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Evaluation of the Seroprevalence of Cystic Echinococcosis by the Indirect Hemagglutination (IHA) Method at Balıkesir University Faculty of Medicine: A Seven-year Analysis

Balıkesir Üniversitesi Tıp Fakültesi'nde İndirekt Hemaglütinasyon (IHA) Yöntemi ile Kistik Ekinokokkoz Seroprevalansının Değerlendirilmesi: Yedi Yıllık Analiz

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ABSTRACT

Objective: Echinococcosis is a zoonotic infection caused by cestodes of the *Echinococcus* genus. The most common form, cystic echinococcosis, is a chronic parasitic disease caused by the *Echinococcus granulosus sensu lato* species complex. The aim of our study is to determine the prevalence of cystic echinococcosis in our hospital and to reveal the regional epidemiological characteristics of the disease based on the obtained data.
Methods: In this study, 526 serum samples sent to the medical microbiology laboratory from various hospital departments and outpatient clinics with suspected cystic echinococcosis between 01.07.2018 and 01.07.2025 were evaluated. A commercial ELI.H.A *Echinococcus* (ELITech Microbio, France) kit was used. The clinical diagnosis, gender, age, delivery department, and demographic characteristics of the patients were retrospectively analyzed.
Results: Among the 526 patients evaluated in the study, 302 (57.4%) were female. Of the total applications, 60.5% originated from the general surgery outpatient clinic. Using the IHA test, seropositivity was detected in 21.5% of the 526 patients. When the distribution according to antibody titers was examined, the antibody levels detected in the serum samples were as follows: 6.8% (n=36) at 1/80, 6.1% (n=32) at 1/160, 4% (n=21) at 1/320, 4.9% (n=26) at 1/640, 3.8% (n=20) at 1/1280, 2.8% (n=15) at 1/2560, and 5.9% (n=31) at 1/5120. Among the 90 patients with available clinical data for cystic echinococcosis, the liver was identified as the most frequently involved organ (95.5%). Among 76 patients with available cyst size measurements, most cysts measured 51-100 mm (48.6%). According to the World Health Organization-IWGE classification, CE3b was the most common stage (23.6%).
Conclusion: The obtained data indicate that cystic echinococcosis remains an important public health problem in Balıkesir. Therefore, in light of these findings, it is necessary to strengthen healthcare services for the diagnosis and follow-up of the disease and to place greater emphasis on preventive measures.
Keywords: Echinococcosis, prevalence, zoonotic

ÖZ

Amaç: Ekinokokkoz, *Echinococcus* türlerine ait sestodların yol açtığı bir zoonotik enfeksiyondur. En yaygın görülen form olan kistik ekinokokkoz, *Echinococcus granulosus sensu lato* tür kompleksi tarafından oluşturulan, kronik seyirli ilerleyen bir paraziter hastalıktır. Çalışmamızın amacı hastanemizdeki kistik ekinokokkoz sıklığını belirlemek, elde edilen veriler doğrultusunda hastalığın bölgesel epidemiyolojik özelliklerini ortaya koymaktır.
Yöntemler: Çalışmada, 01.07.2018 ve 01.07.2025 tarihleri arasında kistik ekinokokkoz şüphesiyle çeşitli servis ve polikliniklerden tıbbi mikrobiyoloji laboratuvarına gönderilen 526 serum örneği incelendi. Ticari bir ELI.H.A *Echinococcus* (ELITech Microbio, Fransa) kiti kullanıldı. Hastaların klinik tanısı, cinsiyet, yaş, örneğin gönderildiği bölüm ve demografik özellikleri retrospektif olarak değerlendirildi.

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Bulgular: Çalışmada değerlendirilen 526 hastanın 302'si kadın (%57,4) olarak belirlenmiştir. Toplam 526 başvurunun %60,5'i genel cerrahi polikliniğindendir. IHA testi ile 526 hastanın %21,5'inde seropozitiflik saptanmıştır. Titrelere göre dağılım incelendiğinde, serum örneklerinde saptanan antikor düzeyleri 1/80 titrede %6,8 (n=36), 1/160 titrede %6,1 (n=32), 1/320 titrede %4 (n=21), 1/640 titrede %4,9 (n=26), 1/1280 titrede %3,8 (n=20), 1/2560 titrede %2,8 (n=15) ve 1/5120 titrede %5,9 (n=31) bulunmuştur. Kistik ekinokokkoz için klinik verilerine ulaşılabilen 90 hasta arasında en sık tutulan organ karaciğer (%95,5) olarak saptanmıştır. Kist boyutu ölçümleri mevcut olan 76 hastada ise kistlerin çoğunluğunun 51-100 mm (%48,6) arasında olduğu belirlenmiştir. Dünya Sağlık Örgütü-IWGE sınıflamasına göre en sık görülen evre CE3b (%23,6) olmuştur.
Sonuç: Elde edilen veriler Balıkesir'de kistik ekinokokkozun önemli bir halk sağlığı sorunu olduğunu ortaya koymaktadır. Bu nedenle, bu veriler ışığında hastalığın tanı ve takibine yönelik sağlık hizmetlerinin güçlendirilmesi ve koruyucu önlemlere daha fazla önem verilmesi gerekmektedir.
Anahtar Kelimeler: Ekinokokkoz, prevalans, zoonotik

INTRODUCTION

Echinococcosis in humans is a zoonotic infection caused by cestodes belonging to the *Echinococcus* species. The most commonly observed form, cystic echinococcosis, is a chronic parasitic disease caused by the *Echinococcus granulosus sensu lato* species complex. Alveolar echinococcosis, on the other hand, is associated with *Echinococcus multilocularis*. Additionally, two species endemic to the New World—*Echinococcus vogeli* and *Echinococcus oligarthrus*—are responsible for a form referred to as "neotropical echinococcosis". Among these, *Echinococcus vogeli* causes a disease with polycystic structures, whereas *Echinococcus oligarthrus* leads to a rare clinical presentation with a single cyst (1).

For the detection of antibodies in the serum samples of patients with cystic echinococcosis, commonly used serological methods include indirect hemagglutination assay (IHA), indirect fluorescent antibody test, and enzyme-linked immunosorbent assays (ELISA) (2). These tests exhibit high sensitivity, generally ranging from 60% to 90%, depending on the disease stage and the type of antigen used (3). ELISA or IHA tests can be employed for screening purposes; however, it is recommended that positive results be confirmed by methods such as immunoblotting (4).

The aim of our study is to determine the prevalence of cystic echinococcosis in our hospital, to identify the epidemiological characteristics of the disease based on the obtained data, and thereby to contribute to diagnosis, follow-up, and preventive healthcare services.

METHODS

Between 1 July 2018 and 1 July 2025, a total of 526 serum samples were analyzed in this study. These serum samples were obtained from patients who presented to Balıkesir University Health Practice and Research Hospital were sent to the Medical Microbiology Laboratory from various clinics and departments with a preliminary diagnosis of cystic echinococcosis. Patient data were retrospectively analyzed from the hospital information system records. The study included adult patients aged between 18 and 90 years, and only the first test result for each patient was considered. The clinical diagnosis, sex, department from which the sample was sent, demographic features, and clinical parameters of the included patients were retrospectively evaluated. For statistical analysis, patients were categorized into six age groups: 18-30,31-40,41-50,51-60,61-70, and 71 years and above.

As part of the study, specific antibodies against *Echinococcus granulosus* were semi-quantitatively detected in serum samples using the commercial kit ELI.H.A *Echinococcus* (ELITech Microbio, France), based on the principle of indirect hemagglutination. The test is based on the reaction of sheep erythrocytes coated with antigen and serum samples. The serum samples were diluted at a ratio of 1:40 using buffer solution. Fifty microliters (50 µL) of buffer solution was added to each of the nine wells. Subsequently, 50 µL of diluted serum and antigen-coated erythrocyte suspension (R1 reagent) was added to the first seven wells. Serial dilutions ranging from 1/80 to 1/5120 were prepared for each sample, and the test was applied accordingly. The eighth well served as a control for natural anti-sheep agglutinins and contained a mixture of serum and uncoated erythrocytes (R2 reagent). The nineth well, containing only buffer and R1 reagent, was used as a reagent control. After a 2-hour incubation at room temperature, the results were evaluated macroscopically. In the presence of specific antibodies, agglutinated sensitized erythrocytes formed a reddish, homogeneous layer on the microplate surface, indicating a positive reaction. In contrast, a ring-shaped sediment at the bottom of the well indicated a negative result. Serologically, titers below 1/80 were considered negative, titers of 1/80 and 1/160 were classified as borderline or equivocal, and titers of 1/320 or higher were accepted as positive (5). The validity of the test was confirmed using both positive and negative control sera (Figure 1).

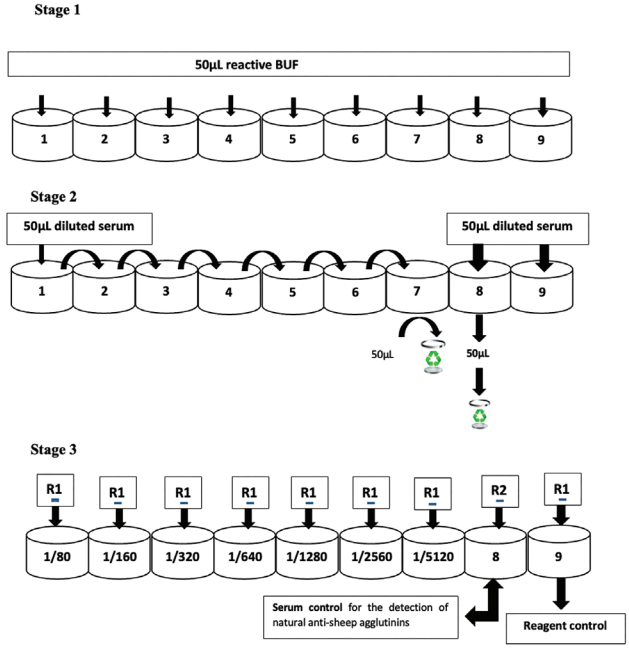

Figure 1. Schematic abstract of the working principle of the indirect hemagglutination test
BUF: Buffer solution

Statistical Analysis

The data obtained in the study were recorded and statistically analyzed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Numerical data were presented as percentages and mean ± standard deviation. Categorical data were expressed as percentages. The chi-square test was used to compare independent groups containing categorical variables. A p-value of less than 0.05 was considered statistically significant.

Ethical Approval

Ethical approval for this study was obtained from the Non-Interventional Clinical Research Ethics Committee of Balıkesir University, dated June 3, 2025, with the approval number E.526429, decision number 2025/220.

RESULTS

Of the 526 patients tested for cystic echinococcosis using the ELI.H.A *Echinococcus* IHA, 302 (57.4%) were female. The mean age of the patients included in the study was 55.5±16.3 years. Among the total 526 cases evaluated, 60.5% were referred from the general surgery outpatient clinic, followed by internal medicine (12.7%), gastroenterology (6.6%), inpatient wards (5.1%), and infectious diseases (4.9%).

The distribution of antibody titers in the serum samples was as follows: 1/80: 6.8% (n=36), 1/160: 6.1% (n=32), 1/320: 4.0% (n=21), 1/640: 4.9% (n=26), 1/1280: 3.8% (n=20), 1/2560: 2.8% (n=15), 1/5120: 5.9% (n=31) (Figure 2). According to the results of the indirect hemagglutination test, 21.5% (n=113) of the patients had seropositivity at a titer of 1/320 or higher. The mean age of seropositive patients was 50.2±16.5 years. When positivity rates were compared across age groups, the highest rates were observed in the 41-50 and 61-70 age groups, each with 19.5% (n=22), while the lowest rate was seen in patients aged 71 and older, with 11.5% (n=13). A statistically significant difference in positivity rates was found between age groups (p=0.003). Among the patients who tested positive, 58 (51.3%) were female. There was no statistically significant difference in seropositivity rates between females and males (p=0.086).

The cyst characteristics and demographic features of patients with cystic echinococcosis with antibody titers ≥1:320 are presented in Table 1. Radiological findings were available for a subset of patients. The liver was the most frequently involved organ (95.5%) among 90 patients with available localization data. Among 76 patients with available cyst size measurements, most cysts measured 51-100 mm (48.6%). According to the World Health Organization (WHO)-IWGE classification, CE3b was the most common stage (23.6%).

Between 2018 and 2025, serological evaluations for cystic echinococcosis revealed variations in seropositivity rates across the years. When examined by year, the seropositivity rates

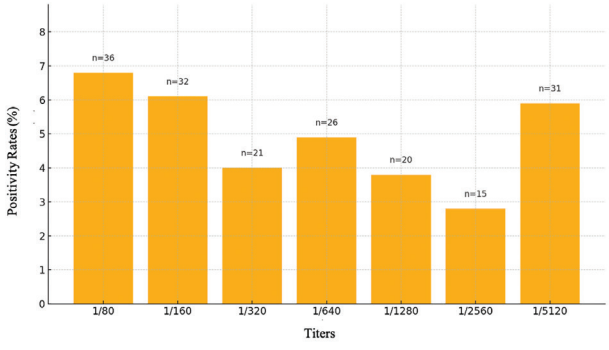

Figure 2. Distribution of positivity rates and titers in serum samples studied by *Echinococcus* indirect hemagglutination test

Table 1. Clinical evaluation of patients with cystic echinococcosis with IHA seropositivity ≥1/320

			n (%)
Demographic characteristics	**Gender**	Female/male	58 (51.3)/55 (48.7)
	Age distribution	18-30	19 (16.8)
		31-40	16 (14.1)
		41-50	22 (19.5)
		51-60	21 (18.5)
		61-70	22 (19.5)
		≥71	13 (11.5)
	Department	General Surgery	84 (74.3)
		Gastroenterology	4 (3.5)
		Infectious Diseases	4 (3.5)
		Internal Medicine	3 (2.6)
		Pulmonology	2 (1.7)
		Other	16 (14.1)
Cyst characteristics*	**Location (site)** (n=90)**	Liver	86 (95.5)
		Lung	7 (7.7)
		CNS	1 (1.1)
		Spleen	4 (4.4)
		Bone	2 (2.2)
		Heart	2 (2.2)
	Dimensions (mm) (n=76)	0-10	1(1.3)
		11-30	11 (14.4)
		31-50	15 (19.7)
		51-100	37 (48.6)
		>100	12 (15.7)
	Stage (WHO-IWGE)* (n=76)	CE1	6 (7.8)
		CE2	5 (6.5)
		CE3a	10 (13.1)
		CE3b	18 (23.6)
		CE4	13 (17.1)
		CE5	6 (7.8)
		Unspecified/ suspected	18 (23.6)

*: The evaluation was performed based on patients whose radiological findings were available, **: More than one category may be present in the same patient, CNS: Central nervous system, WHO-IWGE: The World Health Organization Informal Working Group on Echinococcosis, mm: Millimeter

were 50% in 2018 (4/8), 18.8% in 2019 (17/90), 19.6% in 2020 (11/56), 18.6% in 2021 (11/59), 36.7% in 2022 (18/49), 24.5% in 2023 (25/102), 16.8% in 2024 (18/107), and 19.5% in 2025 (9/46) (Table 2). A statistically significant difference was found among the annual seropositivity rates (p=0.049). These findings indicate marked fluctuations in positivity rates over the years, with a noticeable upward trend in 2022 compared to previous years. In our study, the seropositivity rate during the coronavirus disease 2019 (COVID-19) period (2020-2022) was found to be 32.2% for cystic echinococcosis.

DISCUSSION

Cystic echinococcosis is classified as a neglected tropical disease by the WHO (6). Although cystic echinococcosis is prevalent globally, it is recognized as a significant public health problem particularly in endemic regions such as South America, the Mediterranean Region, Central Asia, Türkiye, Western China, and East Africa (7,8).

In our study, seropositivity rates varied by age group, with high positivity rates detected in the 41-50 and 61-70 age groups. No statistically significant association was found between gender and test results (p=0.086), indicating that positive test results were observed at similar rates in both male and female patients, and that gender did not significantly influence test outcomes. Several studies in Türkiye have shown that cases of cystic echinococcosis typically present with clinical symptoms after the age of 40, with a peak incidence observed between the ages of 50 and 60 (9). In the study conducted by Taşbent et al., (10) the highest IHA positivity was observed in the 41-65 age group, at a rate of 37.7%. In contrast, Başer et al. (11) reported the highest seropositivity in the 21-40 age group.

Table 2. Cystic echinococcosis seropositivity rates between 2018 and 2025 years*

Year	Positive	Negative	Total	Positivity rate (%)
2018	4	4	8	50.0**
2019	17	73	90	18.9
2020	11	45	56	19.6
2021	11	48	59	18.6
2022	18	31	49	36.7
2023	25	77	102	24.5
2024	18	89	107	16.8
2025	9	37	46	19.5
Total	113	413	526	21.5

*: A statistically significant difference was found in the chi-square test, in which the results were compared according to the years (p=0.049), **: In 2018, the positivity rate was found to be high due to the low number of total patients

In our study, the seroprevalance of cystic echinococcosis was found to be 21.5% at Balıkesir, using the IHA method. According to studies conducted in Türkiye, the prevalence of cystic echinococcosis ranges between 5% and 25%. An overview of publications using the IHA method for detecting seropositivity in cystic echinococcosis is presented in Table 3. In a study conducted by Özkeklikçi and Cirit, (12) a seropositivity rate of 28.6% was reported among patients presenting with suspected cystic echinococcosis to the parasitology laboratory in Gaziantep between 2015 and 2022. In another study by Çelik et al., (13) 1,607 serum samples submitted to Adıyaman University Training and Research Hospital between 2013 and 2020 showed a seropositivity rate of 15.1% via the IHA method. Bağcı and Akarsu (14) evaluated serum samples sent to the Central Laboratory of Ankara University Hospital between 2023 and 2024 using the IHA method and reported a seropositivity rate of 5.9% (14). Taşbent et al. (10) also reported a seropositivity rate of 15.2% (n=143) among 938 patients in Konya. In the study by Alver et al., (15) 19.9% (n=213) of the 1,072 patients who presented to Bursa Uludağ University Hospital with suspected cystic echinococcosis were found to be seropositive using the IHA method. Çiftçi et al. (16) reported a seropositivity rate of 25.1% (n=221) in 879 patient serum samples sent to the microbiology laboratory of Selçuk University Faculty of Medicine between 2010 and 2014 with a preliminary diagnosis of cystic echinococcosis. In the study by Behçet and Avcıoğlu (17), which evaluated patients with suspected cystic echinococcosis using the IHA method, a seropositivity rate of 10.6% was observed among 644 patients. In another study by Başer et al., (11) a seropositivity rate of 21.6% was reported among 1,543 patients who presented with suspected cystic echinococcosis at Selçuk University Faculty of Medicine Hospital between 2015 and 2020. In a study conducted at Balıkesir Atatürk State Hospital, 19.8% of 823 serum samples collected between 2011 and 2013 showed positivity at a titre of 1/320 or higher (18).

In the present study, the liver was the most frequently involved organ (95.5%), followed by the lung (7.7%). This finding is consistent with previous studies conducted in Türkiye (19,20). Regarding cyst size, the majority of cysts in our study measured 51-100 mm (48.6%). Similarly, in a multicenter study evaluating 170 patients with cystic echinococcosis in Türkiye, most cysts were reported to measure between 5 and 10 cm (47.1%) (21). In terms of WHO-IWGE classification, CE3b was the most common stage (23.6%) in our study. These findings suggest that many patients are diagnosed when cysts reach moderate to large sizes, likely due to the slow-growing nature of the disease.

The seropositivity rate during the COVID-19 period (2020-2022) was found to be 32.2% for cystic echinococcosis in our study. In the study by Ulusan Bağcı (22), a comparison of pre-pandemic (2019 and earlier) and pandemic (2020-2022) periods in İzmir showed that the seropositivity rate decreased from 18.4% to 14.6% during the pandemic period when evaluated using the IHA method.

Table 3. Analysis of publications containing IHA method for cystic echinococcosis

Researcher	Interval	Province	Positivity rates (%)	Sample number	Methods
Çiftçi et al. (16)	2010- 2014	Konya	25.1	879	IHA (Hydatidose, Fumouze Laboratoires, France)
Şafak (18)	2011-2013	Balıkesir	9.8	823	IHA (Hydatidose, Fumouze Laboratoires, France)
Behçet and Avcıoğlu (17)	2013-2018	Bolu	10.6	644	IHA (Fumouze Diagnostics, France)
Çelik et al. (13)	2013-2020	Adıyaman	15.1	1607	IHA (Fumouze Laboratoires, France)
Taşbent et al. (10)	2014- 2018	Konya	15.2	938	IHA (Hydatidose, Fumouze Laboratoires, France)
Başer et al. (11)	2015-2020	Konya	21.6	1543	IHA (Fumouze Laboratoires, France)
Özkeklikçi and Cirit (12)	2015-2022	Gaziantep	28.6	1811	IHA (ELITech Microbio, France)
Alver et al. (15)	2017-2018	Bursa	19.9	1072	IHA (Siemens Healthcare Diagnostics, Germany)
Ulusan Bağcı (22) Pre-COVID	10.09.2017-10.03.2020	İzmir	18.4	1444	IHA (Hydatidose, Fumouze Laboratoires, France)
Ulusan Bağcı (22) COVID	11.03.2020-11.09.2022	İzmir	14.6	877	IHA (Hydatidose, Fumouze Laboratoires, France)
Bağcı and Akarsu (14)	01.01.2023-01.09.2024	Ankara	5.9	636	*Echinococcus* IHA (Fumouze Diagnostics, France)
Present study	2018-2025	Balıkesir	21.5	526	IHA (ELITech Microbio, France)

IHA: Indirect hemagglutination assay

A total of 6.8% of the serum samples included in the study showed a suspicious reaction at a 1/80 titre, and 6.1% at a 1/160 titre. The IHA method can be used for the screening and diagnosis of cystic echinococcosis. In cases of low-titre suspicious positivity detected by IHA, it is recommended that the test be repeated after two or three weeks (5). As a secondary confirmatory test, Western blot or immunoblot methods are preferred to verify the diagnosis when IHA results are borderline or suspicious (23).

Study Limitations

The limitations of this study include its retrospective design and being conducted at a single center, which restricts the generalizability of the results. The sensitivity and specificity of the indirect hemagglutination test used in the study may vary depending on laboratory conditions and the stage of the disease. In our study, no secondary confirmatory method was used for samples with suspicious titres. In addition, radiological data for some patients could not be accessed. Therefore, multicenter and prospective studies are needed for a more robust analysis of epidemiological data at regional or national levels.

CONCLUSION

The findings of this study indicate that cystic echinococcosis remains a significant public health issue in Balıkesir, a region with intensive agricultural and livestock activities. Consequently, it is essential to strengthen healthcare services related to the diagnosis and follow-up of the disease and to place greater emphasis on preventive measures. For effective control of cystic echinococcosis, early diagnosis, efficient monitoring, and increased public awareness initiatives are of critical importance.

*Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Non-Interventional Clinical Research Ethics Committee of Balıkesir University, dated June 3, 2025, with the approval number E.526429, decision number 2025/220.

Informed Consent: Informed consent was no received due to the retrospective nature of the study.

Footnotes

*Authorship Contributions

Concept: N.İ., Design: N.İ., Data Collection or Processing: N.İ., Y.Ö., Analysis or Interpretation: N.İ., Y.Ö., T.K.A., A.G.Ş., Literature Search: N.İ., Y.Ö., T.K.A., Writing: N.İ., Y.Ö., T.K.A., A.G.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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