen extracts from both sources. Antigen B is highly immunogenic and was described in previous reports under reduced condition on SDS–PAGE, with three bands with molecular sizes of approximately 8 or 12, 16, and 24 kDa (Leggatt et al., 1992; Lightowers et al., 1989; Oriol et al., 1971). Other researchers reported also that, the smallest subunit of antigen B (8 kDa) is highly sensitive and is more specific than the others (Ali-Khan, 1974; Maddison et al., 1989).

Hence, generally, the smallest subunit has proved the most useful target in diagnostic studies (Ortona et al., 2000; Rott et al., 2000). Some of the antigenic bands detected in the present study by EITB (16 and 8 kDa using HCF crude extracts from sheep and 24–22 kDa using HCF crude extracts either from camel or sheep) might be used as a confirmatory test in suspected cases of human infection with hydatid disease.

In conclusion, this study has provided interesting data on the use of EITB as a powerful tool in the context of serodiagnosis of human hydatidosis.

References


