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Research Article

Seroprevalence of Cattle Hydatidosis in Some Districts in the East Anatolian Region of Turkey

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Abstract: Hydatidosis, caused by hydatid cysts, is a widespread and hazardous disease in humans and animals worldwide. The disease is very common in Turkey, causing serious economic losses. In this study, the seroprevalence of hydatidosis was determined by enzyme-linked immunosorbent assay (ELISA) and indirect fluorescence antibody technique (IFAT). A total of 597 serum samples were collected from cattle of various breeds and ages in 8 provinces in Eastern Turkey. Partially purified cyst fluid antigen from sheep hydatid cyst fluid was used as antigen in ELISA and whole protoscolex antigens were used in IFAT. The results showed that 63.3% and 54.9% of 597 cattle were positive with ELISA and IFAT, respectively. The highest seroprevalence was in Muş (85.3%) and the lowest was in Kars (46.6%) by ELISA. The seroprevalence was between 77.3% (Muş) and 35.1% (Erzurum) by IFAT.

Key Words: Hydatidosis, cattle, ELISA, IFAT, seroprevalence

Doğu Anadolu Bölgesindeki Bazı İllerde Sığırlarda Hidatidosisin Seroprevalansı

Özet: Hidatidosis, insan ve hayvan sağlığını yakından ilgilendiren önemli bir paraziter zoonozdur. Dünya'da birçok ülkede yaygın olarak görülen bu hastalık, Türkiye'de de gerek halk sağlığı, gerekse ekonomik açıdan önemli bir sorun oluşturmaktadır. Bu çalışmada, paraziter hastalıkların teşhisinde yaygın olarak kullanılan ELISA (Enzyme-linked Immunosorbent Assay) ve IFAT (Indirekt Floresan Antikor Tekniği) testleriyle hidatidosisin seroprevalansı araştırılmıştır. Bu amaçla, Doğu Anadolu Bölgesinde bulunan 8 farklı ilden toplam 597 sığır serumu çalışmaya dahil edilmiştir. ELISA testinde antijen olarak koyun hidatik kist sıvısından hazırlanan kısmi purifiye kist sıvısı antijeni, IFAT'ta ise bütün protoskoleks antijeni kullanılmıştır. Çalışma sonucunda; 597 serum örneğinin ELISA ve IFAT ile testi neticesinde sırasıyla % 63,3 ve % 54,7 oranlarında seropozitiflik tespit edilmiştir. ELISA ile en yüksek seroprevalans Muş ilinde belirlenmiş (% 85,3) olup, en düşük seroprevalans Kars (% 46,6) ilinde tespit edilmiştir. Bununla birlikte, IFAT ile seroprevalans % 77,3 (Muş) ile % 35,1 (Erzurum) arasında tespit edilmiştir.

Anahtar Sözcükler: Hidatidosis, sığır, ELISA, IFAT, seroprevalans

Introduction

Echinococcosis is a zoonotic disease that occurs throughout the world and causes economic losses and public health problems in many countries. Domestic intermediate hosts (cattle, sheep, goats) are major reservoirs for the disease in humans in Turkey (1). Infection of humans occurs during the natural transmission of the parasite between the canid definitive hosts and domestic livestock intermediate hosts. Together with other Echinococcus spp., it is recognised as one of the most important parasitic zoonoses (2). In addition, large hydatid cysts in the liver and lungs of sheep and cattle can result in significant economic loss to the meat industry through condemnation of the infected organs (3).

The infection data of hydatidosis in livestock are most commonly collected at slaughterhouses. Abattoirs which are strictly regulated can often provide acceptable prevalence data. However, in many areas where the disease is endemic, home slaughter is practised and few abattoirs have adequate veterinary supervision. In addition, postmortem diagnosis of the disease is of little use in areas of low prevalence, where segregation of infected animals is essential for disease control. The

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development of a sensitive, specific, and reproducible serological assay for livestock would provide useful epidemiological data for the antemortem study and control of hydatid disease (4).

Currently, indirect haemagglutination (IHA), indirect immunoelectrophoresis (IEP), counter immunoelectrophoresis (CIEP), and double diffusion (DD) are used in the serodiagnosis of hydatidosis, but they have some disadvantages such as cross-reactions with some Taeniid cestodes (5).

Enzyme-linked immunosorbent assay (ELISA) is an important serological test for the diagnosis of hydatidosis. Although there are several conflicting reports on the suitability of ELISA for the diagnosis of hydatidosis (6,7), some researchers showed that it may be successfully adapted for the serological diagnosis of hydatidosis (5,8). The sensitivity and accuracy of ELISA depend on the compositon, concentration and stability of the antigen used (8).

In the diagnosis of parasitic diseases, important improvements have been achieved by applying the indirect fluorescence antibody test (IFAT). Therefore, this technique has become an important diagnostic tool in parasitology laboratories. In the diagnosis of hydatidosis by IFAT, frozen parasite tissue sections, the whole scolex, and germinal membranes were used as antigen (9,10).

Hira et al. (11) used arc 5 antigen in serodiagnosis of human hydatidosis and reported 98.18% sensitivity and 98.28% specificity by ELISA. Şimsek and Köroğlu (12) determined the sensitivity and specificity of ELISA in sheep hydatidosis to be 60% and 94%, respectively. Şenlik (13) tested 300 sheep sera by IFAT and reported that a sensitivity of 78.95% and specificity of 92.57% were obtained by IFAT at 1/128 and higher dilutions. There are many studies about the prevalence of cattle hydatidosis in Eastern Anatolia, but many of them are based on abattoir inspections. The prevalence of hydatidosis was reported to be 19.5%-90% in Erzurum (14,15), 24.7%-50% in Kars (14,16), and 19.4%-37.8% in Van (17,18).

The aim of this work was to determine the seroprevalence of cattle hydatidosis in Eastern Anatolia with ELISA and IFAT. This study also served to correlate sex, breed, and age with ELISA and IFAT results in cattle.

Materials and Methods

Sample Collection

This study was carried out between May and October 2001 in towns in Elazığ, Malatya, Bingöl, Muş, Van, Erzurum, Erzincan, and Kars provinces and their surrounding villages, in Eastern Anatolia. A total of 597 blood samples were obtained from the jugular vein in sterile air-vacutained tubes in these locations. All the cattle were 1 year old or older and usually grazed in the pasture for at least one season. The cattle consisted of the Brown Swiss breed, the Holstein breed, a cross breed, and a local breed.

Antigen Preparation for ELISA

An *Echinococcus granulosus* antigen B (AgB) enriched fraction was prepared from hydatid cyst fluid (HCF) obtained from sheep infected with hydatid cysts. The procedure was performed as previously described by Oriol et al. (19). Briefly, HCF was aspirated under sterile conditions and examined microscopically for the presence of protoscoleces.

HCF was clarified by centrifugation at 2000 g for 15 min to remove the protoscoleces and any other solid materials and 100 ml of the clarified supernatant fluid was first dialysed overnight at 4 ºC against 0.005 M acetate buffer, pH 5.0. The cyst fluid was then centrifugated at 15,000 g for 30 min at 4 ºC. The pellet was collected and dissolved in 10 ml of 0.2 M phosphate buffer, pH 8.0, boiled in a water bath for 15 min and re-centrifugated at 20,000 g for 1 h at 4 ºC. The precipitate pellet was discarded, and the supernatant containing antigen B was assayed for protein concentration and reactivity, and stored in aliquots at -20 ºC until used. Protein concentration was determined by the method described by Lowry et al. (20).

Antigen Preparation for IFAT

HCF was collected from liver or lung(s) hydatid cysts of cattle that exhibited multiple cyst infection at post-mortem examination. HCF was aspirated under sterile conditions and examined microscopically for the presence of protoscoleces. HCF was clarified by centrifugation at 2000 g for 15 min to remove the protoscoleces. Protoscoleces were collected and washed 3 times with normal physiological saline. Then protoscoleces were diluted with 10% formalin (avarage 5000 protoscoleces in 1 ml). Whole scolex antigen was fixed on non-fluorescent microscope slides and stored at -20 ºC until used.

Control sera

Positive control serum obtained from a cow diagnosed with hydatidosis postmortem was positive in the IFA (1/512) and IgG-ELISA (1/2560) tests. Negative control serum was obtained from an uninfected calf which was negative in the IFA (1/4) and IgG-ELISA (1/20) tests and postmortem inspection.

ELISA

ELISA was performed essentially as described by Hira et al. (8). Briefly, 5 µg/ml of antigens were coated onto the wells of ELISA plates (Linbro EIA microtitration plate 96 flat bottom Lot No: 805202) in a carbonate/bicarbonate buffer, pH 9.6, overnight at +4 °C. After thorough washing (PBS containing 0.05% Tween 20), the blocking step was performed. Skimmed–milk powder at 5% (w/v) was used for the blocking step. After 2 h incubation at 37 °C the plates were washed twice. Sera were added at dilutions from 1/50 and the plates were incubated for 2 h at 37 °C. After the incubation, the plates were washed at least 5 times. The conjugate, peroxidase labelled antibodies against bovine IgG (anti-bovine IgG peroxidase conjugate, Sigma Immunochemicals Cat No: A7414, St. Louis, MO, USA), was then added at a concentration of 1/1000. After 2 h incubation (37 °C) and further washing to remove unbound conjugate, the amount of enzyme bound was assayed using a o-phenylene diamine (OPD) and hydrogen peroxide (H_2O_2) in citrate/phosphate buffer as substrate. Conveniently readable results were obtained after 10 min incubation at room temperature (21-25 °C). Absorbance values were read at 450 nm using an ELISA reader (Medispec ESR 200). The cut-off value was calculated as the mean of the negative control sera absorbance values plus 2 standard deviations.

IFAT

Twofold serial dilutions (1/4 to 1/512) of negative and positive sera were prepared in PBS (pH 7.2) and allowed to react seperately with whole scolex antigen. This was carried out at 37 °C for 30 min with anti bovine IgG (antibovine IgG FITC conjugated, Sigma Chem, Cat No: F7887, St. Louis, MO, USA) at a concentration of 1/40. After the incubation slides were again washed 4 to 5 times with PBS (pH 7.2). Evans blue was used as a counterstain to minimise autofluorescence at 1/5000 dilution. PBS washing was performed again and glycerine/PBS (1/9) was added to the slide, which was then examined under a fluorescent microscope (Olympus CX31). The optimum titre was evaluated as 1/128 and all sera were evaluated according to this titre. The antigen-antibody reaction was characterised by the appearance of a specific brilliant greenish-yellow fluorescence on the whole protoscoleces. Negative samples were observed by the appearence of a red fluorescence on the protoscoleces (13).

Data Analysis

The data were analysed with SPSS and a chi-square test was used to evaluate all the data.

Results

In the ELISA and IFAT analysis of serum samples of 597 cattle from 8 provinces in Eastern Anatolia, 63.6% and 54.9% seroprevalence was obtained, respectively ($P < 0.01$). The results of ELISA and IFAT by location in Eastern Turkey are summarised in Table 1. In the ELISA analysis, while the highest positivity was obtained from Muş (85.3%), the lowest (46.6%) was in Kars. In the IFAT, the highest seropositivity level was also observed in Muş (77.3%), whereas the lowest seropositivity was observed in Erzurum (35.1%). The mean prevalence was as 59.2% in all 8 provinces.

The overall prevalence figures of hydatidosis were estimated as 63.6% (380/597) and 54.9% (328/597) by ELISA and IFAT ($P < 0.01$), respectively. Proportions obtained by ELISA were higher in Elazığ, Malatya, Muş, Van, Erzincan, Erzurum and Kars, whereas they were lower in Bingöl when compared with IFAT results.

ELISA and IFAT results of all sera are shown in Table 2. In 288 samples, both ELISA and IFAT were positive; however in 175 samples both were negative. In 92 samples ELISA was positive and IFAT was negative, while in 42 samples ELISA was negative and IFAT was positive.

The seroprevalence shown by ELISA and IFAT by sex, breed and age is shown in Table 3. The seroprevalence shown by both tests was the same for females and males ($P > 0.05$). At the same time, seroprevalence was higher in the cross-breed than in the culture breed with ELISA. However, there were no differences among the breeds in the IFAT results ($P > 0.05$). As shown in the same table, the highest seroprevalence was obtained from cattle at 4 years of age by ELISA and at 2 years of age by IFAT.

Table 1. ELISA and IFAT results by location in Eastern Turkey.

Location	Positive (n)		Negative (n)		Prevalence (%)		Mean Prevalence (%)
	ELISA	IFAT	ELISA	IFAT	ELISA	IFAT	
Elazığ	50	40	25	35	66.6[a]	53.3[a]	59.9
Malatya	43	37	31	37	58.1[b]	50[b]	54.1
Muş	64	58	11	17	85.3[c]	77.3[c]	81.3
Bingöl	46	51	29	24	61.3[d]	68[d]	64.6
Van	59	44	15	30	79.7[A]	59.4[B]	69.5
Erzincan	44	42	31	33	58.6[e]	56[e]	57.3
Erzurum	39	26	35	48	52.7[C]	35.1[D]	43.9
Kars	35	30	40	45	46.6[f]	40[f]	43.3
Total	380	328	217	269	63.6[E]	54.9[F]	59.2

a-f, values with the same letter within the same line are not significantly different ($P > 0.05$)
A-F, values with the same letters within the same line are significantly different ($P < 0.01$)

Table 2. Distribution of the results of the samples examined by both tests by location.

Location	ELISA, IFAT				Total
	+, +	+, -	-, +	-, -	
Elazığ	38	12	2	23	75
Malatya	32	11	5	26	74
Muş	53	11	5	6	75
Bingöl	45	1	7	22	75
Van	39	20	5	10	74
Erzincan	34	9	8	24	75
Erzurum	23	16	3	32	74
Kars	24	12	7	32	75
Total	288	92	42	175	597

+,+: positive by ELISA and IFAT; +: positive by at least one test
-,-: negative by both tests; -: negative by at least one test

Discussion

Hydatidosis is one of the most important and hazardous helminth infections in humans and livestock, but it has proved difficult to establish an accurate prevalence status in intermediate hosts in any continent. This is partly due to optimisation difficulties of the avaliable diagnostic tests and the high costs of performing these tests under field conditions. For this reason, most prevalence studies have relied on slaughter data (21). This study employed a serological survey to try and establish the seroprevalence status of hydatidosis in cattle in Eastern Turkey.

HCF has been found to be a good source of antigen for the serodiagnosis of hydatid disease (22). The use of purified antigenic fractions in highly sensitive techniques has obviated cross-reactions with most of the other infections (23). Partially purified HCF of sheep was used as a source of antigen in the ELISA test in the present

Table 3. Seroprevalence shown by ELISA and IFAT by sex, breed, and age.

		ELISA			IFAT		
		Examined (n)	Infected (n)	Prevalence (%)	Examined (n)	Infected (n)	Prevalence (%)
Sex	Male	147	87	59.1^{A}	147	75	51^{B}
	Female	450	293	65.1^{A}	450	253	56.2^{B}
Breed	Culture	348	210	60.3^{b}	348	184	52.8^{C}
	Local	174	113	64.9^{ab}	174	97	55.7^{C}
	Crossbred	78	57	73.1^{a}	78	47	60.2^{C}
Age	1	65	33	50.7^{e}	65	30	46.1^{g}
	2	168	116	69^{cd}	168	103	61.3^{f}
	3	96	55	57.3^{de}	96	46	47.9^{g}
	4	84	64	76.2^{c}	84	50	59.5^{fg}
	5	54	32	59.2^{de}	54	26	48.1^{fg}
	6≤	130	80	61.5^{de}	130	73	56.1^{fg}

A, B, C, values with the same letter within the same column are not significantly different ($P > 0.05$)
a, b, values with the same letters within the same column are significantly different ($P < 0.01$)
c, d, e, values with the same letters within the same column are significantly different ($P < 0.01$)
f, g, values with the same letters within the same column are significantly different ($P < 0.01$)

study. In the diagnosis of hydatidosis by IFAT, frozen parasitise tissue sections, whole scolex and germinal membranes have been used as antigen (9,10) In the present study, whole-scolex was used as antigen in IFAT.

Furthermore in both tests, the cattle sera collected from the city of Muş and surroundings had the highest level of hydatid antibodies. This may be explained by the fact that Muş province has larger pastures and livestock population than the other provinces. The mean prevalence of cattle hydatidosis has been reported as 19.4%-90% (14-18) in various parts of Eastern Turkey. Our findings resemble those in previous works. However, all the other studies are based on abattoir inspections only. The mean prevalence in Kars and Erzurum was lower than that in the other provinces. Both of them have longer winters and the weather is snowy (at least 6 months a year). Therefore, all livestock animals are fed on the farm throughout the long winter. In addition, Echinococcus eggs die during this period and the pastures are clean the following spring.

Some researchers (13,24) reported that hydatidosis was more prevalant in females than in males while Şimşek and Köroğlu (12) reported the opposite. El-Badawi et al. (25) found that there was no difference between males and females consistent with our results.

Many studies about age-prevalence relations in hydatidosis reported that prevalence increased with age (12,13,24,26,27). The present work shows that seroprevalence increases with age, in general. The highest prevalence was determined at age 4 with ELISA and age 2 with IFAT. In addition, the second highest prevalence ratio was found at age 4 with IFAT and this was similar to the ratio at age 2. Therefore, the seroprevalence ratios are high in cattle aged 4 with both tests.

The results obtained in the present work confirm that cattle hydatidosis is quite endemic in Eastern Turkey. Furthermore, both ELISA and IFAT are suitable for seroepidemiological investigations, especially when prepared with purified or partially purified antigen.

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