

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.

Résumé : DIAGNOSTIC DE L'ÉCHINOCOCCOSE CANINE : COMPARAISON ENTRE LA DÉTECTION DE COPROANTIGÈNES ET LA NÉCROPSIE CHEZ DES CHIENS ERRANTS ET DES RENARDS ROUGES DU NORD DE LA JORDANIE

La méthode ELISA de type sandwich a été utilisée pour le diagnostic de l'infection à *Echinococcus granulosus* par la détection de coproantigènes chez 94 chiens errants et huit renards rouges (*Vulpes vulpes*) provenant du nord de la Jordanie. Les résultats ont été comparés avec ceux obtenus par nécropsie. Chez les chiens, le taux d'infection par *E. granulosus* était de 13,8 % avec une charge parasitaire de trois à plus de 10000 vers par animal. En revanche, sur les 13 chiens infectés, huit étaient coproantigène positifs (sensibilité de 61,5 %). La sensibilité croît à 87,5 % et 100 % chez les chiens porteurs respectivement de plus de 20 et de plus de 100 vers. La spécificité du test ELISA-coproantigène était de 91 %. La réaction croisée la plus importante a été observée chez les chiens porteurs de *Dipylidium caninum*. Les valeurs prédictives positives et négatives pour le test ELISA-coproantigène étaient respectivement de 50 et 94,2 %. Ainsi, un chien coproantigène négatif est très probablement non infecté par *E. granulosus*. A contrario, un chien coproantigène positif n'est peut-être pas réellement infecté par *E. granulosus*, sauf si sa charge parasitaire est élevée, supérieure à 100 vers.

MOTS CLÉS : *Echinococcus granulosus*, échinococcose canine, coproantigène, Jordanie.

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.*,

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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 by 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

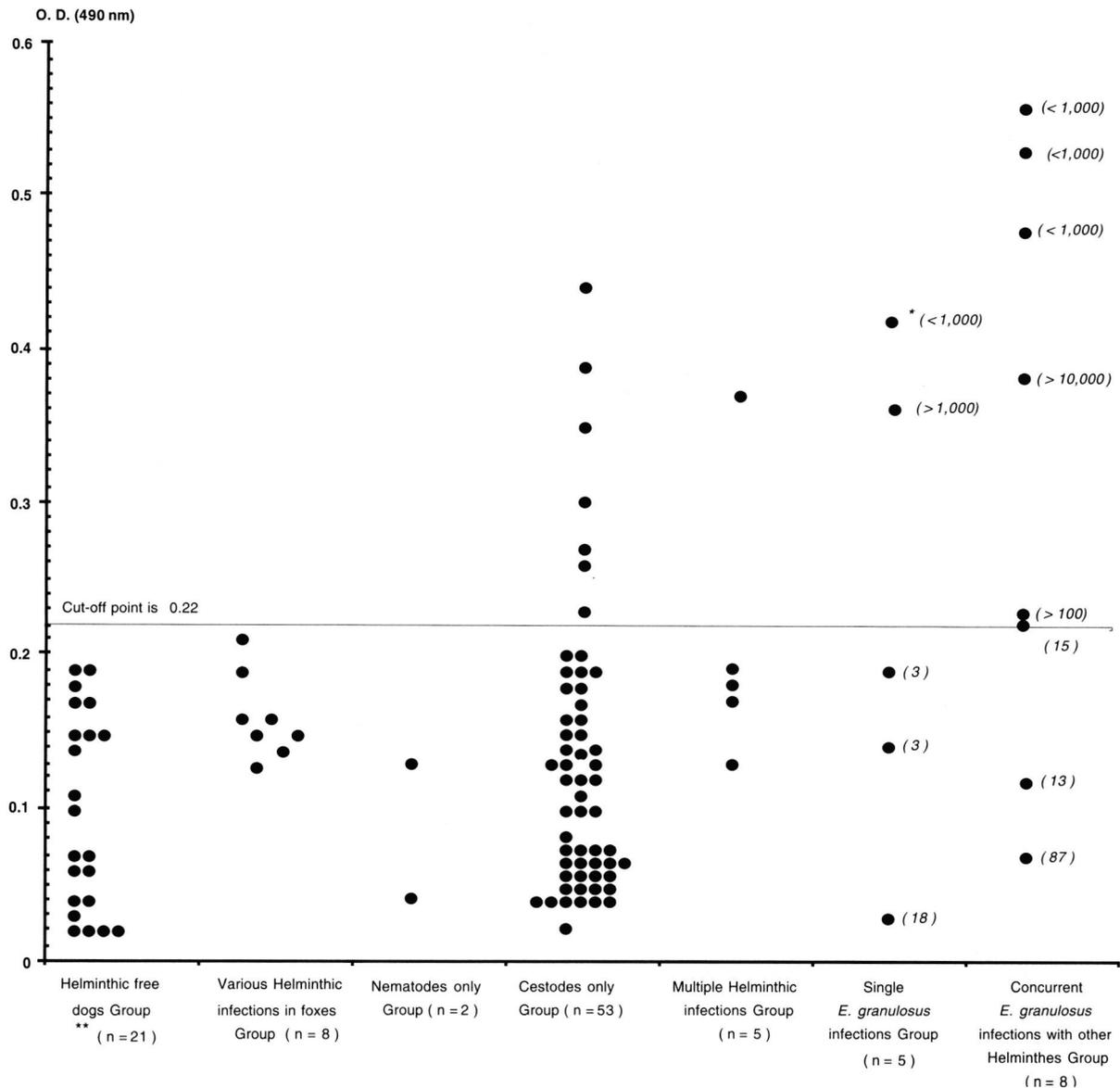


Fig. 1. – Detection of *E. granulosus* coproantigens in dogs and foxes by IgG capture ELISA. The cut-off point value (—) was determined by calculating the mean optical density (O.D.) value at λ 490 nm for 21 fecal samples of helminth free dogs + 3 S.D.

* 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.

fetal calf serum (FCS) at 1:1 v/v ratio (Sigma, USA), and 100 μ l of each sample was added per well and incubated for one hour at RT. After three washings, 100 μ l of horseradish peroxidase (HRP) conjugated with anti EgACSA IgG at a dilution of 1:200 was added and incubated for one hour. After three washings, 100 μ l of substrate solution consists of H_2O_2 in 0.1 M citrate buffer, pH 4.5 containing ortho-phenyldiamine (OPD) chromogen (Sigma, USA) was incubated for 30 minutes at RT in dark. Finally, the optical density of each well was read at a wavelength of 490 nm using Micro-ELISA auto-reader (Dynatech, Virginia, USA). Positive control wells contained 5 μ g/ml EgACSA ins-

tead of fecal sample. Negative control wells contained pooled fecal materials from helminth free dogs. External marginal wells of each micro-titer plate were excluded. The cut-off point was calculated by measuring the mean optical density (OD) at 490 nm of helminth free dog fecal samples (no = 21) + 3 SD. All fecal samples were tested in triplicate.

EVALUATION OF COPROANTIGEN-ELISA TEST

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

Infection mode	No. of infected dogs	
	No.	%
Single <i>E. granulosus</i> infection	5	5.3
Concurrent infection with:		
<i>Taenia</i> spp. ^(a)	2	2.1
Dipylidids ^(b)	1	1.1
<i>Taenia</i> spp. ^(a) and Dipylidids ^(b)	2	2.1
Dipylidids ^(b) and Nematodes ^(c)	1	1.1
<i>Taenia</i> spp. ^(a) , Dipylidids ^(b) and Nematodes ^(c)	1	1.1
<i>Taenia</i> spp. ^(a) , Dipylidids ^(b) and <i>Mesocostoides</i> sp.	1	1.1
Total infection	13	13.8

^(a) *Taenia* spp. included *T. pistiformis*, *T. taeniaeformis* and other unidentified *Taenia* species.

^(b) Dipylidids included *Dipylidium caninum*, *Diplopylidium* sp. and *Joyeuxiella* sp.

^(c) Nematodes included *Toxocara* sp. or *Toxascaris* sp.

Table I. – Rates of dog infection with *E. granulosus* either alone (single infection) or concurrent infections with other helminths in 94 dogs from northern Jordan.

Worm burden of <i>E. granulosus</i>	No. and percentage of infected dogs	
	No.	%
< 20	5	38.5
20-< 100	1	7.7
100-< 500	1	7.7
500-< 1,000	4	30.7
1,000-< 5,000	1	7.7
> 10,000	1	7.7
Total	13	100.0

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

Type of infection ¹	Dogs				Foxes ²	
	Necropsy		Copro-ELISA		Necropsy	
	No.	%	No.	%	No.	%
Helminth free	21	22.3	0	0.0	0	0.0
<i>E. granulosus</i> alone	5	5.3	2	2.1	0	0.0
<i>E. granulosus</i> & other ces.	6	6.4	5	5.3	0	0.0
<i>E. granulosus</i> , other ces. & nem.	2	2.1	1	1.1	0	0.0
Other ces.	53	56.4	7	7.4	3	37.5
Cestodes, nem. & /or acantho.	5	5.3	1	1.1	3	37.5
Other nem. & /or acantho.	2	2.1	0	0.0	2	25.0
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 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.

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.

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

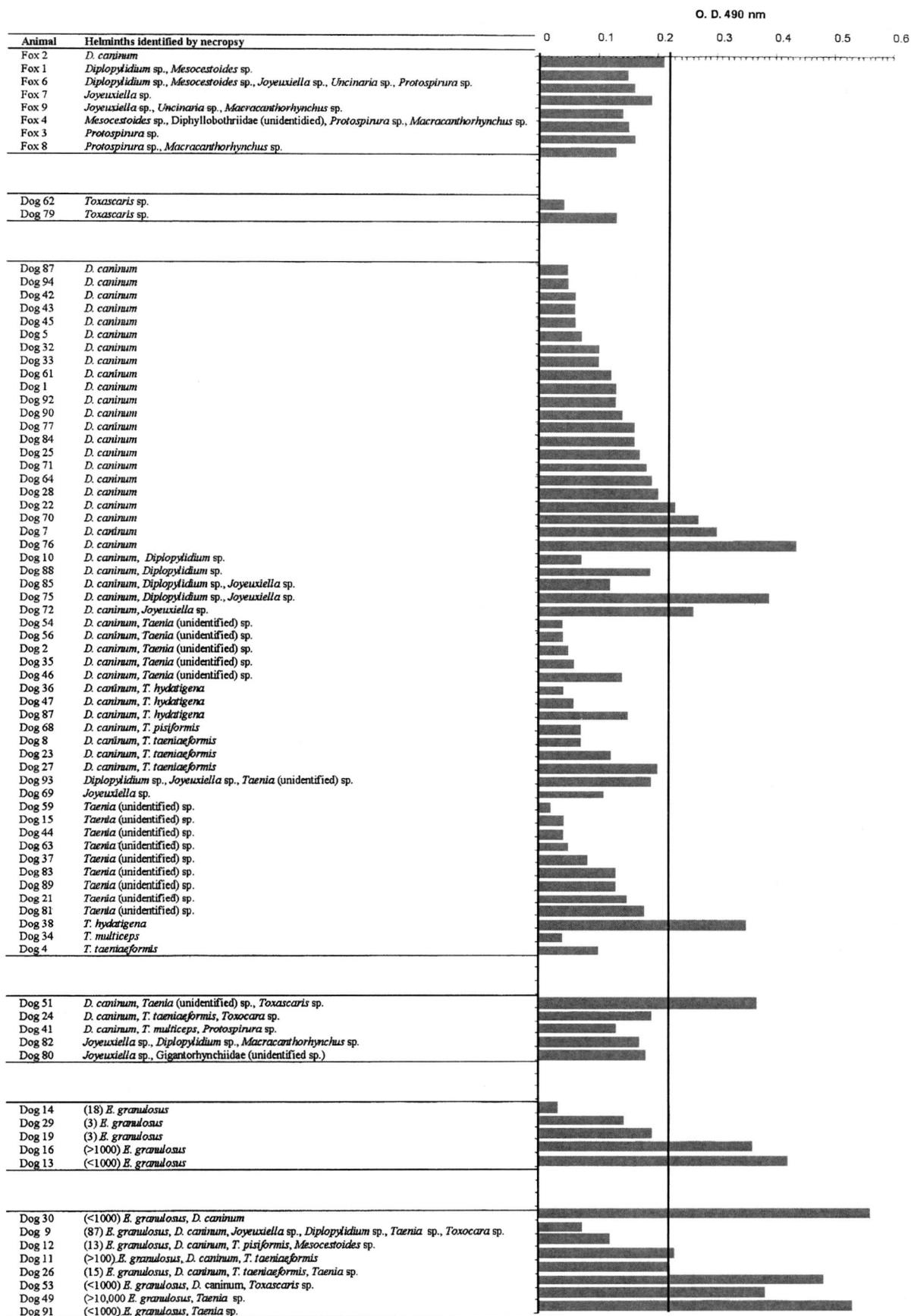


Fig. 2. – Detection of *E. granulosus* coproantigens by IgG capture ELISA in 81 fecal samples from 73 stray dogs and eight red foxes, all of which were infected with at least one species of intestinal *helminths*. The cut-off point value (—) was calculated as in Figure 1.

Parameter	Coproantigen-ELISA	%
Sensitivity ¹	8/13	61.5
Specificity ²	81/89	91.0
Positive predictive value ³	8/16	50.0
Negative predictive value ⁴	81/86	94.2

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.

Table IV. – Sensitivity, specificity, positive and negative predictive values of coproantigen-ELISA test for *E. granulosus* detection in 94 stray dogs and eight red foxes from northern Jordan.

to 87.5 % and 100.0 %, respectively. Figure 2 depicts the ELISA OD readings for coproantigens in the fecal samples of all dogs and foxes infected with intestinal helminths as compared with the necropsy data. Evidently, higher OD values were mostly seen in dogs infected with high *E. granulosus* burdens whether singly or concurrently with other helminth parasites. Out of the eight dog fecal samples that were false coproantigen positive, six were for dogs infected with *D. caninum* alone or with other Dipylidid species (i.e. *Joyeuxiella* and/or *Diplopylidium* 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.

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-Shehabi *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 (Ajjlouni *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 worms. This contrasts earlier observations in which most false coproantigens-ELISA positive samples were seen in *Taenia hydatigena* infections (Allan *et al.*, 1992; Deplazes *et al.*, 1992; Molgar *et al.*, 1997) Here, one of the eight false coproantigens positive samples was for a dog infected with *T. hydatigena* alone. Indeed, *T. hydatigena* infection was lower than *D. caninum* in the present series. This reflects the strayhood of the dogs in contrast to home owned dogs that are routinely dosed for fleas and other ectoparasites. Thus, the consideration of coproantigens as being genus-specific (Craig *et al.*, 1995) must be re-examined. Apparently, some somatic antigens may be shared between Dipylidid and Taeniid worms including *E. granulosus*. The use of excretory/secretory (E/S) antigens instead of adult somatic crude antigens to hyperimmunize rabbit for the preparation of reagents to be used in coproantigens-ELISA may result in more genus-specific antibodies and diminish the cross-reactivity between different genera of cestodes (Deplazes *et al.*, 1992). However, the true cross-reactivity between *D. caninum* and *E. granulosus* can only be proven by

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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REFERENCES

- ABDEL-HAFEZ S.K., AL-YAMAN F.M. & SAID I.M. Further studies on prevalence of hydatidosis in slaughtered animals from North Jordan. *Zeitschrift für Parasitenkunde*, 1986, 72, 89-96.
- ABDEL-HAFEZ S.K. & KAMHAWI S.A. Cystic echinococcosis in Levant countries (Jordan, Palestinian Autonomy, Israel, Syria and Lebanon), *in*: Compendium on Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Reference to Morocco. Andersen F.L., Ouhelli H. & Kachani M. (eds), Brigham Young University, Provo, Utah, 1997, 292-316.
- AHMAD G. & NIZAMI W.A. Coproantigens: early detection and suitability of an immunodiagnostic method for echinococcosis in dogs. *Veterinary Parasitology*, 1998, 77, 237-244.
- AJLOUNI A.Q., SALIBA E.K. & DISI A.M. Intestinal cestodes of stray dogs in Jordan. *Zeitschrift für Parasitenkunde*, 1984, 70, 203-210.
- ALLAN J.C. & CRAIG P.S. Coproantigens in gut tapeworm infection: *Hymenolepis diminuta* in rats. *Parasitology Research*, 1989, 76, 68-73.
- ALLAN J.C., CRAIG P.S., GARCIA NOVAL J., MENCOS F., LIU D., WANG Y., WEN H., ZHOU P., STINGER R., ROGAN M. & ZEYHLE E. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology*, 1992, 104, 347-356.
- BRADFORD M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the protein-dye binding. *Analytical Biochemistry*, 1976, 72, 248-254.
- CRAIG P.S. Immunodiagnosis of *Echinococcus granulosus*, *in*: Compendium on cystic Echinococcosis with special reference to the Xinjiang Uygur autonomous region, the People's Republic of China. Andersen F.L. (ed), Brigham Young University, Provo, Utah, 1993, 85-118.
- CRAIG P.S. Immunodiagnosis of *Echinococcus granulosus* and a comparison of techniques for diagnosis of canine echinococcosis, *in*: Compendium on cystic Echinococcosis in Africa and in Middle Eastern countries with special reference to Morocco. Andersen F.L., Ouhelli H. & Kachani M. (eds), Brigham Young University, Provo, Utah, 1997, 85-118.
- CRAIG P.S., GASSER R.B., PARADA L., CABRERA P., PARIETTI S., BORGUES C., ACUTTIS A., AGULLA J., SNOWDEN K. & PAOLILLO E. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. *Veterinary Parasitology*, 1995, 56, 293-301.
- DEPLAZES P., GOTTSTEIN B., ECKERT J., JENKINS D.J., EWALD D. & JIMENEZ-PALACOS S. Detection of *Echinococcus* coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. *Parasitology Research*, 1992, 78, 303-308.
- DEPLAZES P., JIMENEZ-PALACIOS S., GOTTSTEIN B., SKAGGS J. & ECKERT J. Detection of *Echinococcus* coproantigens in stray dogs of northern Spain. *Applied Parasitology*, 1994, 35, 297-301.
- ECKERT J., GEMMELL M.A., SOULSBY E.J.L. & MATYAS Z. Guide-line for surveillance, prevention and control of Echinococcosis/Hydatidosis. World Health Organization, 1984, 147 p.
- EL-SHEHABI F.S., ABDEL-HAFEZ S.K. & KAMHAWI S.A. Prevalence of intestinal helminths of dogs and foxes from Jordan. *Parasitology Research*, 1999, 85, 928-934.
- GASSER R.B., LIGHTOWLERS M.W., OBENDORF D.L., JENKINS D.J. & RICKARD M.D. Evaluation of a serological test system for the diagnosis of natural *Echinococcus granulosus* infection in dogs using *E. granulosus* protoscolex and oncospherical antigens. *Australian Veterinary Journal*, 1988, 65, 369-373.
- GASSER R.B., JENKINS D.J., PAOLILLO E., PARADA L., CABRERA P. & CRAIG P.S. Serum antibodies in canine echinococcosis. *International Journal for Parasitology*, 1993, 23, 579-586.
- GEMMELL M.A. Quantifying the transmission dynamics of the family Taeniidae with particular reference to *Echinococcus* spp.: an update, *in*: Compendium on cystic Echinococcosis in Africa and in Middle Eastern countries with special reference to Morocco. Andersen F.L., Ouhelli H. & Kachani M. (eds), Brigham Young University, Provo, Utah, 1997, 54-71.
- JENKINS D.J. & RICKARD M.D. Specific antibody responses to *Taenia hydatigena*, *Taenia pisiformis* and *Echinococcus granulosus* infection in dogs. *Australian Veterinary Journal*, 1985, 62, 72-78.

- JENKINS D.J. & RICKARD M.D. Specific antibody responses in dogs experimentally infected with *Echinococcus granulosus*. *American Journal of Tropical Medicine and Hygiene*, 1986, 35, 345-349.
- MALGOR R., NONAKA N., BASMADJIAN I., SAKAI H., CARAMBULA B., OKU Y., CARMONA C. & KAMIYA M. Coproantigen detection in dogs experimentally and naturally infected with *Echinococcus granulosus* by a monoclonal antibody-based enzyme-linked immunosorbent assay. *International Journal for Parasitology*, 1997, 27, 1605-1612.
- MATOSSIAN R.M., RICKARD M.D. & SMYTH J.D. Hydatidosis: a global problem of increasing importance. *Bulletin of World Health Organization*, 1977, 55, 499-507.
- NONAKA N., IIDA M., YAGI K., ITO T., OOI H.K., OKU Y. & KAMIYA M. Time course of coproantigen excretion in *Echinococcus multilocularis* infections in foxes and alternative definitive host, golden hamsters. *International Journal for Parasitology*, 1996, 26, 1271-1278.
- SCHANTZ P.M., CHAI J., CRAIG P.S., ECKERT J., JENKINS D.J., MACPHERSON C.N.L. & THAKUR A. Epidemiology and control of hydatid disease, *in: Echinococcus and hydatid disease*. Thompson R.C.A. & Lymbery A.J. (eds), CAB International, Wallingford, 1995, 233-331.
- SCHANTZ P.M. & KRAMER M.H.J. Larval cestode infections: cysticercosis and echinococcosis. *Current Opinions in Infectious Diseases*, 1995, 8, 342-350.
- Schantz P.M. Sources and uses of surveillance data for cystic echinococcosis, *in: Compendium on cystic Echinococcosis in Africa and in Middle Eastern countries with special reference to Morocco*. Andersen F.L., Ouhelli H. & Kachani M. (eds), Brigham Young University, Provo, Utah, 1997, 72-84.

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