

Direct immunodiagnosis of *Echinococcus granulosus* in feces of stray, companion and policy dogs in Baghdad province - Iraq

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Abstract

Echinococcus granulosus is a cosmopolitan parasite affected mainly the dogs and can afflicted most animals resulting in an echinococcosis that persist endemically or high endemically in Middle Eastern countries involved Iraq. The main goal of this study was to use the direct ELISA as a diagnostic tool in detection of the causative agent in only lived dogs of some regions in Baghdad province-Iraq. The fecal samples were collected from a totally (70) dogs (comprised; 38 stray, 22 companion and 10 policy dogs) during seven months. The direct ELISA techniques revealed that the infection rates were (18.42%) and (4.55%) in stray and companion dogs respectively, with absence of infection in policy dogs. Also, the degree of infection in seropositive dogs was (62.5%), (12.5%) and (25%) for mild, moderate and strong infections respectively. Statistically the significant differences had been reported between the examined dogs and within the positive dogs at ($P<0.05$).

Key words: *Echinococcus granulosus*, direct ELISA, dogs (stray, companion, policy), fecal samples.

التشخيص المناعي المباشر للمشوكات الحبيبية في براز الكلاب السائبة و كلاب الرفقة والكلاب البوليسية في محافظة بغداد - العراق

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الخلاصة

المشوكات الحبيبية هي طفيلي عالمي الانتشار يصيب بشكل اساسي الكلاب ويمكن ان تتأثر به معظم الحيوانات مسببا مرض المشوكات الذي يتواجد في معظم بلدان الشرق الاوسط ومن ضمنها العراق بشكل مستوطن او عالي الاستيطان. الهدف الرئيسي من هذه الدراسة هو استخدام اختبار التلازن المناعي المباشر (ELISA) كأداة تشخيصية في تحديد العامل المسبب في الكلاب الحية فقط في بعض مناطق محافظة بغداد - العراق. جمعت عينات البراز بالإجمال من (70) كلباً تضمنت (38 كلب سائب و 22 كلب رفقة و 10 كلاب بوليسية) خلال سبعة اشهر. كشفت تقنية الاليزا المباشر ان معدل الاصابة كان (18.42%) و (4.55%) في الكلاب السائبة و كلاب الرفقة على التوالي ، مع عدم وجود اصابة في الكلاب البوليسية. كذلك كانت درجة الاصابة في الكلاب الموجبة مصليا (62.5%) و (12.5%) و (25%) للإصابات الخفيفة والمتوسطة والقوية على التوالي. احصائيا سجلت الاختلافات المهمة بين الكلاب الخاضعة للدراسة وبين الكلاب الموجبة للإصابة عند مستوى ($P<0.05$).

الكلمات المفتاحية: الاليزا العدرية ، كلاب (سائبة ورفقة وبوليسية) ، عينات براز .

Introduction

Echinococcus granulosus is a parasitic pathogen belongs to metacestode of Taeniidae family, which divided to different genotypes, indicated by numbers (G1-G10) and by the intermediate host, although each genotype may infect a multiple intermediate hosts (1, 2). However, *E. granulosus* required

two mammalian hosts (definitive and intermediate) to complete its life cycle (3). The global prevalence of *Echinococcus* in different parts of world resulted in a major public health problems in animals and humans, not only in endemic areas but also in regions that with low or without infection

due to the potential transmission of infected livestock to create new endemic area (4,5). In dogs the infestation with parasite doesn't cause infection, while in intermediate hosts, the hydatid cysts can be serious and the problem is that they developed very slowly over years without signs; and mostly discovered too late time (6). If the vital organs (such as lung, liver, kidney, brain, heart) are affected, the hurt in many cases cannot be repaired and fatal (7). As well as, if the cysts are ruptured, the strong anaphylactic reactions or death may occur (8). The accurate diagnosis of echinococcosis had an important component in establishment of the epidemiological parameters and for preventing the livestock and human from infection (9). Recently, and due to the desperately need to use of the efficient diagnostic methods in order to reflect a real level of infection and to establish accurately the infection with *E. granulosus* in dog; many immunodiagnostic methods were developed to fulfill a better sensitivity and specificity, and without threatening the animal's life (10). ELISA test has been designed for detection of the quantitative level of specific antibodies against *E. granulosus* in serum and plasma (indirect ELISA), and for determination of the parasitic antigens in feces (coproELISA) with variable sensitivity and specificity (11). However the detection of specific antigens in fecal samples has an advantage over serum antibody detection in high probability of correlation with current infection (12). Also, coproantigen ELISA appears to be valuable in detecting of infection in the definitive host with high specificity and sensitivity (13). Prathiush *et al.*, (2008) defined the parasite coproantigens as "a specific parasitic product that associated with parasite metabolism and appear in host's feces" (14). Also, these products are flexible to immunological detection and present independently on the reproductive material of parasite (like eggs or proglottids) and disappear shortly from the feces after removal of the intestinal infection (15). The goals of this study were to: 1. Detection the prevalence of *E. granulosus* in (stray, companion and polices) dogs in some regions of Baghdad province, by using the

coproELISA technique for first time in Iraq. 2. Resolving the degree of infection in positive dogs by an establishment the levels of antigen-antibody reaction according to the result's interpretation of ELISA kit titration.

Materials and methods

1. Study area, period and samples collection

At different districts of Baghdad province, a total of 70 adult dogs (38 strays, 22 companions and 10 polices) of both sexes, were submitted to samples collection during the period (from July 2015 to January 2016). From each one about 5 grams of feces were collected rectally, and only 1 gram was kept in 35 ml tubes that contained a 0.3% phosphate buffered saline (PBS) with 10% formalin and stored at (-80°C) for a minimum of five days in order to kill off any *Echinococcus* spp. infective eggs. Then the samples were defrosted, homogenized with wooden spatulas, shaken, and centrifuged at 2500 rpm for five minutes. The supernatants were filled up into a bijoux sample tubes and stored at -20°C until they used for analysis (16).

2. CoproELISA

The direct coproELISA kit that produced by Shenzhen Lvshiyuan Biotechnology Co., Ltd / China, was used to detect an *Echinococcus granulosus* antigen in feces of infected dogs. The current manufacturer's recommendations for interpretation of control and sample results test were detailed in (Table 1). Also, depending on the antigens-antibodies titration, the severity of infection was divided into three levels included the mild, moderate and strong infection.

Table (1): Interpretation of results

Controls and sample	ODR	Interpretation
OD Positive control	= 1.00	
OD Negative control	= 0.10	
CUTOFF Value	Negative control + 0.15	
Samples	< CUTOFF Value	Negative sample
	= CUTOFF Value	Positive sample

3. Statistical analysis

All data was labeled and analyzed by a computerized Microsoft office excel (2013) and IBM SPSS programs (V.23). Chi-square

and t-test were used to detection the significant differences between the positive infected stray, companion and police dogs, as

well as between the levels of infection in positive dogs. Statistically, the significant differences were at level ($P < 0.05$) (17).

Results

Out of a total 70 dogs, 8 (11.43%) were the overall seropositive rate of *E. granulosus* infection, which included 7/38 (18.42%), 1/22 (4.55%) and 0/10(0%) in stray, companion and polices dogs respectively

(Table 2). According to test kit titration, the seropositive infections had been divided into three levels, mild, moderate and strong infection, and the results were 5 (62.5%), 1 (12.5%) and 2 (25%) respectively (Table 3).

Table (2): Seropositive results by coproELISA Test

Tested Dogs	Total No.	Seropositive		Seronegative	
		No.	%	No.	%
Stray	38	7	18.42 ^a	31	81.58
Companion	22	1	4.55 ^b	21	95.45
Policy	10	0	0 ^c	10	100
Total	70	8	11.43	62	88.57

Differences in small letters vertically, referred to a significant difference at level $P < 0.05$

Table (3): Degree of infection in seropositive dogs

Degree of infection	Total (8)	
	No.	%
Mild	5	62.5 ^a
Moderate	1	12.5 ^c
Strong	2	25 ^b

Differences in small letters vertically, referred to significant differences at level $P < 0.05$

Discussion

Currently, Echinococcosis was tagged as a disease of unrecognized increasing importance and the application of coproantigen and/or serodiagnostic techniques in epidemiological studies might provide more accurate information about the prevalence of this infection because the technique could detect asymptomatic cyst carriers (18, 19). In Iraq, several studies were concerned with the detection of *E. granulosus* in stray dogs, only, in northern, middle and southern parts of it, reporting different rates of infection that ranged from (20%) to (100%) in tested regions, by using a necropsy method (20, 21, 22). The present study demonstrated that the parasite was concentrated in stray dogs more than others and several factors can play an important role in the high increasing of echinococcosis prevalence such as the poor sanitation, the incorrect elimination of slaughter domestic's residues, the employ of potentially contaminated water, absence of control schemes by losing of stray dogs and apply of vaccination that supply a proper protection, physical effects such as stress, and lack of chemotherapy application (23, 24, 25). Therefore, the scarcity or absence of the disease in companion and policy dogs respectively, may be referred to the superior

attention in related to a clean environment, low exposure to pollutants, sanitary nutrition, artificial insemination, and owner's care (26, 27, 28). Although, the most dependable technique in detection of *E. granulosus* in dogs is by necropsy, due to the parasite burdens can be accurately collected and the parasites can be estimated; but this method have several disadvantages like the biohazard safety measures that should be taken in place during the process, ethical justification as well as the samples that obtained at necropsy are potentially biased because this material is not generally accessible to dogs (23, 29, 9). Therefore, the using of antibody and coproantigen ELISAs in diagnosis of canine Echinococcosis has been recommended and shown a great improvement in diagnosis over the traditional methods (arecoline purge and necropsy) (30). CoproELISAs that developed and validated primarily to test an infection with *E. granulosus*, are usually genus-specific for *Echinococcus* spp., and depending on the endemic region and the aims of a study (16). Also, most studies reported a feasible sensitivity ranged (78-100) % and good pattern of specificity ranged (85-95)% as well as a high degree of pre-visible detection even the dogs were infected with

other intestinal parasites (3, 31, 32). The diminishing in sensitivity and specificity was in general corresponding with low severity of infections that may appear a false negative result (33).

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