DIAGNOSIS OF CANINE ECHINOCOCCOSIS:
COMPARISON OF COPROANTIGEN DETECTION WITH NECROPSY
IN STRAY DOGS AND RED FOXES FROM NORTHERN JORDAN

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Summary:
The sandwich enzyme linked immunosorbent assay (ELISA) was used as a diagnostic test for Echinococcus granulosus infection by detecting coproantigens in 94 stray dogs Canis familiaris and eight red foxes (Vulpes vulpes) from northern Jordan. The results were analyzed in relation to actual helminth infection as revealed by necropsy. The infection rate of dogs with E. granulosus was 13.8 % with a worm load ranging between 3 - > 10,000 per infected dog. In contrast, eight of 13 E. granulosus infected dogs were coproantigen positive (overall sensitivity 61.5 %). The sensitivity increased to 87.5 % and 100 % in dogs harboring > 20 and > 100 worms/dog, respectively. The specificity of coproantigen-ELISA was 91 %. The greatest cross-reactivity was found in dogs infected with Dipylidium caninum. The positive and negative predictive values for the coproantigen-ELISA test were 50 % and 94.2 %, respectively. Thus, a coproantigen negative dog is most probably truly negative for E. granulosus. In contrast, a coproantigen positive dog may not be truly positive for E. granulosus, except if it has a high worm burden of > 100 worms/animal.

KEY WORDS: Echinococcus granulosus, canine echinococcosis, coproantigens, Jordan.

INTRODUCTION

Unilocular hydatidosis or cystic echinococcosis (CE) is a cosmopolitan cyclozoonotic disease caused by the taeniid cestode Echinococcus granulosus and is one of the major parasitic diseases of public health importance (Mattosian et al., 1977; Schantz & Kramer, 1995; Schantz et al., 1995). The disease is endemic or highly endemic in Middle Eastern countries including Lebanon, Syria, Palestinian Authority, Israel, and Jordan (See review by Abdel-Hafez & Kamhawi, 1997). The stability of CE in Jordan is multi-factorial as pertaining to improper slaughtering and human practices as well as to the abundance of stray dogs (Abdel-Hafez et al., 1986; Abdel-Hafez & Kamhawi, 1997).

The epidemiology of CE requires the consideration of three host components: the ungulate herbivore intermediate hosts, humans as accidental hosts and dogs as definitive hosts. Determination of prevalence and incidence in these hosts should precede any planning of a control program. Direct identification of hydatid cysts in various organs of slaughtered intermediate hosts can determine the level of endemicity of the disease in a given area (Gemmell, 1997). Both serological and imaging techniques are used to diagnose human CE infections and to determine the prevalence and incidence in endemic countries (Schantz & Kramer, 1995). Direct and indirect methods can determine the prevalence of canine echinococcosis. Direct methods rely on the examination of purgative samples or contents of small intestine following necropsy as well as of fecal specimens to identify whole worms, proglottids and/or eggs (Eckert et al., 1984; Allan et al., 1984; Amin et al., 1991; Amin et al., 1997).
1992; Craig, 1993). These methods not only are time-consuming, but also suffer from low sensitivity and specificity (Craig, 1997). Eggs are not released during the pre-patent period and their release is irregular during patency (Nonaka et al., 1996). Morphological similarities among eggs of all the taeniid species that may infect dogs simultaneously limit the specificity of direct methods. In addition, these tests are quite hazardous to both animals and examiners. Indirect methods are based on the identification of copro-DNA, anti-adult worms antibodies in the serum and feces as well as coproantigens detection. Serodiagnosis is accompanied with false negative results at the commencement of infection and false positive ones due to cross reactivity with other helminth infections (Jenkins & Rickard, 1985 and 1986; Gasser et al., 1988 and 1993).

The sandwich enzyme linked immunosorbent assay (ELISA) has recently been applied for diagnosis of *E. granulosus* and *E. multilocularis* in canines through the detection of coproantigens (Allan et al., 1992; Deplazes et al., 1992 and 1994; Craig et al., 1995; Nonaka et al., 1996; Malgor et al., 1997; Ahmad & Nizami, 1998). The coproantigen test has the advantage of early detection of the infection during prepatency, in 4-10 days post-infection (Deplazes et al., 1992). Moreover, positive results indicate current infection because coproantigens are derived from adult worms and would disappear as soon as the parasites are eliminated.

In this study, the *E. granulosus* coproantigen test was used to assess the sensitivity, specificity and the positive and negative predictive values of this test under field conditions in stray dogs and red foxes from northern Jordan.

### MATERIALS AND METHODS

Ninety four stray dogs (*Canis familiaris* Linnaeus, 1758) and eight red foxes (*Vulpes vulpes* Linnaeus (1758)) from Irbid and Mafraq Governorates, northern Jordan were shot in the field between June, 1994-July, 1995.

The necropsy of the animals was carried out in the field as described by El-Shihabi et al. (1999). Briefly, an abdominal cut was made in each animal and the intestine was tied from the pyloric and anal ends and collected in a bag. Bags were stored in an icebox and carried to the laboratory within three hours. The carcasses were burned in the field to ensure no contamination of the environment. In the laboratory, each intestine was divided into four pieces of equal length. Each piece was cut longitudinally and soaked in 0.15 M phosphate buffer saline (PBS, pH 7.2) for five minutes. The mucosal lining was gently scraped with a spatula into clean glass dishes and the collected intestinal contents were allowed to settle in 1,000 ml conical Nalgene graduates (Nalge Company, Rochester, USA). Following several washes with PBS, aliquots were examined under a dissecting microscope. The intestinal helminth parasites were identified as described earlier by El-Shihabi et al. (1999).

#### *E. GRANULOSUS ADULT WORM CRUDE SOMATIC ANTIGEN EXTRACT*

*E. granulosus* adult worm crude somatic antigen (EgACSA) extract was prepared as described earlier (Allan et al., 1992). Briefly, about 500-600 adult *E. granulosus* worms were isolated from the small intestines of infected animals. They were washed three times with 0.15 M Streptomycin containing phosphate buffer saline (PBS, pH 7.2) for 30 minutes. Thereafter, they were frozen at –20°C, thawed twice and homogenized using 2 ml capacity glass homogenizer in ice bucket for five minutes. Finally, the homogenate was centrifuged at 1,500 g for 20 minutes at 4°C and the protein content of the supernatant was determined by Bradford method (Bradford, 1976).

#### COPROANTIGEN PREPARATION

Fresh fecal materials were collected from each dog and fox and mixed in 1:1 w/v ratio with 0.15 M PBS (pH 7.2) containing 0.3 % Tween 20. The mixture was shaken vigorously using a Vortex shaker and then centrifuged at 2,000 g for 30 minutes at 4°C. The supernatants were frozen at –20°C and stored until further use.

#### COPROANTIGEN-ELISA

Hyperimmune rabbit anti EgACSA antiserum was prepared as described previously (Allan & Craig, 1989). The IgG fraction was purified using protein A as a ligand in affinity chromatography of protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Conjugation of the IgG fraction with horseradish peroxidase type VI enzyme (Sigma, USA) and the IgG capture ELISA were carried out as described previously (Allan and Craig, 1989) with modification. Briefly, wells of microtiter plates (Greiner F ELISA plates, Frickenhausen, Germany) were coated overnight with 100 μl of rabbit anti EgACSA IgG at a dilution of 1:800 in 0.05 M carbonate buffer, pH 9.6 at 4°C using rocking plate shaker (Denley, England). The wells were washed three times with 0.1 % Tween 20 in 0.15 M PBS, pH 7.2 and blocked with 4 % bovine serum albumin (fraction V, PARK, UK) or 12 % skimmed milk (Regilait instant dried skimmed milk, France) in 0.15 M PBS, pH 7.2 for one hour at room temperature (RT). Following washing the wells three times, the fecal supernatants were mixed individually with heat inactivated
Fig. 1. Detection of *E. granulosus* coproantigens in dogs and foxes by IgG capture ELISA. The cut-off point value (—) was determined by calculating the mean optical density (O.D.) value at λ 490 nm for 21 fecal samples of helminth free dogs + 3 S.D. * Number in parenthesis indicates the worm burden of *E. granulosus* in infected dogs as determined by necropsy. ** Number in parenthesis denotes number of animals examined in each group.

To assess the efficacy of the coproantigen ELISA, the sensitivity, specificity, positive predictive and negative predictive values of the test were determined using necropsy data as a golden standard (Schantz, 1997).
RESULTS

NECROPSY DATA

Table I shows that 13 of 94 (13.8 %) stray dogs were infected with E. granulosus either as single or concurrent infections with other helminthic species. Single infection with E. granulosus accounted for 5.3 % of the dogs (38.5 % of infected dogs). None of eight foxes was found infected with E. granulosus. Worm burden with E. granulosus ranged from 3->10,000 worms per dog, with 46.2 % of infected dogs having a worm burden of < 100 worms/animal (Table II). All eight foxes and 77.7 % of the dogs were infected with at least one intestinal helminth species (Table III). Moreover, single or concurrent cestode infections accounted for 71/73 (97.3 %) of the infected dogs and six out of the eight foxes. The most predominant helminth species encountered in stray dogs was Dipylidium caninum alone or in combination with other Dipylidids and/or other cestodes. The infection rate with this species was 51.1 % (48 out of total 94 necropsied dogs) and 65.8 % of the total number of dogs infected with helminths. All foxes were found infected with one or more species of cestodes, nematodes and/or acanthocephalans but never with either E. granulosus or Taenia species (Table III). Five of the foxes were found infected with Dipylidids, particularly Diplopylidium and Joyeuxiella species. Three foxes were infected concurrently with Macracanthorhynchus acanthocephalan and other cestodes and/or nematodes.

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Necropsy</th>
<th>Copro-ELISA</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helminth free</td>
<td>21</td>
<td>22.3</td>
<td>0</td>
</tr>
<tr>
<td>E. granulosus alone</td>
<td>5</td>
<td>5.3</td>
<td>2</td>
</tr>
<tr>
<td>E. granulosus &amp; other ces.</td>
<td>6</td>
<td>6.4</td>
<td>5</td>
</tr>
<tr>
<td>E. granulosus, other ces. &amp; nem.</td>
<td>2</td>
<td>2.1</td>
<td>1</td>
</tr>
<tr>
<td>Other ces.</td>
<td>53</td>
<td>56.4</td>
<td>7</td>
</tr>
<tr>
<td>Cestodes, nem. &amp; /or acanth.</td>
<td>5</td>
<td>5.3</td>
<td>1</td>
</tr>
<tr>
<td>Other nem. &amp; /or acanth.</td>
<td>2</td>
<td>2.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Total infection | 94 | 100.0 | 16 | 17.0 | 8 | 100.0 |

1 Abbreviations: ces., cestodes; nem., nematodes; acanth., acanthocephalans.
2 Faecal specimens of all foxes were copro-ELISA negative.

Table II. - Worm burden of E. granulosus in infected dogs from northern Jordan.

<table>
<thead>
<tr>
<th>Worm burden of E. granulosus</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>5</td>
<td>38.5</td>
</tr>
<tr>
<td>20-&lt; 100</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>100-&lt; 500</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>500-&lt; 1,000</td>
<td>4</td>
<td>30.7</td>
</tr>
<tr>
<td>1,000-&lt; 5,000</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>&gt; 10,000</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table III. - Intestinal helminth fauna and infection rates in dogs and foxes from northern Jordan. Necropsy data were used to assess the diagnosis of E. granulosus coproantigens by capture ELISA.

SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES OF COPROANTIGEN-ELISA

Table III compares the coproantigen-ELISA results with necropsy data of dogs and foxes. While none of the fox fecal samples were coproantigen positive, 16 of 94 (17.0 %) of the dog fecal samples were coproantigen positive. Eight of 13 E. granulosus positive dogs as revealed by necropsy were also coproantigen positive. In contrast, out of 89 canines (dogs and foxes) which were negative for E. granulosus infections by necropsy, eight were positive in the coproantigen assay. In this way, the sensitivity of coproantigen-ELISA test for 94 dogs and eight foxes was 61.5 %, while the specificity of this test was 91.0 % (Table IV). The positive predictive value of this test was 50 % while the negative predictive value was as high as 94.2 %. All of the five false-coproantigen negative samples were for dogs harboring an E. granulosus worm burden of < 100 worms (Fig. 1). Moreover, seven out of eight dogs harboring > 20 E. granulosus worms were coproantigen positive. This increases the sensitivity values of the test for dogs with E. granulosus burden of > 20 and > 100 worms.
Fig. 2. – Detection of *E. granulosus* coproantigens by IgG capture ELISA in 81 fecal samples from 73 stray dogs and eight red foxes, all of which were infected with at least one species of intestinal *helminths*. The cut-off point value (---) was calculated as in Figure 1.
Table IV. - Sensitivity, specificity, positive and negative predictive values of coproantigen-ELISA test for E. granulosus detection in stray dogs from northern Jordan.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coproantigen-ELISA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity1</td>
<td>8/13</td>
<td>61.5</td>
</tr>
<tr>
<td>Specificity2</td>
<td>81/89</td>
<td>91.0</td>
</tr>
<tr>
<td>Positive predictive value3</td>
<td>8/16</td>
<td>50.0</td>
</tr>
<tr>
<td>Negative predictive value4</td>
<td>81/86</td>
<td>91.2</td>
</tr>
</tbody>
</table>

1Sensitivity of coproantigen-ELISA was calculated as % E. granulosus copro-positive animals/all positives by necropsy.
2Specificity of coproantigen-ELISA was calculated as % E. granulosus copro-negative animals/all negatives by necropsy.
3Positive predictive value of coproantigen-ELISA was calculated as % E. granulosus positive animals by both necropsy and coproantigen-ELISA/all copro-positives by coproantigen-ELISA alone.
4Negative predictive value of coproantigen-ELISA was calculated as % E. granulosus negative animals by both necropsy and coproantigen-ELISA/all copro-negatives by coproantigen-ELISA alone.

DISCUSSION

In the present study, the IgG capture ELISA for the detection of coproantigens was used in conjunction with necropsy to determine the infection rate of E. granulosus and other intestinal helminths in stray dogs and red foxes from northern Jordan. The infection rate with E. granulosus as determined by necropsy was 13.8%. This conforms with our earlier report (El-Shehab et al., 1999), but was lower than what had been reported over 15 years ago in which 19.6% of stray dogs from Irbid Governorate were found infected (Ajlouni et al., 1984).

The sensitivity of coproantigens ELISA was found to be highly dependent on the E. granulosus worm burden. The overall sensitivity of the test was relatively low at 61.5% regardless of the worm load. However, the sensitivity reached as high as 87.5% and 100.0% for dogs harboring > 20 and > 100 worms/dog, respectively. It is this category of animals, which is most important epidemiologically as a source of livestock and human infection. Seven out of 13 (53.8%) E. granulosus infected dogs had a worm burden of > 100 worms/dog (Table II). Thus, the test is quite useful to monitor dog infection rates in surveillance and control program. It is simple, safe, cheap and less time-consuming than the other standard techniques that determine canine echinococcosis by necropsy or purgation with arecoline hydrobromide.

The positive predictive value of the test, as calculated here, appears to be quite low (Table IV) and denotes a reflection of the detectability level of the worm load. Lower E. granulosus infections (i.e. < 100 worms/animal) yielded mostly negative coproantigen-ELISA results. The positive predictive value increased significantly with higher worm burdens. The detectable worm load by coproantigens-ELISA has influenced the positive predictive value of the test as only eight of total 16 coproantigen positive dogs were actually E. granulosus infected animals. Seven out of these eight animals had a worm burden > 100 worms/dog. Thus, one can not rely on coproantigen-ELISA alone to diagnose low worm burden of E. granulosus in dogs. In contrast, the negative predictive value was high (Table IV). Thus, a negative result confirms that a dog is either truly non-infected or harbors a very low worm burden infection, both conditions are epidemiologically not significant.

The specificity of this test was high (91.0%) and is comparable to what has been reported earlier (Allan et al., 1992; Deplazes et al., 1992; Craig et al., 1995). In the present investigation, most of the cross reactivity was noted in dogs infected with D. caninum alone or with other Dipylidid species (i.e. Joyeuxiella and/or Dipylidium species). One of the other two samples was infected with Taenia sp. and the other with both D. caninum and Taenia spp. (Fig. 2).

In this way, out of 48 dogs which were D. caninum positive as revealed by necropsy, 41 (85.4%) were coproantigen negative.
experimental infection studies with the two-helminth species. It should be pointed out that only 14.6 % of 48 *D. caninum* infected dogs were coproantigen positive. One can not rule out that such dogs might have had recent *E. granulosus* infection that had been cleared out. Alternatively, a very small worm load of *E. granulosus* might have been missed during necropsy of some of these dogs and thus resulted in coproantigen positivity.

The ultimate conclusion of this study is that coproantigen-ELISA is a suitable test that can be used in epidemiological surveys. Its sensitivity is very high in dogs harboring > 20 worms/animal and is absolute in those with > 100 worm load. Currently, this test has been adopted as a routine technique to investigate canine echinococcosis prevalence in various parts of the country.

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