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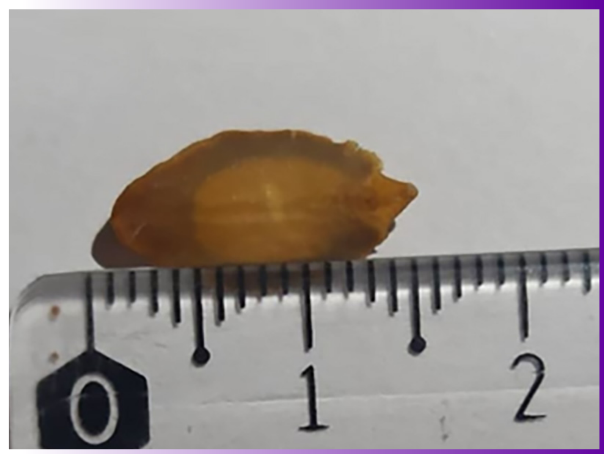

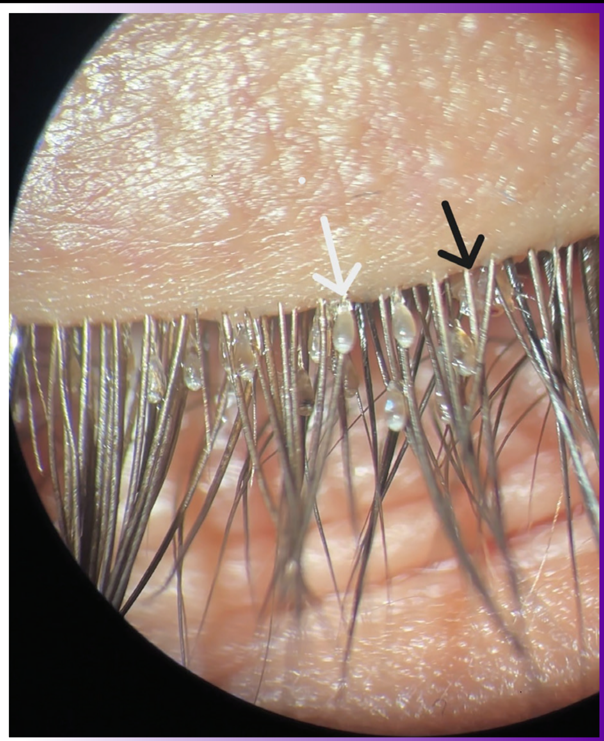

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EDİTÖRDEN

2026 yılının ilk sayısını biri yurt dışından olmak üzere 6 özgün araştırma makalesi ve 2 olgu sunumu ile çıkarmaktayız. Özgün çalışmalar arasında, Bolu ve Düzce illerimizdeki kişilerde *Microsporidium* türlerinin bildirildiği bir çalışma, Amebiasisli olgulardaki tam kan parametrelerini inceleyen bir çalışma, Kist Hidatik tanısında önemli yöntem olan seroloji ve radyolojinin uyumlarını irdeleyen retrospektif bir analiz ile toplum sağlığı açısından önem taşıyan ve üniversite öğrencilerinin parazit hastalıkları hakkındaki bilgi düzeylerini ölçen bir anket çalışması, ayrıca tıbbi sülüklerle yapılan çalışmalarda önem taşıyan sağlıklı ve deforme olmuş sülüklerdeki durumu histolojik düzeyde inceleyen bir araştırma yer almaktadır. Yurt dışından ise Cezayir'de ruminantlarda fascioliasis insidansı hakkında bir çalışma bu sayımıza eklenmiştir.

Olgu sunumları olarak; nadir de olsa görülebilen bilateral *Phthiriasis* olgusu ile yoğun bakımdaki bir hastada rastlanan miyazis olgusuna yer verilmiştir.

Dergimizin ESCI'ye başvurusu yeniden yapılmış olup sonucu beklenmektedir. Bu sürece büyük katkısı olan ve gönderilen makalelere özveri ile hakemlik yapan, her yıl dördüncü sayının sonunda da listesi yayınlanan akademisyenlerimize de teşekkür etmek ve minnetlerimi sunmak isterim. Ayrıca, inceleme için makale gönderilen hocalarımızın incelemeyi "kabul" veya "ret" ettiklerini bildiren mesaja en kısa zamanda cevap vermeleri de sürecin hızlanması açısından oldukça önem taşıdığını siz değerli hocalarımıza hatırlatmak isterim.

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Prof. Dr. Yusuf Özbel
Baş Editör

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An Investigation of Microsporidia Species in Humans Living in Düzce and Bolu Provinces of Türkiye

Düzce ve Bolu İllerinde Yaşayan İnsanlarda Mikrosporidia Türlerinin Araştırılması

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ABSTRACT

Objective: The literature about microsporidia clearly shows that the prevalence of microsporidia varies at different rates depending on the sampling status and region in the studies. However, the majority of studies have been conducted in immunocompromised individuals. It is vital to determine the prevalence of pathogens in society in order to protect immunocompromised individuals from opportunistic pathogens. Consequently, the present study investigated stool samples from Düzce and Bolu provinces for microsporidia, irrespective of disease group.
Methods: A study of 400 stool samples from Düzce and Bolu was performed. The samples were first stained with trichrome and examined under a microscope at 100x magnification. DNA was then isolated, amplified with polymerase chain reaction primers, and the samples were identified. The relationship between microsporidia presence and age, sex, and diarrheal status was statistically investigated.
Results: Following microscopic examination, microsporidia spores were detected in 25 of the 400 (6.25%). Microsporidia spores were detected in 6.6% (14/212) of the Düzce samples and 5.8% (11/188) of the Bolu samples. Consequently, molecular analysis revealed the presence of microsporidia in 49 of the 400 (12.25%). The positivity rates were found to be 11.3% (24/212) for Düzce and 13.3% (25/188) for Bolu. The spores detected in this study were identified as belonging to the species *Enterocytozoon bieneusi*. A statistical analysis was conducted, revealing no significant association between the presence of the pathogen and diarrhea complaints, gender, or age.
Conclusion: It is seen that the presence of microsporidia in our study is at a level similar to rates reported in immunocompetent individuals around the world. Additionally, a correlation between the presence of microsporidia and complaints has been reported, especially in immunocompromised individuals, but no relationship has been determined with complaints in this study. Furthermore, the present study demonstrated that neither age nor gender exhibited any correlation with the presence of microsporidia.
Keywords: Microsporidia, *Enterocytozoon bieneusi*, prevalence, diarrhea, molecular

ÖZ

Amaç: Literatüre bakıldığında, fırsatçı patojen mikrosporidiaların yaygınlığının çalışmalardaki örneklem durumuna ve bölgelere göre farklı oranlarda raporlandığı görülmektedir. Bununla birlikte çalışmaların büyük bir çoğunluğu bağışıklığı yetersiz kişilerde gerçekleştirilmiştir. Bağışıklığı yetersiz kişilerin fırsatçı patojenlerden korunması için toplumda o patojenin yaygınlığının belirlenmesinin çok önemli olduğunu düşünmekteyiz. Bu sebeple, mevcut çalışmada hastalık grubu gözetmeksizin Düzce ve Bolu illerinden gaita numuneleri mikrosporidia açısından araştırılmıştır.

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Türkiye PARAZİTOLOJİ Dergisi

Yöntemler: Bu çalışmada Düzce ve Bolu illerinde yaşayan insanlardan toplam 400 gaita numunesi temin edilmiştir. Örnekler ilk olarak trikrom boyama ile boyanarak mikroskopta 100x büyütmede incelenmiştir. Yine her örnekten DNA izolasyonu yapılmış ve sonrasında türlere özgü primeler kullanılarak polimeraz zincir reaksiyonu ile tür tespiti gerçekleştirilmiştir. Mikrosporidia varlığının yaş, cinsiyet ve ishal durumu ile ilişkisi istatistiksel olarak araştırılmıştır.
Bulgular: Mikroskobik incelemeler sonucunda 400 örneğin 25'inde (6,25) mikrosporidia sporları saptanmıştır. İllerin kendi içerisinde değerlendirilmesi sonucunda Düzce örneklerinin %6,6'sında (14/212) Bolu örneklerinin %5,8'inde (11/188) mikrosporidia sporları görülmüştür. Moleküler analizin sonucunda 400 örnekten 49 tanesinde (12,25) mikrosporidia saptanmıştır. Pozitiflik oranlarının Düzce için %11,3 (24/212) ve Bolu için %13,3 (25/188) olduğu görülmüştür. Tespit edilen sporların *Enterocytozoon bieneusi* türüne ait olduğu saptanmıştır. Yapılan istatistiksel analiz sonucunda patojenin varlığı ile ishal şikayeti, cinsiyet ve yaşın arasında anlamlı bir ilişki olmadığı görülmüştür.
Sonuç: Çalışmamızdaki mikrosporidia varlığının bağışıklığı yeterli kişilerde raporlanan oranlarla benzer düzeyde olduğu görülmektedir. Bu durum, mikrosporidia türlerine maruz kalmanın yaygın olduğunu ve sağlıklı bireylerde herhangi klinik belirti göstermeden enfeksiyonun toplumda sürekli devam ettiğini göstermiştir. Literatürde mikrosporidia varlığı ile gastrointestinal şikayetlerin ilişkili olduğunu bildiren çalışmalar varken tam tersi durumun da varlığı raporlanmıştır. Özellikle bağışıklık sistemi açısından kronik bir rahatsızlığı olmayan kişilerde mikrosporidia türleri kendini sınırlayan ve klinik belirti göstermeyen enfeksiyonlar oluşturması sebebiyle bazı çalışmalarda şikayetlerle mikrosporidia ilişkisinin olmadığının raporlanması normal bir durumdur.
Anahtar Kelimeler: Mikrosporidia, *Enterocytozoon bieneusi*, prevalans, ishal, moleküler

INTRODUCTION

Microsporidia species are spore-forming unicellular eukaryotes and are obligate intracellular pathogens. To date, more than 1500 species belonging to 200 genera have been identified within phylum microsporidia which have broad spectrum of host. Of the 17 human pathogen microsporidia species, *Enterocytozoon bieneusi* is most common agent in humans followed by *Encephalitozoon intestinalis*. They are responsible for more than 90% of human microsporidosis and are often associated with gastrointestinal tract infections (1,2).

It is established that microsporidia species are responsible for opportunistic infections and severe clinical symptoms, particularly in individuals with suppressed or insufficient immune systems, such as organ transplant recipients and human immunodeficiency virus (HIV) positive individuals. Consequently, majority of studies have been conducted on immunocompromised individuals (3-6).

It is greatly important to ascertain the prevalence of the pathogen in society and the environment in order to protect immunocompromised individuals afflicted with diseases such as HIV and cancer. We think that determining the frequency of microsporidia species in the society and the environment through studies conducted irrespective of disease group will help the implementation of appropriate measure for protection of immunodeficient people.

For this reason, stool samples were obtained from individuals residing in Düzce and Bolu provinces, irrespective of their disease status, and subjected to microscopic and molecular examination to ascertain the prevalence of microsporidia species in the aforementioned provinces.

METHODS

In this study 400 fecal samples were obtained from Düzce University (DU) Health Application and Research Facility (212 samples) and the Bolu Abant İzzet Baysal University Training and Research Hospital (188 samples) between April 2021 and July 2021. The sample amounts obtained from the provinces were calculated in proportion to their population. Samples were collected irrespective of any disease group to determine prevalence of microsporidia in society. However, stool samples were obtained from subjects who were not immunocompromised and did not receive either antibiotic or antiparasitic medication. Samples were examined using both microscopic and molecular methods. We commenced the research following the ethics approval from the "DU Non-Invasive Health Research Ethics Committee" (decision no: 2019/41 and date: 16.12.2019). Stool samples were promptly conveyed to the parasitology laboratory, where they were separated into two groups, within every fecal sample, both for microscopic and molecular examinations.

Microscopic Examinations

Parted number of samples for microscopy are put to the formol-ether sedimentation method and subsequently modified trichrome staining (MTS) for determining parasites under microscope at 100X magnification (2).

Molecular Methods

DNA extraction: Genomic DNA was extracted directly from fecal specimens using "GeneAll Stool DNA Minikit" (GeneAll, Seoul, South Korea) following the manufacturer's instruction. All reagents necessary for the extraction process were present within the kit.

Polymerase chain reaction (PCR) amplification: C1 and C2 primers were selected for use in to amplifying a con-served region of the small-subunit rRNA of four microsporidia species namely *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *E. cuniculi* and *E. hellem* (7). Subsequently, a second PCR reaction was conducted using an alternative primer pair with positive specimens from the first PCR application for the purpose of identifying the species (Table 1). The ATB 2X PCR Master Mix brand mixture was used in the PCR reaction and the final volume was calculated to be 50 µL. The size of the bases was determined with the Thermo Scientific GeneRuler 1 kb Plus DNA Ladder marker (Figure 1).

PCR reactions were run using the "BİO-RAD T100" thermal cycler device. Following the denaturation of the DNA at 94 °C for a period of 5 minutes, 35 cycles were conducted in following manner: Denaturation at 94 °C for 1 minute, annealing 56 °C for 1 minute, and elongation at 72 °C for 1 minute. Following the completion of 35 cycles of process, PCR reactions were terminated at 72 °C for a period of 5 minutes. The amplified products were subjected to electrophoresis on an agarose gel and stained with ethidium bromide. The stained gel was examined under ultraviolet light.

Statistical Analysis

Chi-square tests were employed to evaluate the correlation between microsporidia positivity and factors such as age and gender. A Kappa test was also applied to ascertain the sensitivity and specificity of the methods. A p-value of less than 0.05 was considered to indicate a statistically significant result.

Table 1. Primer pairs sequences

Primers	Base squences	Species
C1 (F)	5'-CACCAGGTTGATTCTGCC-3'	*Enterocytozoon bieneusi, Encephalitozoon intestinalis, E. cuniculi* and *E. hellem*
C2 (R)	5'-GTGACGGGCGGTGTGTAC-3'	
EBIEF1 (F)	5'-GAAACTTGTCCACTCCTTACG-3'	*Enterocytozoon bieneusi*
EBIEF1 (R)	5'-CCATGCACCACTCCTGCCATT-3'	
EIMCF (F)	5'-GTCTATGGTGAATGCTGCAGTT-3'	*Encephalitozoon intestinalis*
EIMCR (R)	5'-ATTTCGATCCTCTAGCCTTCGT-3'	

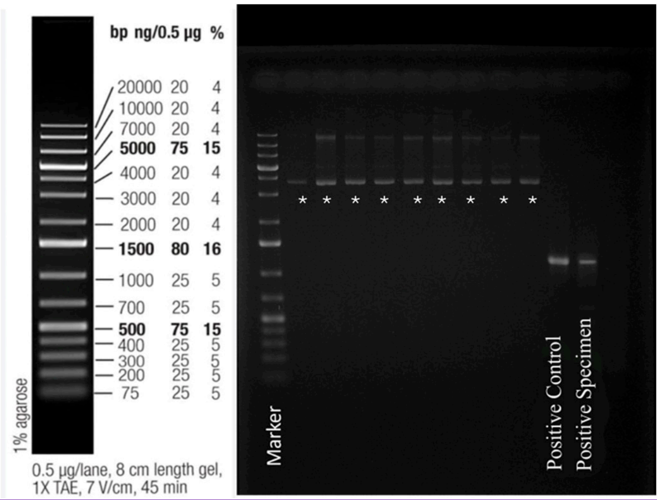

Figure 1. Image of PCR products of 1% agarose gel electrophoresis (*signed strips belong another study)
PCR: Polymerase chain reaction

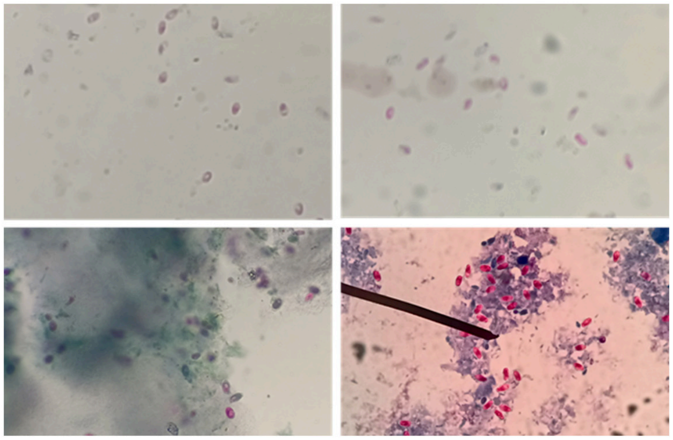

Figure 2. MTS-stained microsporidia spores from different individuals (100x magnification)
MTS: Modified trichrome staining

RESULTS

Microscopic Results

As a result of examination of the MTS-stained slides at 100X magnification, microsporidia spores were observed in 25 out of 400 (6.25%) samples (Figure 2). Upon separation of the cities where the patients come from, the positivity rate in Düzce was determined to be 6.6% (14/212) while in Bolu it was 5.8% (11/188).

Molecular Results

As a result of electrophoresis for PCR products, strips of parasite DNA were observed in 49 out of 400 (12.25%) samples in total. When the cities were evaluated separately, the prevalence in Düzce was found to be 24/212 (11.3%) while in Bolu was 25/188 (13.3%). Following the PCR reactions conducted with species-specific primers (*E. bieneusi* and *E. intestinalis*), the presence of strips was observed exclusively in those, primers selected for *Enterocytozoon bieneusi*.

Statistical Results

In this study, the relationship between the presence of microsporidia and age, gender and diarrhea status was evaluated based on PCR results because of its reliability. Although certain differences were observed among the factors compared in terms of presence of microsporidia, these differences were not found to be statistically significant.

While 26 of 204 samples (12.7%) obtained from women were found to be microsporidia positive, 23 of 196 samples (11.7%) from men were found to be positive in total. In the separate evaluation of the cities, positivity was observed in 15 of 118 women samples (12.7%) and 9 of 94 men samples (9.6%) in Düzce. Furthermore, 11 of the 86 women samples (12.8%) and 14 of the 102 men samples (13.72%) were positive in Bolu. The overall level of positivity is notably elevated among the sampled female population. Nevertheless, while the positivity rate is high in females within the Düzce province, it exhibits a higher frequency among males residing in the Bolu province.

In one sample, microsporidia positive was found among 16 diarrheic individuals (6.3%) while 48 of 384 (12.5%) non-diarrheal patients were positive. Microsporidia were identified in one of 7 diarrheal samples (14.3%) and 24 of 181 non-diarrheal samples (13.3%) in Bolu province. The presence of microsporidia was not detected in any of the nine diarrheal samples from Düzce province, yet 24 of 203 (11.8%) non-diarrheal samples were positive. It is noteworthy that the prevalence of microsporidia in individuals exhibiting diarrhea is lower in comparison to non-diarrheal. Nevertheless, the limited number of diarrheal samples collected precludes the ability to make precise comparisons.

When the results were evaluated in terms of age groups, it was seen that 3 of 32 (9.4%) in the "1-14" age group, 5 of 50 (10%) in the "15-24" age group, 27 of 214 (12.6%) in the "25-64" age group and 14 of 104 (13.5%) in the "65 and over" age group. An examination of these results demonstrates a clear increase in the frequency of microsporidia as the age of the subjects increases but the chi-square analysis revealed that there was no statistically significant relationship between gender, diarrhea and age groups with the presence of microsporidia in this study (Table 2).

Table 2. Distribution of microsporidia according to gender, age and diarrhea

Groups		Total	Positive	Percentage	p-value
Gender	Women	204	26	12.7%	0.439
	Men	196	23	11.7%	
Age	1-14	32	3	9.4%	0.705
	15-24	50	5	10%	
	25-64	214	27	12.6%	
	65 and over	104	14	13.5%	
Diarrhea	Yes	16	1	6.25%	0.885
	No	384	48	12.5%	

Table 3. Crosstab of molecular and microscopic results

Groups		Molecular				
		Positive		Negative		Total
		n	%	n	%	
Microscopic	**Positive**	25	51	0	0	6.3
	Negative	24	49	351	100	93.7
Total		49	100	351	100	100

Considering the potential limitations of a single-method approach, this study employed a dual-method design, assessing the compatibility of these methods. As a result of analysis, the sensitivity and specificity of MTS was calculated to be 51% and 100%, respectively (Table 3). The Kappa test yielded a compatibility score of 0.646 for the two methods employed in this study. This result indicates that the compatibility between the two methods is moderate.

DISCUSSION

The majority of research conducted on these microorganisms has been carried out on immunocompromised people, as microsporidia species are known to cause significant issues in those with these, mentioned individuals. It is of great importance to determine the prevalence of the pathogen in society and the environment in order to ensure the protection of immunocompromised individuals. It is our contention that an investigation of the prevalence of microsporidia in society, conducted without regard to disease group, will facilitate the implementation of appropriate measures for the protection of individuals, especially those with compromised immune systems from these pathogens. Accordingly, samples provided and included in this research were irrespective of any special circumstances.

As a result of microscopic and molecular examination, microsporidia positivity rates were 6.25% and 12.25%, respectively in current study. A review of other studies conducted in Türkiye reveals that the positivity rates of microscopic examinations vary between 5 and 10%, while those of molecular methods range from 10 to 69%. Microsporodiosis rates are higher in immunocompromised individuals, and the course of disease has also been reported to be more serious. Moreover, the researchers provided an explanation for the elevated positivity rates observed in molecular techniques, citing the enhanced reliability of such techniques and the characteristics of the patient cohort in question. A review of the relevant studies reveals that microsporidia positivity in immune sufficient people is close to our study (4,5,8-11). In contrast to the findings of previous studies conducted in our country, which identified *E. intestinalis* as the primary infectious agent, our study observed *E. bieneusi* as the causative agent (4,5,10). This indicates that the predominant microsporidia species may vary according to factors such as geographical location. An analysis of the prevalence of microsporidiosis in the country demonstrates the presence of the pathogen in the population at a certain rate. Indeed, in their meta-analysis of studies conducted within our country, Çetinkaya and Caner (12) documented the mean frequency of microsporidia as 13.4% in human subjects and 15.2% in other vertebrate hosts. This review provides an adequate framework for the frequency of microsporidia in our country and clearly demonstrates the importance of increasing studies on this subject.

The prevalence of human microsporidiosis in European countries is reported to vary between 1-25% (6,13,14). It is noteworthy that the positivity rate is higher in HIV+ patients and organ transplant recipients. The reported positivity rates are suggestive of the prevalence and ubiquity of microsporidia species, which are able to persist in the community without any clinical symptoms in healthy individuals. A survey of European literature reveals the presence of studies that identify *E. bieneusi* as the predominant species, a finding consistent with the results of the present study. However, other studies report a higher prevalence of *E. intestinalis* (6,13). The prevalence of microsporidiosis in Asian countries is approximately 10% (15-17). It can be posited that the reasons for the low prevalence in some studies are the investigation of a single pathogen species and utilization of PCR analysis exclusively in samples that have been microscopically identified as positive (18). The use of single species-specific primer in molecular-based methods precludes the detection of other microsporidia species. Nevertheless, if PCR were performed solely on microscopically positive samples, the resulting prevalence figures would be lower than the actual rates. In the present study, we used species-specific primers and performed PCR on each sample, even if microscopically negative, to try to eliminate these problems. Following a comprehensive meta-analysis of 84 studies investigating the presence of microsporidia in China, Qiu et al. (19) reported that the positivity rate in living creatures (mammals such as humans, cattle, dogs etc.) was 20%, and the rate in water samples was 64.5%. The authors concluded that microsporidia are present at a considerable level in Asian countries (19). Most of the microsporidia prevalence studies conducted on the Africa continent have been conducted in Central African countries and the prevalence appears to vary between 3-67.5%. Furthermore, observed increase in prevalence during rainy seasons is also one of the issues that should be taken into consideration, because the importance of socio-economic status in terms of microsporidiosis is more effective in African countries (20-23). A consideration of the available literature reveals a decline in microsporidia rates particularly in individuals with HIV positivity. There is a consensus among researchers that the use of highly effective antiretroviral therapy is one of the most significant factors contributing to this condition (6,21). From a global perspective, as Wang et al. (20) have noted, microsporidia have the potential to induce health complications not only in individuals with compromised immune systems (e.g., the elderly, children, and HIV patients) but also in those residing in various regions worldwide. Indeed, an analysis of the results of the present study, as well as those previously conducted in the country, reveals that the frequency of microsporidia in immunocompetent individuals is significant.

In one sample, microsporidia positive was found among 16 diarrheic individuals (6.3%) while 48 of 384 (12.5%) non-diarrheal patients were positive. The results of the statistical analysis conducted in the present study indicate that there is no statistically significant difference in the prevalence of microsporidia between individuals with or without diarrhea. The observed phenomenon can be attributed to the limited number of individuals affected by diarrhea. While there are studies showing that diarrhea, indigestion and fatigue are associated with the presence of microsporidia, some studies have reported that there is no relationship between the presence of the pathogen and these complaints (11,24,25). The defining characteristics of microsporidia as an opportunistic pathogen is the ability of the parasite to cause infection and manifest clinical symptoms in individuals with compromised immune systems. Consequently, results of some studies focusing on specific patient groups demonstrate that incidence of complaints is risen in the presence of microsporidia species.

The present study revealed that prevalence of microsporidia in women was 12.7% (26/204) while in men it was 11.7% (23/196). It was observed that the rate of microsporidia was higher in women in the Düzce province (12.7%) and in men (13.72%) in the Bolu province. However, these differences were not statistically significant. As evidenced by studies made worldwide, there is no significant correlation between gender and presence of pathogen. However, it has been observed that in some regions, the rates are higher than one gender or the other, depending on the environmental and working conditions (18,22,25).

It is established that the prevalence of microsporidia differs with age intervals. Especially in childhood and old age, the rate of infection rises because of the lack of personal hygiene and weak immune status (26,27). Despite the absence of a statistically significant difference between age groups in our study, a higher prevalence of microsporidia positivity was observed in individuals aged 65 and above (13.5%).

In many studies, including our own, there have been discrepancies between the rates of microsporidiosis reported by microscopic and molecular methods. This is due to the fact that microsporidia are very small and are frequently overlooked in microscopic examinations when experienced personnel are not available. Furthermore, although fluorescent dyes such as Uvitex 2B and Calcofluor, which bind to the chitin structure in the cell, are employed, it is imperative to exercise caution to avoid misidentifying fungal spores with microsporidia (2,28). The results of our study demonstrated that the positivity of microsporidia was 6.25% by microscopy and 12.25% by PCR. The Kappa analysis yielded a moderate compatibility score (0.646) between both methods. The specificity of MTS was calculated as 100%. The presented results show that the MTS method effectively reduces the occurrence of false positive outcomes. However, it should be noted that this method exhibits moderate sensitivity, necessitating greater care during microscopic examinations to prevent false-negative results.

It is obvious that the positivity rates reported by microscopy are lower than those in molecular methods. While this is linked to the enhanced sensitivity and reliability of molecular methods, it is crucial to proceed with utmost caution in procedures such as DNA isolation and primer selection. Even if the processes are carried out correctly, incorrect results may be obtained from PCR analysis. Indeed, as reported by Ghoyounchi et al. (18) reported the microsporidia spores observed under a microscope could not be detected by PCR. This may be attributed to the inappropriate or thin staining in the microscopic examination or the existence of inhibitor materials in the feces during DNA isolation.

Consequently, it is important to consider the limitations of the methods when designing the study and, if feasible, employ at least two methods to ensure accurate and reliable results.

Study Limitations

In the field of epidemiological studies, the power analysis is of critical importance for the accurate estimation of the prevalence of a pathogen or disease. In the present study, the sample size was set at 400, due to the high costs of molecular methods, and no power analysis was performed. This is considered a limitation of the present study.

CONCLUSION

We believe that our findings contribute to our knowledge about the prevalence of microsporidia in our country. However, it is our contention that a greater understanding can be gained by determining the prevalence of microsporidia in animals and the environmental samples in addition to human specimens. Thus, we posit that valuable insights can be gleaned regarding the phylogenetics of microsporidia species and their transmission routes through ITS genotyping. It is clear that further research on microsporidia is required in our country, where studies on this subject are scarce.

*Ethics

Ethics Committee Approval: We commenced the research following the ethics approval from the "DU Non-Invasive Health Research Ethics Committee" (decision no: 2019/41 and date: 16.12.2019).

Informed Consent: Informed consent was obtained from all subjects involved in the study.

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Footnotes

*Authorship Contributions

Design: M.Ç., E.A., Ş.Ö., Data Collection or Processing: M.Ç, B.D.G., N.E., Analysis or Interpretation: M.Ç., Ş.Ö., Literature Search: M.Ç., E.A., Writing: M.Ç., E.A., Ş.Ö.

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Original Investigation
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Investigation of Systemic Immune Inflammatory Index and Other Complete Blood Parameters in Cases with Amoebiasis

Amoebiasisli Olgularda Sistemik İmmüno Enflamatuvar İndeks ve Diğer Tam Kan Parametrelerinin Araştırılması

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ABSTRACT

Objective: *Entamoeba histolytica* is a major parasitic cause of acute gastroenteritis. In this study, hematological inflammatory indices were assessed in adhesin-positive amoebiasis cases.
Methods: Adhesin test results and hemogram parameters were evaluated simultaneously in cases who were referred to Necmettin Erbakan University Faculty of Medicine, Medical Parasitology laboratory with suspicion of amoebiasis and whose *Entamoeba histolytica* specific adhesin enzyme-linked immunosorbent assay test was found to be positive between January 2022 and December 2023. In this study, the indices were calculated using haemogram parameters. Age- and sex-matched groups were formed, consisting of cases with adhesin test-positive acute gastroenteritis (APAG) and those with adhesin test-negative acute gastroenteritis (ANAG). In addition to common statistical analyses, the diagnostic performance of various hematologic inflammatory parameters in predicting adhesin positivity was evaluated by receiver operating characteristic analysis.
Results: The results of 340 cases were analyzed, including 136 cases under the age of 18. Blood lymphocyte and monocyte levels were significantly lower in the APAG group compared to the ANAG group (p=0.004 and p=0.048, respectively), while no significant differences were observed in the remaining haemogram parameters. There was also no statistically significant difference in C-reactive protein levels between the groups (p=0.061). Among the calculated indices, only the platelet-to-lymphocyte ratio (PLR) showed a significant difference between groups (p=0.017). In the gender-based subgroup analysis of the APAG group, red blood cell levels were found to be lower in female cases (p=0.026), while no significant differences were observed in the calculated indices.
Conclusion: This study evaluated the predictive performance of various hematologic inflammatory parameters in determining adhesin test positivity. Although the PLR showed a statistically significant difference between groups, the positive and negative predictive values of all evaluated parameters remained moderate. These findings suggest that the diagnostic utility of these biomarkers is limited.
Keywords: Amoebiasis, adhesin test, SIRI, PLR, NLR

ÖZ

Amaç: *Entamoeba histolytica*, akut gastroenteritlerin önde gelen paraziter etkenlerinden biridir. Bu çalışmada, adezin pozitif amoebiasis olgularında hematolojik enflamatuvar indeksler değerlendirilmiştir.
Yöntemler: Ocak 2022-Aralık 2023 tarihleri arasında Necmettin Erbakan Üniversitesi Tıp Fakültesi, Tıbbi Parazitoloji laboratuvarına amibiyazis şüphesi ile gönderilen ve *Entamoeba histolytica* spesifik adezin enzim bağlı immünosorbent analiz testi pozitif bulunan hastalarda adezin test sonuçları ve hemogram parametreleri eş zamanlı olarak değerlendirilmiştir. Bu çalışmada, indeksler hemogram parametreleri kullanılarak hesaplanmıştır. Adezin testi pozitif akut gastroenterit (APAG) ve adezin testi negatif akut gastroenterit (ANAG) hastalarından oluşan, yaş ve cinsiyet açısından eşleştirilmiş gruplar oluşturulmuştur. Genel istatistiksel analizlere ek olarak, çeşitli hematolojik enflamatuvar parametrelerin adezin pozitifliğini öngörmedeki tanısal performansı alıcı işletim karakteristiği analizi ile değerlendirilmiştir.
Bulgular: Çalışmada 136'sı 18 yaş altı olmak üzere 340 hastanın sonucu incelenmiştir. APAG grupta kan lenfosit ve monosit düzeyleri ANAG gruba göre anlamlı olarak (p=0,004, p=0,048, sırasıyla) düşük bulunurken, kalan hemogram parametrelerinde farklılığa rastlanmamıştır. C-reaktif protein düzeyinde de istatistiksel olarak fark tespit edilmemiştir (p=0,061). Platelet lenfosit

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oranı (PLR) indeksinde iki grup arasındaki fark anlamlı bulunurken (p=0,017) diğer indekslerde gruplar arasında anlamlı farklılık saptanmamıştır. APAG grubunun cinsiyete dayalı alt grup analizinde, kırmızı kan hücresi seviyeleri kadın hastalarda daha düşük bulunurken (p=0,026), hesaplanan diğer indekslerde anlamlı bir farklılık gözlenmemiştir.

Sonuç: Bu çalışmada, çeşitli hematolojik enflamatuvar parametrelerin adezin pozitifliğini öngörme performansları değerlendirilmiştir. Her ne kadar PLR indeksinde gruplar arası fark saptansa da genel olarak tüm parametrelerde pozitif ve negatif prediktif değerler orta düzeyde kalmaktadır. Bu sebeple, söz konusu biyobelirteçlerin tanısal performansının kısıtlı olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Amibiyazis, adezin test, SIRI, PLR, NLR

INTRODUCTION

Amebiasis is a communicable enteric parasitic infection caused by *Entamoeba histolytica (E. histolytica)* (1). It is predicted that approximately 50 million individuals worldwide, particularly in developing countries, are affected by *E. histolytica*, with almost 100,000 of these resulting in death (1,2). The primary route of transmission of *E. histolytica* is the ingestion of food or water contaminated with amebic cysts (1). In developed countries, where water- and food-borne transmission is rare, sexually transmitted infections among men who have sex with men have also been reported (3). Most *E. histolytica* infections are asymptomatic; however, individuals on immunosuppressive therapy, pregnant women, and infants are at higher risk of developing invasive disease, which may include dysentery or extra-intestinal manifestations such as amebic liver abscesses (1,3).

Asymptomatic patients typically show normal colonoscopic findings and no history of bloody stools. Diagnostic microscopy, including observation of erythrocyte-engulfed trophozoites, may not always detect infection (4). Direct microscopy is often used as the initial diagnostic step due to its low cost, practicality, and accessibility. However, differentiation of pathogenic *E. histolytica* from the non-pathogenic *E. dispar* is essential, as reliance on microscopy alone can result in diagnostic errors (4,5). Modern serological tests offer high sensitivity and specificity, allow differentiation from non-pathogenic species, and can process large numbers of samples rapidly, making them especially useful during outbreaks (6).

Prompt diagnosis and treatment of *E. histolytica* are essential to prevent complications and reduce mortality (7,8). Although serological and molecular methods with higher sensitivity and specificity are available, traditional fecal microscopy remains the most commonly used diagnostic approach worldwide (9). However, this method cannot reliably distinguish *E. histolytica* from other non-pathogenic Entamoeba species, leading to false-positive results and missed asymptomatic cases. Therefore, practical and cost-effective supplementary diagnostic parameters are needed (6-9).

Hemogram-derived indices, including mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and systemic inflammation response index (SIRI), have emerged as accessible and cost-effective markers of systemic inflammation (10-12). These parameters have been increasingly studied in various infectious and inflammatory conditions due to their ability to reflect the balance between different immune cell populations and platelet activity. Evidence on the utility of these indices in parasitic infections is limited (13-15), though they could help assess inflammation and severity in amoebiasis.

In *E. histolytica* infection, neutrophils constitute the first line of defense, contributing to parasite clearance through phagocytosis, degranulation, and the formation of neutrophil extracellular traps (16). However, trophozoites can interact with β_2 integrins on the neutrophil surface, triggering a phosphatidylinositol 3-kinase dependent pathway that leads to excessive reactive oxygen species generation and subsequent neutrophil apoptosis. This mechanism allows the parasite to effectively counteract neutrophil-mediated killing, facilitating tissue invasion and immune evasion. Monocytes also play a central role in the early innate immune response against *E. histolytica*, contributing to phagocytosis, cytokine-mediated inflammation, and the recruitment of other immune cells, while the parasite employs monocyte locomotion inhibitory factor and additional mechanisms to suppress monocyte activity and facilitate tissue colonization (17).

Despite the established role of neutrophils in amoebiasis (16,17), no studies have directly evaluated NLR or related indices as diagnostic or prognostic markers. Since excessive neutrophil activation contributes to tissue injury and inflammation, composite markers such as SII (platelet×neutrophil/lymphocyte) and SIRI (neutrophil×monocyte/lymphocyte) (11-14) may serve as practical tools to estimate inflammatory burden and disease severity in amoebiasis, especially in resource-limited settings.

In this planned study, the properties of haemogram-derived indices as inflammatory markers in adult and paediatric cases with adhesin test positive *E. histolytica* infection will be investigated.

METHODS

Population of Study and The Ethical Approval

This study was conducted with the approval of Necmettin Erbakan University Drug and Non-Medical Device Research Ethics Committee (decision no: 2025/5656, date: 21.03.2025).

In this study, the adhesin test results and hemogram parameters [e.g., neutrophils ($10^3/\mu L$), lymphocytes ($10^3/\mu L$), monocytes ($10^3/\mu L$), platelets ($10^3/\mu L$)] of the cases who were referred to the Medical Parasitology Laboratory of Necmettin Erbakan University Medical Faculty for testing between January 2022 and December 2023 with suspicion of amoebiasis were examined simultaneously.

Between these dates, cases with acute gastroenteritis were assigned to the adhesin test-positive acute gastroenteritis (APAG) or adhesin test-negative acute gastroenteritis (ANAG) group according to fecal adhesin antigen results. Cases with positive adhesin test results were evaluated with age- and sex-matched controls and compared with blood parameters. Fecal samples from (range 0-86 years) 204 adult and 136 paediatric cases were included in the study. The study design was retrospective, and the necessary information was obtained from the hospital record system.

E. histolytica was detected in fresh stool samples using the commercial immunoassay Entamoeba CELISA Path (Cellabs Pty Ltd., Australia), which has high sensitivity and specificity. Stool samples were analyzed strictly according to the manufacturer's instructions without any modifications. The kit's performance has been previously validated, showing sensitivities ranging from 93% to 100% and specificities from 93% to 100% across studies involving 44 to 757 fecal specimens, with comparisons made against culture or Zymodeme methods. Results were interpreted following the recommended cut-offs, and all assays were performed under standardized laboratory conditions. This test provides rapid detection of the adhesin of *E. histolytica* in fecal samples. In spectrophotometric measurement, samples with optical density$_{450}$≥0.150 are considered "positive." An automated analyzer was used to detect hemogram parameters.

The indices were calculated using the following formulas (11-14):

NLR: Neutrophil count/lymphocyte count

LMR: Lymphocyte count/monocyte count

Monocyte-to-lymphocyte ratio (MLR): Monocyte count/lymphocyte count

PLR: Platelet count/lymphocyte count

SIRI: Neutrophil count×monocyte count/lymphocyte count

SII: Platelet count×neutrophil count)/lymphocyte count

MPVPCR: Mean platelet volume/platelet count ratio

Statistical Analysis

Tests were selected based on the normality of the numerical data (assessed by the Shapiro-Wilk test) and whether the data were paired. For numerical data, t-tests were used to compare two independent groups if the data were normally distributed, and ANOVA was used to compare more than two groups. In paired data, repeated measures ANOVA was used. When data were not normally distributed, the Wilcoxon rank test for two groups or the Kruskal-Wallis test for more groups was used. For categorical data, chi-square tests were used if the number of cell counts was sufficient, and Fisher's exact test was implemented for small sample sizes. In all tests, p<0.05 is statistically significant was considered statistically significant.

In this study, the diagnostic performances of various haematological inflammatory parameters in predicting adhesin positivity were evaluated by receiver operating characteristic (ROC) analysis. In ROC analysis, the diagnostic discriminative power of a parameter is expressed by the "area under the curve (AUC)" value. An AUC value close to 0.5 indicates that the question test is indistinguishable from random guessing in terms of discriminative power, while an AUC value close to 1.0 indicates high discriminative power.

RESULTS

The study included 340 participants, 169 women (49.71%) and 171 men (50.29%). The mean age of those included in the study was 29.83±24.83 years (range 0-86; median 26), 136 were <18 years, and 204 were ≥18 years. No significant difference was found in terms of age and gender differences for amoebiasis cases (p>0.999). A total of 340 cases were included in the study and equally divided into two groups according to the adhesin test results: adhesin-negative (ANAG, n=170) and adhesin-positive (APAG, n=170). The comparison of hematological and inflammatory parameters between the groups is presented in Table 1. Although the C-reactive protein (CRP) level was slightly higher in the APAG group compared to the ANAG group, the difference was not statistically significant (p=0.061). Lymphocyte counts (×10³/μL) were significantly higher in the adhesin-negative group compared to the adhesin-positive group (p=0.004). Monocyte counts (×10³/μL) were also significantly higher in the adhesin-negative group (0.79±0.54) than in the adhesin-positive group (0.69±0.39) (p=0.048). No statistically significant differences were observed between the groups for other hematological parameters, including white blood cell (WBC), neutrophil, red blood cell (RBC), hematocrit, mean corpuscular volume (MCV), red cell distribution width (RDW), platelet, MPV, hemoglobin (Hb), and platelet distribution width values (p>0.05 for all).

When the indexes in both groups are examined, PLR was found to be significantly higher in the APAG group compared to those with the ANAG group [Wilcoxon test (W)=12212, p=0.017]. Nevertheless, there was no statistically significant difference in the LMR, MLR, MPVPCR, NLR, SII, and SIRI indexes obtained from haemogram between APAG and ANAG groups (Table 2).

In the adhesin-negative group, there were 84 females (49.7%) and 86 males (50.3%), while in the adhesin-positive group, there were 85 females (50.3%) and 85 males (49.7%). Considering the values of haemogram parameters among the amoebiasis cases, only the RBC value was found to be significantly higher in male sex than in female sex (W=2897, p=0.026). Similarly, there was no significant difference in CRP values between male and female cases with amoebiasis (p=0.849). There was no difference in the values of haemogram derivative indexes; NLR (p=0.747), PLR (p=0.855), MLR (p=0.286), LMR (p=0.176), MPVPCR (p=0.421), SII (p=0.756), and SIRI (p=0.807) between male and female amoebiasis cases.

Of 170 cases with positive adhesin test, 102 were ≥18 years of age and 68 were <18 years of age. Significant differences were observed between adult and paediatric amoebiasis cases in neutrophil (p=0.029), lymphocyte (p=0.013), eosinophil (p=0.031), MCV (<0.001), and MPV (p=0.04) values. Lymphocytes were higher in patients under 18, while the other parameters were higher in adults. Significant age-related differences were found in all haemogram-derived indices. NLR (W=2253, p=0.000), PLR (W=2841, p=0.046), MLR (W=2355.5, p=0.000), MPVPCR (W=2630, p=0.008), SII (W=2447, p=0.001), and SIRI (W=2276, p=0.000) were significantly higher in adults, while LMR was higher in patients under 18 years (W=4376.5, p=0.001).

According to the findings of the analysis, PLR showed a statistically significant difference with AUC=0.576 (p=0.016). The 95% confidence interval of PLR is between 0.515-0.637, and although it has statistical significance, the low AUC value indicates that it is not a clinically powerful marker. When the AUC values of other indexes were examined, AUC=0.551 for NLR (p=0.103), AUC=0.530 for MLR (p=0.338), AUC=0.470 for LMR (p=0.338), AUC=0.518 for MPVPCR (p=0.565), AUC=0.529 for SII (p=0.354), and AUC=0.513 for SIRI (p=0.689). In all of these parameters, AUC values did not show statistical significance and were found to be very close to 0.5 (Table 3 and Figure 1).

Additionally, this study evaluated the predictive performance of various haematological inflammatory parameters for adhesin positivity by ROC analysis. The most appropriate cut-off points for each parameter were determined by ROC analysis according to Youden index and diagnostic accuracy measures were calculated. In the results obtained with ROC analysis, SII has the highest sensitivity with 50.6%, and LMR has the highest specificity with

Table 1. Comparison of haemogram values according to adhesin test results

	Overall (n=340)	ANAG (n=170)	APAG (n=170)	p
CRP				0.061
Mean	28.59±50.33	26.57±50.53	30.61±50.19	
WBC				0.425
Mean	8.79±4.2	9.04±4.25	8.54±4.16	
Neutrophils (10^3/uL)				0.706
Mean	5.45±3.43	5.55±3.55	5.36±3.32	
Neutrophils (%)				0.166
Mean	59.24±17.46	58.14±16.57	60.35±18.28	
Lymphocytes (10^3/uL)				0.004
Mean	2.4±1.74	2.58±1.73	2.23±1.73	
Lymphocytes (%)				0.034
Mean	29.25±15.78	30.83±15.33	27.67±16.11	
Monocytes (10^3/uL)				0.048
Mean	0.74±0.47	0.79±0.54	0.69±0.39	
Monocytes (%)				0.312
Mean	9.28±7.94	9.88±9.65	8.68±5.7	
Eosinophils (10^3/uL)				0.514
Mean	0.18±0.4	0.16±0.17	0.21±0.53	
Eosinophils (%)				0.722
Mean	2.09±3.22	1.92±2.15	2.26±4.01	
Hb (g/dL)				0.325
Mean	12.35±2.33	12.47±2.33	12.22±2.33	
RBC (10^6/uL)				0.106
Mean	4.51±0.84	4.57±0.8	4.44±0.87	
HCT (%)				0.067
Mean	37.96±8.14	38.79±9.04	37.13±7.06	
MCV (fL)				0.911
Mean	84.31±8.94	84.09±8.85	84.54±9.05	
RDW (%)				0.414
Mean	16.04±21.82	14.96±2.81	17.11±30.74	
PLT (10^3/uL)				0.164
Mean	306.82±133.76	320.17±141.45	293.55±124.65	
MPV (10^3/uL)				0.087
Mean	10.08±1.33	10.21±1.06	9.96±1.56	
PDW (fL)				0.150
Mean	11.53±2.46	11.67±2.19	11.39±2.71	

ANAG: Adhesin test-negative acute gastroenteritis, APAG: Adhesin test-positive acute gastroenteritis, CRP: C-reactive protein, WBC: White blood cell, Hb: Hemoglobin, RBC: Red blood cell, HCT: Haemotocrit, MCV: Mean corpuscular volume, RDW: Red cell distribution width, PLT: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width

95.3% among the other indexes. Positive and negative predictive values were calculated at rates close to each other for all indexes (Table 4).

DISCUSSION

E. histolytica is an important cause of morbidity and mortality, especially in developing tropical regions with poor sanitation (18). There is currently no effective vaccine for prevention. The diagnosis of intestinal amoebiasis is based on clinical symptoms and laboratory diagnosis. Traditional methods, immunological tests, and molecular modalities are used in diagnosis. Microscopic evaluation is the primary used method throughout the world because it is relatively cheap, easy, and accessible. Another commonly used method is the identification of *E. histolytica* specific antigens by enzyme-linked immunosorbent assay (ELISA)

Table 2. The comparison of haemogram-derived inflammatory indices according to adhesin test result

	Overall (n=340)	ANAG (n=170)	APAG (n=170)	p
NLR				0.091
Mean	8.2±73.75	11.21±103.9	5.19±9.91	
PLR				0.017
Mean	211.59±415.96	156.45±101.86	266.41±574.16	
MLR				0.394
Mean	0.51±1.08	0.39±0.32	0.64±1.48	
LMR				0.338
Mean	3.78±2.82	3.85±2.98	3.71±2.65	
MPVPCR				0.578
Mean	0.04±0.04	0.04±0.05	0.04±0.03	
SII				0.323
Mean	2611.33±26836.57	3807.11±37810.59	1415.56±3485.33	
SIRI				0.764
Mean	5.06±34.55	6.13±47.78	3.99±10.49	

ANAG: Adhesin test-negative acute gastroenteritis, APAG: Adhesin test-positive acute gastroenteritis, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, MPVPCR: Mean platelet volume/platelet count ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammation response index

Table 3. Area under the curve values calculated for haemogram-derived indices

Test result variable(s)	Area	Standard error	p	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
NLR	0.551	0.031	0.103	0.490	0.613
PLR	0.576	0.031	0.016	0.515	0.637
MLR	0.530	0.032	0.338	0.468	0.592
LMR	0.470	0.032	0.338	0.408	0.532
MPVPCR	0.518	0.032	0.565	0.456	0.580
SII	0.529	0.031	0.354	0.468	0.591
SIRI	0.513	0.032	0.689	0.451	0.574

NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, MPVPCR: Mean platelet volume/platelet count ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammation response index

tests. The advantage of these tests is that they are largely capable of distinguishing between *E. histolytica* and non-pathogenic *E. dispar.* In addition, the sensitivity of ELISA tests is 80-94% and specificity ranges from 94-100%, which means that perform better than microscopic and culture methods (19-20). Although the sensitivity of molecular and serological diagnostic methods for the determination of intestinal amoebiasis is quite high, microscopic examination is widely used especially in developing countries. Haemogram parameters may be useful in diagnosing symptomatic cases of amoebiasis wherever access to advanced laboratory facilities is limited (19).

Platelet size has been shown to represent platelet function. It appears to be a beneficial predictive and prognostic biomarker in cardiovascular disorders, rheumatological diseases and cancers. Evidence from the literature demonstrates that MPV can reveal valuable data on the outcome and prognosis of various pathological disorders, for instance cardiovascular illnesses, respiratory disorders, rheumatological diseases and malignancies (21). In preliminary studies, MPV was evaluated as a positive acute phase reactant, whereas in some studies it was reported as a negative acute phase reactant. Turhan et al. (22) detected much higher MPV levels in the inactive hepatitis B group compared to the controls. In one former investigation, it was observed that while platelet count decreased in the first three days of infection, MPV value increased significantly in patients with sepsis caused by Gram-positive bacteria (23). On the contrary, Mete et al. (24) reported that MPV value was significantly lower in children with rotavirus gastroenteritis compared to the control group. In another study, increased platelet counts and lower MPV levels were determined in patients with amoebiasis compared to the control group (25). In a current study conducted in Türkiye, with adult amoebiasis cases, MPV level was detected to be lower than the control, but the difference found statistically insignificant (26). In our study, similar to the previous study, mean MPV levels were found decreased in the APAG group compared to the ANAG group, but this difference was not statistically significant (p=0.087). This result suggests that low MPV level may be associated with increased platelet activation in cases with amoebiasis, but it is not possible to reach a definite conclusion due to the small sample size in order to reach statistically significant results. Similar to the data of Tatliparmak et al. (26), no significant difference was found between APAGs and ANAG group in terms of CRP value and WBC count in our study. Furthermore, in our study, blood monocyte, and lymphocyte levels among haemogram parameters were found to be statistically lower in the APAG group. These findings could suggest that host innate and adaptive immune system cells play important roles together in the elimination of *E. histoloytica* trophozoites. Macrophages, well known as the tissue form of monocyte cells in the blood, are actively involved in host defense in intestinal amoebiasis. Macrophages activated with interferon-gamma released by lymphocytes, show amoebicidal activity with the nitric oxide that they produce (27). It is thought that macrophages are localized in the intestinal tissue and therefore blood monocyte levels are low in our cases with intestinal amoebiasis.

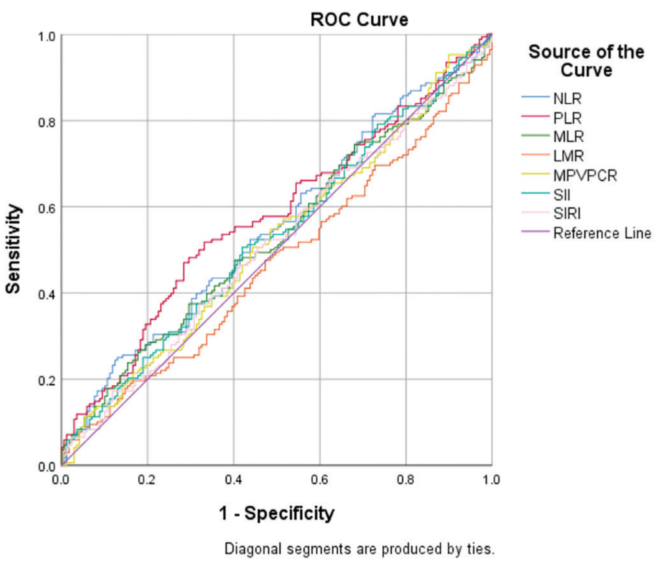

Figure 1. Predictive values of some complete blood count parameters and hemogram-derived markers for amoebiasis

ROC: Receiver operating characteristic, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, MPVPCR: Mean platelet volume/platelet count ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammation response index

The difference in RBC values between male and female patients is most likely due to physiological and hormonal factors rather than amoebiasis. Males generally have higher erythrocyte and Hb levels because androgens stimulate erythropoiesis, while menstrual blood loss plays only a minor role (28,29). Therefore, the sex-based variation in RBC values observed in this study is considered a normal physiological finding rather than a disease-related alteration.

In accordance with the recent literature findings, the levels of indices such as SII, SIRI, PLR and NLR, which are calculated from hemogram parameters, has shown an elevated level in bacterial infections (12,23,30). In one recent study, NLR was reported as a potential biomarker for disease severity and mortality in COVID-19 patients (31). In a former study including viral, bacterial and parasitic gastroenteritis cases, the lowest NLR value was found in the viral group, while the difference in level of the NLR between the groups was not significant (32). A recent study in children with rotavirus gastroenteritis reported that higher values of NLR and PLR were related to hospitalization severe clinical course and enhanced inflammation (33). Studies in parasitic infections have demonstrated the clinical relevance of hemogram-derived indices. In children with malaria, NLR and MLR were significantly higher in complicated cases compared to uncomplicated ($p=0.023$) and healthy groups ($p<0.001$), and both indices correlated positively with parasite density ($r=0.623$, $p=0.022$) (13).

Similarly, another study (34) showed that malaria patients had higher systemic inflammatory biomarkers (NLR, MLR, PLR) than healthy individuals, with NLR exhibiting excellent diagnostic accuracy (AUC=0.937; sensitivity 86.7%, specificity 92.0%), highlighting its potential as a simple and cost-effective marker of inflammation and disease severity in parasitic infections.

Additionally, platelet parameters such as platelet count and plateletcrit (PCT) were significantly altered, with platelet count and PCT together serving as highly sensitive and specific markers for malaria diagnosis (99.0% and 95.0%, respectively), underscoring the utility of hemogram and platelet indices as cost-effective indicators of disease severity in endemic areas (35). In patients with schistosomiasis mansoni, splenomegaly was found to be directly associated with hematological alterations, including thrombocytopenia, leukopenia, and anemia, indicating the diagnostic and prognostic value of blood indices in parasitic diseases (36).

It has been reported that cases with hepatic alveolar echinococcosis with low preoperative NLR and PLR values have a superior 5-year survival in compared to cases with high values. In these cases, the lower SII values have been associated with a better prognosis (37).

Table 4. Sensitivity, specificity, PPV and NPV values of diagnostic parameters according to ROC analysis results

Parametre	Cut-off	Sensitivite	Spesifite	PPV	NPV
NLR	5.019	0.247	0.876	0.667	0.538
PLR	164.796	0.471	0.716	0.625	0.573
MLR	0.506	0.259	0.824	0.595	0.526
LMR	7.581	0.089	0.953	0.652	0.513
MPVPCR	0.034	0.5	0.559	0.531	0.528
SII	696.576	0.506	0.582	0.548	0.541
SIRI	2.002	0.371	0.682	0.538	0.52

PPV: Positive predictive value, NPV: Negative predictive value, ROC: Receiver operating characteristic, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, MPVPCR: Mean platelet volume/platelet count ratio, SII: Systemic immune-inflammation index, SIRI: Systemic inflammation response index

In previous research, NLR, MLR, PLR, and SII rates were observed to be positively correlated with malaria parasitaemia (38). In a retrospective study investigating *Enterobius vermicularis*-associated appendicitis in comparison to acute appendicitis, no statistically significant differences were identified in commonly used inflammatory markers, including CRP, WBC, RDW, lymphocyte, and neutrophil counts, NLR, monocyte and eosinophil counts, platelet, and PLR (p>0.05). However, the SII was found to be significantly lower in pediatric patients with Enterobius-associated appendicitis. Histopathological examination further revealed that most of these patients exhibited no evidence of inflammation in their biopsy specimens (39). Hematological indices such as NLR and PLR, previously shown to correlate with disease severity in malaria, also hold potential as accessible markers in amebiasis. In malaria, elevated NLR and MLR were associated with higher parasite densities and complicated cases, demonstrating their utility in early risk stratification and monitoring therapeutic response (13).

Similarly, in amebiasis, these ratios may reflect systemic inflammation and immune-mediated alterations, providing adjunctive information beyond conventional diagnostic methods. However, their clinical applicability is limited. Despite PLR reaching statistical significance in our study (AUC=0.576, p=0.016), the overall low AUC values of PLR and other hematological indices (NLR, MLR, LMR, MPV/PC, SII, SIRI) highlight their limited clinical relevance, indicating that these parameters may assist in evaluating *E. histolytica* infections but are insufficient as independent diagnostic tools. Factors such as variable cut-off values, demographic differences, comorbid conditions, nutritional status and methodological inconsistencies further restrict their standalone use (40). Therefore, while NLR and PLR can serve as supportive markers, they should complement, rather than replace, standard diagnostic approaches, and further research is needed to validate their routine application in parasitic infections like amebiasis.

To our knowledge, there are no previous studies directly comparing age-related differences in immune or inflammatory responses in amoebiasis. However, the observed differences in haemogram-derived indices between paediatric and adult cases in our study may reflect the maturation and functional shifts of the immune system that occur with age. Higher NLR, PLR, MLR, SII, and SIRI values in adults suggest a more pronounced innate and proinflammatory response, whereas higher LMR values in younger patients may indicate a relatively stronger adaptive lymphocyte-mediated activity. These findings may therefore represent age-dependent variations in the host's immune response to *E. histolytica* infection.

Numerous studies are available for the prognostic use of indices derived from haemogram parameters in a wide range of conditions, including bacterial infections, sepsis, cancer, and autoimmune diseases (11,12,23,30,31). Nevertheless, there are limited number of analyses investigating these parameters in parasitic infections (13-15). To the best of our knowledge, this is the first study evaluating the biomarker potential of NLR, PLR, MLR, LMR SII, SIRI indices in the differentiation of cases with amoebiasis from healthy controls. In the present study, PLR was observed to be significantly elevated in the APAG group, aligning with the findings of Idemudia et al. (38), who demonstrated a positive correlation between PLR and malaria parasitemia. The lack of significant differences in parameters such as NLR, SII, SIRI, MLR, and LMR may be attributed to the fact that intestinal amebiasis typically presents as a localized disease, without inducing a marked systemic inflammatory response.

Study Limitations

The study's insights should be interpreted within the context of several inherent limitations, including its retrospective design, limited sample size, and the insufficient data regarding extraintestinal involvement in amoebiasis cases. Furthermore, the lack of standardized cut-off values for inflammatory indices, the single-center nature of the study, and the absence of testing for other viral or bacterial gastroenteritis pathogens may have influenced the results. A further limitation is that observed differences in RBC, neutrophil, lymphocyte, eosinophil, MCV, and MPV across sex and age groups likely reflect normal physiological variation rather than amoebiasis-related changes. Changes in NLR, PLR, SIRI and SII are not specific to *E. histolytica* infection and may be influenced by other conditions. Therefore, relying solely on these biomarkers could lead to misclassification of patients, especially in cases with other enteric infections or inflammatory diseases.

Nevertheless, the key strength of this study is that, to the best of our knowledge, it is the first to investigate contemporary hemogram-derived indices, such as the SIRI and the SII, in the context of diagnosing a pathogen as prevalent as *E. histolytica*. This study can contribute a valuable foundation for future research in the field.

CONCLUSION

In conclusion, while only monocyte, lymphocyte, and PLR levels were found to be statistically significant among the hematological inflammatory parameters evaluated in the study, these biomarkers alone do not demonstrate sufficient sensitivity and specificity in predicting adhesin positivity. Therefore, to increase the prediction accuracy, comprehensive studies are needed in larger patient groups, where clinical findings are standardized and other causes of gastroenteritis are excluded.

*Ethics

Ethics Committee Approval: This study was conducted with the approval of Necmettin Erbakan University Drug and Non-Medical Device Research Ethics Committee (decision no: 2025/5656, date: 21.03.2025).

Informed Consent: Informed consent was not obtained because this was a retrospective study using previously collected data.

*Authorship Contributions

Surgical and Medical Practices: S.U., F.E.T., Concept: S.U., F.E.T., Design: S.U., F.E.T., Data Collection or Processing: S.U., B.Y., Analysis or Interpretation: S.U., F.E.T., Literature Search: S.U., Writing: S.U., F.E.T.

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Evaluation of the Agreement of Indirect Hemagglutination Test with Radiologic Methods in the Diagnosis of Cystic Echinococcosis: A Retrospective Analysis of Two-year Laboratory Results

Kistik Ekinokokkoz Tanısında İndirekt Hemaglütinasyon Testinin Radyolojik Yöntemlerle Uyumunun Değerlendirilmesi: İki Yıllık Laboratuvar Sonuçlarının Retrospektif Analizi

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ABSTRACT

Objective: Many studies have been conducted to determine the distribution map of cystic echinococcosis (CE) in our country. However, there is no data from Uşak province in the literature. One of the aims of our study is to present the first hospital data on CE disease from Uşak province. Clinical findings, radiologic, and serologic methods are used in the diagnosis. Many studies have been conducted on the role of these identification methods in the diagnosis of CE. Another aim of our study was to evaluate the contribution of indirect hemagglutination (IHA) method and radiologic imaging results to the diagnosis of CE.
Methods: In our study, IHA results of 320 patient serum sent to our laboratory with suspected CE were obtained through the hospital information management system. Demographic data such as age, gender and imaging reports such as computed tomography, magnetic resonance imaging and ultrasonography were accessed from the hospital information system. Statistical analysis of the data obtained was evaluated using SPSS 25 package program.
Results: Hospital data from Uşak province were presented for the first time and the prevalence was calculated as 19.37%. Unlike previous studies, IHA positivity was statistically higher ($p<0.001$) in the 21-40 age range compared to other age groups in our study. In the diagnosis of CE, the IHA test was found to be inadequate in early [World Health Organization (WHO) type 1] and late stage (WHO type 4-5) cases, while this correlation was better in active lesions.
Conclusion: We think that it would be useful to recommend titer follow-up instead of reporting patients with IHA results of 1/80 and 1/160 as negative and that the use of serologic methods as a supportive tool for radiologic imaging methods in the diagnosis of CE will contribute to accurate diagnosis and treatment follow-up.
Keywords: Echinococcosus, indirect hemagglutination, ultrasonography

ÖZ

Amaç: Kistik ekinokokkoz (KE) hastalığının ülkemizdeki dağılım haritasının tespit edilmesi için pek çok çalışma yapılmıştır. Ancak literatürde Uşak iline ait bir veri bulunmamaktadır. Çalışmamızın amaçlarından birisi KE hastalığına dair Uşak ilinden ilk hastane verilerini sunmaktır. Tanıda klinik bulgulardan, radyolojik, ve serolojik yöntemlerden faydalanılmaktadır. Bu tanımlama yöntemlerinin KE tanısındaki yerine dair pek çok çalışma yapılmıştır. Çalışmamızın bir diğer amacı ise KE tanısında kullanılan indirekt hemaglütinasyon yöntemi (İHA) ile radyolojik görüntüleme sonuçlarının tanıya katkısını değerlendirmektir.

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Yöntemler: Çalışmamızda KE şüphesi ile laboratuvarımıza gönderilen 320 hasta serumunun İHA sonuçları hastane bilgi yönetim sistemi üzerinden alınmıştır. Hastaların yaş, cinsiyet gibi demografik verilerine ve bilgisayarlı tomografi, manyetik rezonans görüntüleme, ultrasonografi gibi görüntüleme raporlarına hastane bilgi yönetim sisteminden ulaşılmıştır. Elde edilen verilen istatistiksel analizi SPSS 25 paket programı kullanılarak değerlendirilmiştir.

Bulgular: Uşak ilinden ilk kez hastane verileri sunulmuş olup prevalansı %19,37 olarak hesaplanmıştır. Önceki çalışmalardan farklı olarak 21-40 yaş aralığında İHA pozitifliğinin çalışmamızdaki diğer yaş gruplarına göre istatistiksel olarak daha fazla olduğu (p<0,001) görülmüştür. KE tanısında İHA testinin erken [Dünya Sağlık Örgütü (WHO) tip 1] ve geç evre (WHO tip 4-5) olgularda yetersiz olduğu, aktif lezyonlarda ise bu korelasyonun daha iyi olduğu tespit edilmiştir.

Sonuç: İHA sonucu 1/80 ve 1/160 sonuçlanan hastaları negatif raporlamak yerine titre takibinin önerilmesinin faydalı olacağını ve KE tanısında serolojik yöntemlerin radyolojik görüntüleme yöntemlerini destekleyici olarak kullanılmasının doğru tanı ve tedavi takibine katkı sağlayacağını düşünmekteyiz.

Anahtar Kelimeler: Ekinokokkoz, indirekt hemaglütinasyon, ultrasonografi

INTRODUCTION

Echinococcosis is classified by the World Health Organization (WHO) as one of the 17 neglected tropical diseases. It is a zoonotic infection resulting from the larval stages of cestode species belonging to the genus *Echinococcus* (1). The life cycle of *Echinococcus* species involves two mammalian hosts: a carnivorous definitive host (typically a dog) and a herbivorous intermediate host (often a sheep) (2,3). Humans can act as accidental intermediate hosts by ingesting eggs present in dog feces through inadequately washed fruits and vegetables, or by contaminating their hands through contact with contaminated soil or dogs (3,4). In such cases, depending on the species, one of three clinical forms may arise: the cystic form from *Echinococcus granulosus (E. granulosu) sensu lato*, the alveolar form from *Echinococcus multilocularis*, and the polycystic form from *Echinococcus vogeli* (2).

The most common form of echinococcosis is the cystic form, which can be found worldwide except in a few islands. In endemic regions, its incidence can reach as high as 200 cases per 100,000 individuals (5). In mitochondrial DNA sequencing studies, ten genotypes of *E. granulosus* have been identified, with genotype G1 being the most common cause of cystic echinococcosis (CE) in humans and the most widespread type globally (6). The dominant G1 genotype is frequently observed in regions where sheep farming is prevalent, particularly in countries such as western China, Kazakhstan, Kenya, Tunisia, Morocco, and Argentina (5,6).

Recent epidemiological studies have reported that our country is highly endemic for *E. granulosus sensu lato* (5). In a study conducted under the HERACLES project, which investigated the prevalence of CE in Bulgaria, Romania, and Türkiye, abdominal ultrasound (US) screenings were performed on 8,618 volunteers from our country, with 53 individuals (0.59%) found to be positive for abdominal CE (7). Although CE has been a notifiable disease in our country since 2005, the reported case numbers have been significantly lower than expected. Consequently, a revision was made in 2015, and as a result of compiling epidemiological studies conducted between 2009 and 2019, the incidence rate of the disease increased to 8.70 per 100,000 individuals (8).

CE often presents asymptomatically or with non-specific symptoms, leading to most diagnoses being made incidentally during imaging studies. The liver is the most commonly affected organ, making right upper quadrant pain and hepatomegaly the most frequent symptoms (9). The primary diagnostic method for CE is US, which is used for screening in endemic regions, staging, interventional treatments, and follow-up (4,9). Serological methods are frequently used to support the diagnosis. Among these methods, the indirect hemagglutination (IHA) test is often preferred due to its ease of application, low cost, and simplicity in evaluation (4).

In our study, the results of the IHA test for patient sera sent to the Medical Microbiology Laboratory of Uşak Training and Research Hospital with suspected CE were evaluated retrospectively. The demographic data of the patients, such as age and gender, along with reports from US, computed tomography (CT), and magnetic resonance imaging (MRI), were analyzed to investigate the effectiveness of the IHA test in diagnosis and to compile the first hospital data on CE cases observed in Uşak province.

METHODS

Data Collection

The results of the IHA test for patient sera sent to our laboratory with suspected CE between September 2021 and August 2023 were evaluated retrospectively. For duplicate cases, only the first sample was included in the study. Demographic data such as age and gender, as well as reports from US, CT, and MRI, were obtained using the hospital information management system (HIMS).

IHA Test

Anti-*E. granulosus* antibodies were investigated using a commercial IHA test kit (ELITech Microbio, France) in patient serum samples. The test was conducted using U-bottom microplates with serum dilutions of 1/80, 1/160, 1/320, and 1/640. The absence of sediment or the presence of irregular sediment resembling lace in the antigen-coated red blood cell suspension was considered positive, while a point-like sediment at the center of the wells was deemed negative. In accordance with the manufacturer's recommendations, titers of 1:320 and above were reported as positive. A titer of 1:160 was considered suspicious, and it was recommended that samples be repeated 2-3 weeks later. Titers of 1/80 and below were interpreted as negative.

Radiological Evaluation

The most recent US, CT, and MRI examinations of the patients conducted prior to the IHA test requests were reviewed through the HIMS. The US reports were evaluated, while the CT and MRI examinations were assessed through the hospital's picture archiving and communication system using multiplanar analysis in axial, coronal, and sagittal planes. The imaging findings were evaluated retrospectively by the same radiologist. Patients with findings suggestive of CE in the liver were radiologically classified according to the WHO Informal Working Group on Echinococcosis classification (10). According to this classification: type 1 includes unilocular anechoic cysts, cysts containing echogenicities within the hydatid material, or cysts exhibiting a double contour on the wall; type 2 comprises complex cysts with multiple septa that display a rosette or honeycomb appearance; type 3 encompasses cysts with membrane disintegration (lily pad appearance) and lesions containing daughter vesicles within a solid matrix; type 4 refers to solid, heterogeneous lesions that are hypoechoic or hyperechoic, resembling a wool ball; and type 5 includes solid lesions characterized by wall calcifications, all evaluated as indicative of hydatid cyst lesions.

Statistical Analysis

Statistical analyses of the data obtained in this study were conducted using SPSS version 25 (IBM Corp., Armonk, NY, USA). Descriptive statistics were employed to determine the percentage distributions of the data, and frequency tables were generated accordingly. The chi-square test was used to evaluate the statistical significance of the associations between two variables. A significance level of $p<0.05$ was established for all analyses.

Ethical Authorisation

Our study was approved by the decision of Uşak University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision no: 320-320-19, date: 15.02.2024), and the Helsinki Declaration criteria were taken into consideration.

RESULTS

Among the 320 patients tested for suspected CE, 174 (54%) were female and 146 (46%) were male. IHA test was positive in 62 patients, resulting in a seroprevalence rate of 62/320 (19.37%). The seroprevalence rate among female patients was 38/174 (21.84%), while it was 24/146 (16.44%) among male patients. When analyzed by age distribution, the highest seroprevalence was observed in the age group of 21-40 years (26/320, 8.13%). This age group also exhibited the highest prevalence when assessed separately by gender. The distribution of patients tested with the IHA test by age and gender is shown in Table 1.

When the radiological imaging studies of the patients for whom IHA test was requested were evaluated, the imaging studies of 30 patients, most of whom had negative IHA results, could not be reached. Upon reviewing the available radiological imaging, the rate of finding suggestive of CE in cases with negative IHA results was 55/258 (21.3%), whereas this rate was 56/62 (90.3%) in cases with positive IHA results. According to radiological imaging data in this patient population, seropositivity was 111/320 (34.6%). The detailed evaluation of radiological imaging reports according to the IHA results of the patients in whom IHA test was performed is shown in Table 2.

When the organ distribution in the images was analysed; cystic lesion in the liver was observed in 154 patients alone and in 28 patients with an additional organ. The images of 106/182 (58.2%) of these patients were evaluated in suggestive of CE. IHA was positive in 54/106 (51%) of these patients with a titre of 1/320 and above. While the rate of evaluation of radiological imaging of patients with 0-1/40 dilutions in suggestive of CE was quite low, this rate was higher at dilutions of 1/80 and above. The organ localisations in which cystic lesions were seen on radiological imaging according to the results of the IHA test are shown in detail in Table 3. The WHO classification of the lesions in the liver in which CE was considered was made according to the scoring and shown in Table 4.

Table 1. Distribution of samples tested by indirect haemagglutination by age and gender; n (%)

Age group	Gender	0-1/40	1/80	1/160	All negatives	1/320	1/640	All positives	T
0-20	F	15 (8.62)	2 (1.14)	1 (0.57)	18 (10.34)	2 (1.14)	1 (0.57)	3 (1.72)	**21 (12.07)**
	M	20 (13.69)	1 (0.68)	0 (0)	21 (14.38)	1 (0.68)	2 (1.36)	3 (2.05)	**24 (16.44)**
	T	35 (10.93)	3 (0.93)	1 (0.31)	39 (12.18)	3 (0.93)	3 (0.93)	6 (1.87)	**45 (14.06)**
21-40	F	16 (9.19)	4 (2.29)	2 (1.14)	22 (12.64)	4 (2.29)	12 (6.9)	16 (9.2)	**38 (21.84)**
	M	16 (10.95)	2 (1.36)	3 (2.05)	21 (14.38)	2 (1.36)	8 (5.48)	10 (6.85)	**31 (21.23)**
	T	32 (10)	6 (1.87)	5 (1.56)	43 (13.43)	6 (1.87)	20 (6.25)	26 (8.13)	**69 (21.56)**
41-60	F	45 (25.86)	6 (3.44)	5 (2.87)	56 (32.18)	4 (2.29)	9 (5.17)	13 (7.47)	**69 (39.66)**
	M	33 (22.6)	0 (0)	3 (2.05)	36 (24.65)	0 (0)	6 (4.11)	6 (4.11)	**42 (28.77)**
	T	78 (24.37)	6 (1.87)	8 (2.5)	92 (28.75)	4 (1.25)	15 (4.69)	19 (5.94)	**111 (34.69)**
61-80	F	33 (18.96)	2 (1.14)	3 (1.72)	38 (21.83)	0 (0)	6 (3.45)	6 (3.45)	**44 (25.29)**
	M	37 (25.34)	1 (0.68)	2 (1.36)	40 (27.4)	1 (0.68)	4 (2.73)	5 (3.42)	**45 (30.82)**
	T	70 (21.87)	3 (0.93)	5 (1.56)	78 (24.37)	1 (0.31)	10 (3.13)	11 (3.44)	**89 (27.81)**

Table 1. Continued

Age group	Gender	0-1/40	1/80	1/160	All negatives	1/320	1/640	All positives	T
>80	F	2 (1.14)	0 (0)	0 (0)	2 (1.14)	0 (0)	0 (0)	0 (0)	**2 (1.14)**
	M	3 (2.05)	0 (0)	1 (0.68)	4 (2.74)	0 (0)	0 (0)	0 (0)	**4 (2.74)**
	T	5 (1.56)	0 (0)	1 (0.31)	6 (1.87)	0 (0)	0 (0)	0 (0)	**6 (1.88)**
All ages	F	111 (63.79)	14 (8.04)	11 (6.32)	136 (78.16)	10 (5.74)	28 (16.1)	38 (21.84)	**174 (100)**
	M	109 (74.65)	4 (2.73)	9 (6.16)	122 (83.56)	4 (2.73)	20 (13.7)	24 (16.44)	**146 (100)**
	T	**220 (68.75)**	**18 (5.62)**	**20 (6.25)**	**258 (80.65)**	**14 (4.37)**	**48 (15)**	**62 (19.37)**	**320 (100)**

F: Female, M: Male, T: Total

Table 2. Evalution of radiological imaging results of samples tested by indirect haemagglutination; n (%)

IHA results	Samples without radiological imaging	Samples with radiological imaging	Presence of a cyst (+)	Finding suggestive of CE (+)	Total number of samples (n)
0-1/40	24 (10.9)	196 (89.1)	143 (65)	29 (13.1)	220 (100)
1/80	3 (16.7)	15 (83.3)	13 (72.2)	13 (72.2)	18 (100)
1/160	2 (10)	18 (90)	14 (70)	13 (65)	20 (100)
All negatives	**29 (11.2)**	**229 (88.8)**	**170 (65.9)**	**55 (21.3)**	**258 (100)**
1/320	1 (7.1)	13 (92.9)	13 (92.9)	13 (92.9)	14 (100)
1/640	0 (0)	48 (100)	46 (95.8)	43 (89.5)	48 (100)
All positives	**1 (1.6)**	**61 (98.4)**	**59 (95.2)**	**56 (90.3)**	**62 (100)**
Total	**30 (9.4)**	**290 (90.6)**	**229 (71.6)**	**111 (34.6)**	**320 (100)**

IHA: Indirect hemagglutination, CE: Cystic echinococcosis

Table 3. Distribution of organs with cystic lesions in the radiological imaging of IHA-tested specimens with cystic lesions/suggestive of CE

Organ localisation of the lesion	0-1/40	1/80	1/160	1/320	1/640	Total
Liver	84/26	12/12	11/11	11/11	36/36	154/96
Lung	1/0	0	0	0	2/1	3/1
Spleen	7/0	0	0	0	1/1	8/1
Kidney	27/0	0	1/0	0	2/0	30/0
Surrenal gland	1/0	0	0	0	0	1/0
Mediastinum	2/1	0	0	0	0	2/1
Over	1/0	0	0	0	0	1/0
Retroperitoneum	2/1	0	0	0	0	2/1
Liver and lung	1/1	0	0	1/1	2/2	4/4
Liver and retroperitoneum	0	0	0	0	1/1	1/1
Liver and kidney	13/0	0	1/1	0	0	14/1
Liver and spleen	1/0	0	1/1	0	0	2/0
Liver and paracolic area	0	1/1	0	0	0	1/1
Liver and adrenal gland	1/0	0	0	0	0	1/0
Liver and pancreas	1/0	0	0	0	0	1/0
Liver, spleen, intraabdominal	0	0	0	1/1	1/1	2/2
Liver, lung, kidney	1/0	0	0	0	0	1/0
Liver, kidney, subcutaneous fat tissue	0	0	0	0	1/1	1/1
Total	**143/29**	**13/13**	**14/13**	**13/13**	**46/43**	**229/111**

IHA: Indirect hemagglutination, CE: Cystic echinococcosis

Table 4. WHO classifications of lesions with cysts in the liver on radiological imaging and IHA results

	CE (-)		WHO 1		WHO 2		WHO 3		WHO 4		WHO 5		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0-1/40	75	100	3	14.3	5	15.2	0	0	11	61.1	8	36.4	102	56.1
1/80	0	0	4	19	3	9	2	15.4	1	5.6	3	13.6	13	7.1
1/160	0	0	3	14.3	5	15.2	3	23.1	0	0	2	9.1	13	7.1
All negatives	**75**	**100**	**10**	**47.6**	**13**	**39.4**	**5**	**38.5**	**12**	**66.7**	**13**	**59.1**	**128**	**70.3**
1/320	0	0	2	9.5	3	9	1	7.7	3	16.7	4	18.2	13	7.1
1/640	0	0	9	42.9	17	51.6	7	53.8	3	16.7	5	22.7	41	22.6
All positives	**0**	**0**	**11**	**52.4**	**20**	**60.6**	**8**	**61.5**	**6**	**33.3**	**9**	**40.9**	**54**	**29.7**
Total	**75**	**100**	**21**	**100**	**33**	**100**	**13**	**100**	**18**	**100**	**22**	**100**	**182**	**100**

IHA: Indirect hemagglutination, CE: Cystic echinococcosis, WHO: World Health Organization

DISCUSSION

CE is widely distributed disease, with endemic areas on every continent. It is particularly common in Mediterranean countries, specific regions of Russia, Western China, Central Asia, Australia, South America, and many temperate climate zones (6). Although the mortality rate from CE is relatively low (0.2 per 100,000 individuals), the disease contributes substantially to morbidity and results in significant workforce productivity losses. Despite its preventable nature, CE remains underreported and often overlooked. For this reason, the WHO has classified it as a neglected tropical disease, with a goal of control or eradication by 2030. Nevertheless, with an estimated 2-3 million cases occurring annually worldwide, achieving this goal remains a considerable challenge (3).

Ultrasonography is the primary diagnostic method for CE, also serving an essential role in disease staging, interventional treatments, and follow-up. Serological methods are frequently used to support the diagnosis, with the IHA being a preferred option due to its ease of use, low cost, and straightforward evaluation process (4,9). In cases suspected of CE, the IHA test is commonly applied in our country as an auxiliary diagnostic tool. Studies conducted on patients with a preliminary diagnosis of CE report a seropositivity rate of 10-20% (4,11-14). For example, in an 8-year study from Adıyaman covering January 2013 to December 2020, 244 out of 1,607 patients (15.18%) tested positive for CE using the IHA method (11). Similarly, 9.5% of patients tested at Erzurum Regional Training and Research Hospital between 2009 and 2013 were IHA positive for CE (12). Another study in Bolu, conducted between 2013 and 2018, found a 10.6% seropositivity rate among 644 patients whose serum samples were sent for CE testing (13). In Balıkesir, a retrospective study of IHA data from 2011 to 2013 reported a seropositivity rate of 19.8% (14). Our study yielded comparable seropositivity rates, with 62 of 320 patients (19.37%) testing positive at a titer of 1/320 or above by IHA during the two-year period from September 2021 to August 2023.

Many studies have found that the seropositivity rate in the IHA is higher in women compared to men (11-13). An exception to this trend was observed in a study conducted in İzmir, which analyzed two years of data and reported a higher seropositivity rate in men (4). However, considering that the study period coincided with the coronavirus disease-2019 (COVID-19) pandemic, a new study incorporating data from a broader timeframe found that between 2017 and 2020, seropositivity rates in female patients were indeed higher than in male patients. Additionally, a statistically significant decrease in IHA positivity rates during the pandemic was reported (15). In our study, the seropositivity rate was higher in female patients (21.84%) compared to male patients (16.44%). However, no significant difference was observed between the sexes regarding IHA test positivity (r=1.482, p=0.223).

In the study conducted by Topluoğlu (16) which presented the case data of CE reported to the Ministry of Health, the incidence rate exceeded 1.60 per 100,000 in 19 provinces. The top three provinces in this ranking were Van, Ağrı, and Iğdır. A regional analysis of the reported data showed that eight provinces were located in the Eastern Anatolia Region and six in the Central Anatolia Region. Reviews of studies calculating CE frequency based on IHA test results indicated that the findings were independent of geographical regions. For example, Yılmaz et al. (12) identified a seropositivity rate of 9.5% (191/2009) in Erzurum, while Bagcı et al. (4) reported a rate of 14.12% (75/531) in İzmir. These differences could have arisen from reporting issues concerning CE cases and the lack of sufficient data from other provinces. In the Aegean Region, where our study took place, other research showed seropositivity rates of 13.39% (28/209) in Aydın and 10.1% (49/483) in Manisa (17,18). In our study, we detected the first CE seroprevalence data specific to Uşak province, with a rate of 19.37% (62/320), which was higher than those reported in previous regional studies. This higher rate may have come from more common animal husbandry practices in Uşak compared to the two cities that reported data.

Tevfik et al. (19) reported the highest seropositivity rate (24.01%) in the 21-30 age group, a finding corroborated by Aldemir et al. (20) who identified a similar rate of 23.07% in the same age range. Ulusan Bağcı's (15) study also indicated that the most common age range for diagnosis, both before and after the COVID-19 pandemic, was 20-39 years. In our study as well, the highest IHA test positivity rate was found in the 21-40 age group (41.9%). This age group showed a statistically higher IHA positivity rate compared to other age groups (r=20.462, p<0.001).

Among the 320 samples included in the study, 9.4% lacked any recorded radiological imaging in the hospital information system (HIS). Of the 258 samples that tested negative by the IHA test, 170 (65.9%) showed cysts in radiological images. However, only 55 of these (21.3%) were assessed as indicative of CE. This rate was found to be 13.1% at dilutions of 1/40 or lower, 72.2% at a 1/80 dilution, and 65% at a 1/160 dilution. These findings suggest that serological testing can support radiological examinations within the diagnostic algorithm.

In the study, radiological imaging data were unavailable in the HIS for one of the 62 patients who tested positive for IHA at titers of 1/320 or higher. Among patients with a seropositivity of 1/320, all available radiological examinations revealed findings consistent with CE. For the 48 patients with 1/640 seropositivity, two patients had a history of surgery for CE, explaining the absence of cysts in their imaging results. Three additional patients with 1/640 titers and visible cysts on imaging had also undergone surgery for CE, yet their current imaging suggested lesions inconsistent with CE. These cystic lesions were instead classified as Bosniak category 1 cysts in the kidneys.

In the analysis of negative IHA cases, only 13.1% of samples with 0-1/40 titers showed radiological findings indicative of CE, while samples with 1/80 and 1/160 titers had findings at rates of 72.2% and 60%, respectively. Bagcı et al. (4) also noted CE indicators in 60.7% and 73.3% of cases at titers of 1/80 and 1/160, respectively. According to IHA test guidelines, a 1/80 result is considered negative, and patients with 1/160 results are advised to retest in 2-3 weeks. However, our findings show that a notable proportion of patients with 1/80 titers exhibited radiological indicators. Thus, rather than categorizing 1/80 titers as negative, we suggest that reporting these titers-similar to 1/160 results-along with a recommendation for repeat testing in several weeks, would improve the diagnostic and treatment process.

A detailed evaluation of the radiological images of the patients included in the study showed that 154 cases had cysts isolated to the liver, while 28 cases exhibited cysts in the liver along with one or more other organs or tissues. Among the 154 cases with liver-only cysts, 96 (62.3%) were evaluated as indicative of CE. The remaining 58 liver cysts were attributed to other causes, such as simple cysts, biliary hamartomas, polycystic kidney disease-related cysts, and metastases.

For the 28 cases with cysts in both the liver and additional organs or tissues, 10 cases (37%) were considered consistent with CE. Isolated kidney cysts were observed in 30 cases, and in 16 cases, kidney cysts were accompanied by cysts in one or more other organs or tissues. Nearly all kidney cysts were consistent with simple parenchymal cysts, commonly seen in middle-aged and elderly individuals. Among three cases with cysts isolated to the lungs, one case (33.3%) showed findings consistent with CE. In five cases with cysts in the lungs along with other organs or tissues, four cases (80%) were deemed suggestive of CE.

Our study aligns with the literature (4,11,13,21,22), identifying the liver as the most frequently involved organ in CE cases, observed in 96 out of 111 cases (86.5%), followed by combined liver and lung involvement, observed in 4 out of 111 cases (3.6%).

In our study, patients with CE findings in the liver were evaluated, and it was observed that those with a titer of 1/640 were primarily classified under WHO type 2 (53.8%), indicative of viable cysts. Conversely, patients with CE findings in the liver who were seronegative (titer 0-1/40) were predominantly categorized under the inactive cyst groups, WHO type 4 (61.1%) and type 5 (36.4%) (Table 4). Previous studies have reported high false-negative rates for early and late-stage cysts (4,23,24). Consistent with the literature, our study found that most CE patients with negative IHA results were classified under WHO types 1, 4, and 5.

Study Limitations

Our study has some limitations. As a retrospective, single-center study, US data were evaluated based on recorded reports, detailing the localization of CE lesions but not their dimensions.

CONCLUSION

In conclusion, this study provides the first hospital-based data on CE from Uşak province, with a calculated seropositivity rate of 19.37%. Consistent with the literature, no significant difference was found in CE prevalence between genders. Unlike previous studies, however, we observed a statistically higher IHA positivity among those aged 21-40 compared to other age groups in our study ($p<0.001$). The findings also indicate that the IHA test is less effective in diagnosing early (WHO type 1) and late-stage (WHO types 4-5) cases, but shows better correlation with active lesions. Given that CE was confirmed in 111 out of 229 patients with radiologically detected cystic lesions, we believe serological methods should complement radiological imaging to support accurate diagnosis and treatment.

*Ethics

Ethics Committee Approval: Our study was approved by the decision of Uşak University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision no: 320-320-19, date: 15.02.2024), and the Helsinki Declaration criteria were taken into consideration.

Informed Consent: The results of the IHA test for patient sera sent to our laboratory with suspected CE between September 2021 and August 2023 were evaluated retrospectively.

Footnotes

*Authorship Contributions

Design: B.G., Data Collection or Processing: B.G., T.B., Analysis or Interpretation: B.G., H.H.K., T.B., Literature Search: B.G., H.H.K., Writing: B.G., H.H.K., T.B.

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Incidence and Economic Impact of Fasciolosis in Ruminants from Slaughterhouses in Bejaia Province (Northern Algeria): A Retrospective Survey (2017-2023)

Bejaia Kenti (Kuzey Cezayir) Kesimhanelerindeki Ruminantlarda Fasciolozis İnsidansı ve Ekonomik Etkisi: Retrospektif Bir Araştırma (2017-2023)

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ABSTRACT

Objective: Fasciolosis is considered an important parasitic disease, and also a primary source of morbidity and mortality in ruminants with significant economic losses and public health. The objective of the present study was to determine the prevalence and the economic losses due to fasciolosis in ruminants from different local slaughterhouses in Bejaia province during 7-years.

Methods: Data were obtained from different municipal slaughterhouses, supervised by the Provincial Veterinary Inspection of Bejaia, from January 2017 to December 2023. A veterinary inspected routine animal carcasses at each stage of the slaughtering process in the slaughtering units on a regular and systematic basis.

Results: The overall prevalence of fasciolosis detected by post-mortem examination in cattle, sheep, and goats slaughtered during the study period was 1.58%, 0.04% and 0.02%, respectively. The desired absolute precision (d) of cattle, sheep and goats is 0.011%, 0.63% and 0.51%, respectively. In cattle, fasciolosis was significantly high in January, February, and March; and gradually decreased from April to September, then increased in October, November and December. On the other hand, the monthly cumulative prevalence rates of sheep and goats fasciolosis cases recorded was constant throughout the year. The high recorded prevalence was in autumn and winter was 1.92% and 1.78% in cattle, respectively ($p \leq 0.05$). An overall direct economic loss of 5,904,031€ was incurred during the period in this study from a totality of 12,321.2 kg of liver condemned. The indirect economic loss is due to carcass weight reduction as a result of fasciolosis infection was 44,746,454€.

Conclusion: Our study revealed that the prevalence of cattle fasciolosis was generally high compared to sheep and goats in the abattoir of Bejaia province associated with significant financial losses for butchers in the study area. Furthermore, livestock farmers should be made aware of the importance of this parasitose in order to reduce economic losses.

Keywords: Fasciolosis, economic loss, Algeria

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ÖZ

Amaç: Fasciolozis, önemli bir paraziter hastalık olarak kabul edilmektedir. Geviş getiren hayvanlarda morbidite ve mortalitenin birincil kaynağı olarak önemli derecede ekonomik kayıplara ve halk sağlığına yol açmaktadır. Bu çalışmanın amacı, Bejaia şehrindeki farklı yerel mezbahalardan gelen geviş getiren hayvanlarda 7 yıl boyunca fasciolozis prevalansını ve ekonomik kayıpları belirlemektir.

Yöntemler: Veriler, Ocak 2017'den Aralık 2023'e kadar Bejaia İl Veterinerlik Denetim Kurumu'nun denetimindeki farklı belediye mezbahalarından elde edilmiştir. Bir veteriner, mezbaha birimlerinde kesim sürecinin her aşamasında rutin olarak ve sistematik bir şekilde hayvan karkaslarını denetlemiştir.

Bulgular: Çalışma süresince kesilen sığır, koyun ve keçilerde postmortem muayene ile tespit edilen fasciolozis prevalansı sırasıyla %1,58, %0,04 ve %0,02 idi. Sığır, koyun ve keçiler için istenen mutlak kesinlik (d) sırasıyla %0,011, %0,63 ve %0,51'di. Sığırlarda fasciolozis, Ocak, Şubat ve Mart aylarında önemli ölçüde yüksekti; Nisan'dan Eylül'e kadar kademeli olarak azaldı, ardından Ekim, Kasım ve Aralık aylarında arttı. Öte yandan, kaydedilen koyun ve keçilerdeki fasciolozis olgularının aylık kümülatif prevalans oranları yıl boyunca sabit kaldı. Yüksek prevalans sonbahar ve kış aylarında sırasıyla sığırlarda %1,92 ve %1,78 olarak kaydedildi (p≤0,05). Bu çalışmada, toplam 12.321,2 kg karaciğerin imha edilmesi nedeniyle, söz konusu dönemde €5.904.031 tutarında doğrudan ekonomik kayıp meydana gelmiştir. Fasciolozis enfeksiyonu sonucu karkas ağırlığındaki azalma nedeniyle meydana gelen dolaylı ekonomik kayıp ise €44.746.454 olmuştur.

Sonuç: Çalışmamız, Bejaia ilindeki mezbahada sığırlarda fasciolozis prevalansının koyun ve keçilere kıyasla genel olarak yüksek olduğunu ve bu durumun çalışma alanındaki kasaplar için önemli mali kayıplara yol açtığını ortaya koymuştur. Ayrıca, ekonomik kayıpları azaltmak için hayvancılıkla uğraşan çiftçilerin bu parazitozun öneminin farkında olmaları gerekmektedir.

Anahtar Kelimeler: Fasciolozis, ekonomik kayıp, Cezayir

INTRODUCTION

Algeria is known for its diverse livestock population. According to the National Statistics Office, the local livestock is estimated at thirty-eight million of heads, including cattle, sheep, goats, camels, and equines. In addition, an average of five million tons of red meat is recorded per year. However, animal diseases of parasitic origin have a significant impact on animal productivity as well as human health. Furthermore, the Algerian authorities have adopted a policy aimed at increasing animal production through surveillance and animal health control in order to safeguard the animal herd and ensure food security.

Fasciolosis is considered an important parasitic disease, and also a primary source of morbidity and mortality in ruminants, with significant economic losses and public health consequences (1,2). It is brought on by two genuses of *Fasciola*: *F. gantica*, which is restricted to Africa and Asia, and *F. hepatica*, which is found all over the world (3). The route of fasciolosis transmission is ingestion of metacercaria-infested plants or water contaminated with floating metacercariae in animals and humans (4). Fasciolosis is defined by chronic, acute, or subacute inflammation of the liver and bile ducts, as well as submandibular oedema, anemia, general intoxication, or even death of the animal (5). Significant economic losses are huge caused by fasciolosis such as decreased weight gain, poor carcass quality, decreased milk yield, high treatment and control costs, mortality, and as well as the condemnation of infested livers at abattoirs (6-9).

It is reported that fasciolosis affects about 700 million herbivorous domestic animals worldwide and causes three billion US$ or more is the annual loss in farm animals as a sequence of productivity losses (10,11). In Algeria, numerous studies have been conducted on post-mortem fasciolosis disease in ruminants (12-15); however, there is very little investigation regarding the economic impact of fasciolosis in animals slaughtered (16). There is a need to continually evaluate the fasciolosis incidence and to estimate the economic losses of the liver condemnations caused by fasciolosis parasites in order to management of this pathology. Thus, the objective of the present study was to determine the prevalence and the economic losses due to fasciolosis in ruminants from different local slaughterhouses in Bejaia province from 2017 to 2023.

METHODS

Study Area

The investigation was conducted in the Province of Bejaia (36°43'N, 5°04'E), which is located in Northern Algeria and has a land area of 326,826 square kilometer (km^2) (Figure 1). The study area is composed mainly of several species of trees and natural or cultivated herbs, with the climate in the Mediterranean Region. Meteorological data presented in the present study were obtained from the website https://fr.tutiempo.net/climat/ws-604020.html (Table 1) (17).

Slaughterhouse Postmortem Inspection Procedure

Data were obtained from different municipal slaughterhouses, supervised by the Provincial Veterinary Inspection of Bejaia, from January 2017 to December 2023. A veterinary inspector inspected animal carcasses at each stage of the slaughtering process in the slaughtering units on a regular and systematic basis. The routine slaughterhouse inspection as previously described by Bensid (18). The diagnosis of fasciolosis was done by visual examination and palpation of the liver's entire surface. Two incisions (long and shallow; short and deep) on the visceral surface of the liver (right, left, and the base of the caudate lobes) to inspect the bile ducts for cholangitis lesions related to the presence of flukes (Figure 2).

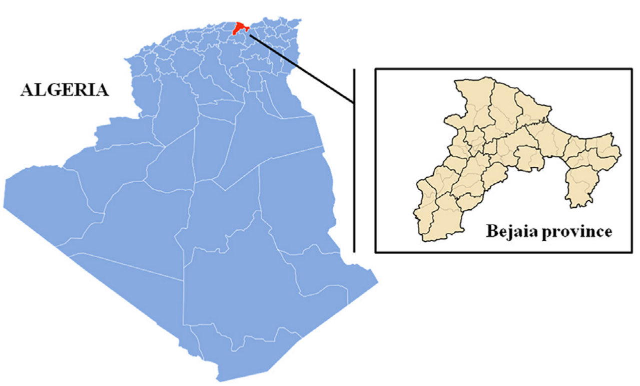

Figure 1. Map of Bejaia province, Algeria

Sample Size Determination

The sample size of the study was determined using the formula given by Thrusfield and Christley (19) with an expected prevalence of 0.6%, since there was no study conducted in the area, a confidence level of 95% and a required absolute precision of 5%.

$N = 1.96^2 * P*(1 - P)/d^2$

Where N = required sample size. P = observed prevalence, d = desired absolute precision at 5%.

Prevalence Determination

The overall fasciolosis prevalence of the three animal species (cattle, sheep, and goats) was calculated from the collected data over the period from 2017 to 2023. The number of slaughtered animals infected with *Fasciola* was reported monthly and annually. The annual fasciolosis prevalence (%) rate was calculated as the number of animals with *Fasciola* parasite divided by the number of animals examined postmortem. The seasonal prevalence (%) was also determined by calculating the total number of animals with the *Fasciola* infection, recorded during all four seasons (spring, summer, autumn, and winter), divided by the total number of animals slaughtered, and examined for each season.

Economic Losses Estimation

The following elements were used to estimate economic losses: average weight of liver, monthly fasciolosis prevalence, and monthly liver price per kg. The economic analysis was conducted by taking the average cost of the monthly selling price of liver in the area study. Financial losses were calculated in Algerian Dinar (DZD) and then converted into Euro (€). The exchange rate considered was different depending on the year. Direct economic losses due to liver condemnation caused by ruminant fasciolosis were assessed by considering the overall prevalence of the disease in the abattoir and the retail market price of an average liver. Indirect economic losses were assessed by estimating

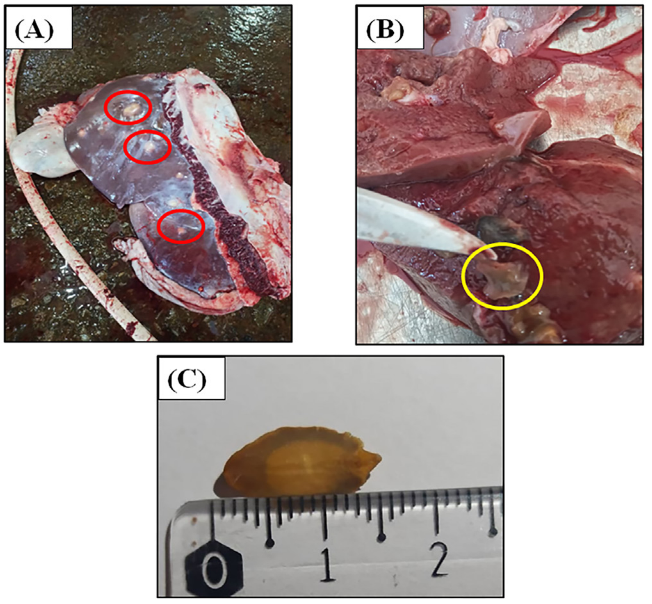

Figure 2. Dorsal side of diseased liver with fluke lesions (A); ventral side of diseased liver with flukes in the bile ducts (B); liver fluke specimen collected in slaughterhouse from infected ruminants of Bejaia province (Algeria) (C)
Yellow circle: Liver with fluke lesions, Red circle: Flukes in the bile ducts

Table 1. Data of temperature, relative humidity and precipitation of different seasons (2017-2023) in Bejaia province, Algeria

Years	2017	2018	2019	2020	2021	2022	2023	All
Winter								
T° min (T °C)	8.27	6.5	7.4	9.1	8.03	7.67	9	**8.00**
T° max (T °C)	17.17	14.8	15.97	18.73	17.7	16.33	18.67	**17.05**
Rainfall (mm)	54.13	78	125.33	81	130.3	111.33	91.87	**95.99**
Humidity (%)	76.17	76.03	77.30	72.40	67.30	72.13	67.10	**72.63**
Spring								
T° min (T °C)	11.1	11.87	13.23	12.63	11.67	11	12	**11.93**
T° max (T °C)	19.8	20.63	22.5	21.13	21.33	21	21	**21.06**
Rainfall (mm)	106.4	74.33	67.67	69.33	79.3	52.6	31.23	**68.69**
Humidity (%)	74.47	77.23	79.73	75.87	74.00	72.60	70.20	**74.87**
Summer								
T° min (T °C)	19.9	19.93	19.83	20.53	20.67	20.67	19.33	**20.12**
T° max (T °C)	29	29.1	29.23	28.87	28.67	30.33	28.67	**29.12**
Rainfall (mm)	6.4	4.67	6.67	15	27.57	22.93	4.2	**12.49**
Humidity (%)	73.53	75.97	74.30	70.20	70.00	70.67	68.77	**71.92**
Autumn								
T° min (T °C)	15.83	15.47	16.4	15.43	16	15.33	15.67	**15.73**
T° max (T °C)	25.53	24.87	26.8	23.73	24	25.33	24.33	**24.94**
Rainfall (mm)	129.13	112	119.67	112	41.7	29.77	29.4	**81.95**
Humidity (%)	72.87	73.23	72.60	70.30	70.63	62.47	64.03	**69.45**

the reduction in carcass yield of animals. Economic losses were calculated using the procedure described by Ogurinade and Ogunrinade (20), explained below:

Direct economic losses due to liver condemnation

LC = CSR * LCo * P

Where: LC = losses due to liver condemnation; CSR = average number of animals slaughtered at abattoir during the study period; LCo = average cost of one liver in Bejaia province, Algeria; P = prevalence of the fasciolosis at the study abattoir.

Indirect economic losses due to ruminant fasciolosis

CW = CSR * CL * MC * P * (n) kg

Where: CW = losses due to carcass weight reduction; CSR = average number of animals slaughtered at abattoirs during the study period; MC = average price for 1 kg of meat in Bejaia province, Algeria; P = prevalence of fasciolosis in the study abattoir; (n) kg = average carcass weight of animal; CL = carcass weight loss (10%) in individual animal due to fasciolosis (21). The adopted carcass weight loss in (kg) was estimated from fresh, normal and health looking, randomly selected from 20 dressed carcasses already passed for human consumption. These were weighed and the average weights of carcasses taken as the estimate weight of a particular carcass. Data on average animal carcass weight and the cost of one kilogram of meat were supplied by Department of Agricultural Statistics, Ministry of Agriculture and Rural Development, Algeria (22).

Statistical Analysis

All the data were entered, stored, and calculated in Microsoft Excel 2007. The retrospective data were analyzed using Statview (Version 4.55) and the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA). The data were also presented using descriptive statistics in the form of a table. Means were compared using One-Way Analysis of Variance to compare differences across multiple groups (years, months and seasons) and Tukey's HSD post-hoc test was applied to identify specific pairwise differences. The independent samples t-test was applied for the purpose of comparison in pairs of groups. Moreover, logistic regression models were additionally performed with infection status as the dependent variable, and species (cattle, sheep, and goats), season (winter, spring, summer, and autumn), year, and meteorological parameters (mean temperature, rainfall, and relative humidity) as independent predictors. In this analysis, a p-value less than 0.05 at the 95% confidence level was considered statistically significant.

RESULTS

Table 2 shows meat inspection data of the number of slaughtered animals (cattle, sheep, and goats) and of fasciolosis detection during a 7-year period (January 2017 to December 2023). A total of 131,961 cattle, 91,658 sheep, and 131,565 goats were slaughtered and inspected during the study period in Bejaia province (i.e., 37.2, 25.8 and 37%, respectively). The rate of slaughtered animals affected by fasciolosis (cattle, sheep, and goats) in the various municipal slaughterhouses in the Bejaia province was 0.6% (i.e., 2146 cases), with a desired absolute precision of 0.32%. The overall prevalence of fasciolosis detected by post-mortem examination in cattle, sheep and goats slaughtered during the study period was 1.58% [95% confidence interval (CI), 1.45-1.82%], 0.04% (95% CI, 0.01-0.08%) and 0.02% (95% CI, 0.006-0.05%), respectively. The distribution of infection by *Fasciola* sp. in slaughtered cattle shows a significant difference when compared to sheep or goats ($p \leq 0.05$). The desired absolute precision (d) of cattle, sheep and goats is 0.011%, 0.63%, and 0.51%, respectively.

A result of annual trends and monthly prevalence of infected animals by fasciolosis during the recording period (2017-2023) is illustrated in Figures 3 and 4, respectively. In cattle, the maximum rate of fasciolosis was 2.39% in the year 2020 and a minimum of 1.09% in the year 2018, as substantiated by analysis of variance followed by Tukey's test ($p \leq 0.05$). The overall annual prevalence rate of the disease showed significant fluctuation ($p \leq 0.05$) in

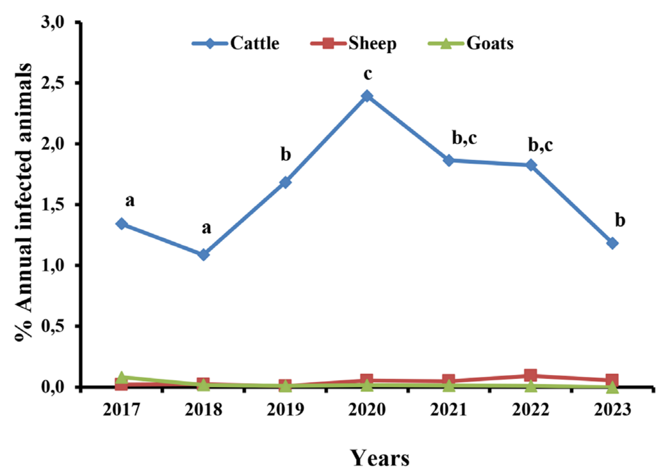

Figure 3. Annually variations in proportions of infected animals for fasciolosis in slaughterhouse during the year 2017-2023 in Bejaia province

a, b, c: Different letters on the same curve indicate a statistically significant difference (Tukey's test, p≤0.05)

Table 2. Slaughter statistics fasciolosis rates of infected animal between 2017 and 2023 in Bejaia province

Species slaughtered	Cattle	Sheep	Goats	Animal total
Number slaughtered **Mean ± SD** **min-max**	131.961 1571±192 646-3685	91.658 1091±286 361-2882	131.565 1566±173 502-2434	355.184 1409±217 361-3685
Percentage slaughtered (%)	37.2	25.8	37	100
Number with fasciolosis infestation	2079	38	29	2146
Global prevalence (%), **95% confidence interval**	1.58[a] 1.45-1.82	0.04[b] 0.01-0.08	0.02[b] 0.006-0.05	0. 0.66%-0.89

a, b: Values with different superscripts in the same raw are significantly different (p≤0.05), SD: Standard deviation levels over the seven-year period of the study. The maximum annual prevalence of fasciolosis cases for sheep and goats was 0.09% and 0.08% in the years 2022 and 2017, respectively. In cattle, fasciolosis was significantly high in January, February, and March and gradually decreased from April to September, then increased in October, November and December. On the other hand, the monthly cumulative prevalence rates of sheep and goats fasciolosis cases recorded were constant throughout the year. The prevalence of fasciolosis was evaluated during four seasons and the results are shown in Table 3. The high recorded prevalence was in winter and autumn, at 1.92% and 1.78% in cattle, respectively ($p \leq 0.05$). Statistical analysis of data on the seasonal prevalence of fasciolosis of small ruminants (sheep and goats) did not show a significant difference (Tukey's test). Logistic regression further corroborated these findings, indicating that the odds of infection were significantly higher in autumn and winter compared to summer, and that cattle were far more likely to be infected than sheep or goats. These analyses provide further support for the hypothesis that there is a clear temporal and seasonal pattern in fasciolosis prevalence, with autumn and winter representing the highest risk periods.

Multivariate logistic regression analyses were used to determine the strength of the association between the occurrence of F. hepatica and its risk factors (Table 4). Cattle exhibited the highest infection risk and served as the reference group. The odds of infection were significantly lower in sheep [odds ratio (OR)=0.03, CI 95%: <0.01-0.05, $p<0.001$] and goats (OR=0.02, CI 95%: <0.01-0.04, $p<0.001$) when compared to cattle. Concerning the seasonality, autumn showed a significantly increased risk of infection (OR=1.45, CI 95%: 1.25-1.68, $p<0.001$), followed by winter (OR=1.32, CI 95%: 1.15-1.52, $p<0.001$). Spring was not significantly associated with infection risk (OR=1.01, NS). Using 2020 as the reference, infection odds were lower in 2017 (OR=0.56), 2018 (OR=0.45), and 2019 (OR=0.70), indicating a rising trend towards the 2020 peak. The post-peak period (2021, 2022, and 2023) exhibited a significantly decline in risk with ORs of 0.78, 0.76, and 0.53, respectively. Increased rainfall was associated with higher infection odds (OR=1.08 per 10 mm, $p=0.01$), while higher temperatures reduced infection risk (OR=0.91 per 1 °C, $p=0.03$). Relative humidity showed a non-significant trend.

Table 5 showed the annual variation of confiscated livers (kilograms) due to *Fasciola* infection (2017-2023) and its financial loss in slaughtered ruminants in Bejaia province abattoirs. The direct economic loss due to condemnation of liver as the result of fasciolosis was done for study animals and a 7-year slaughter rate. More livers were condemned in cattle (12,254 kg) than in sheep (45 kg) and goats (23 kg) during the period of this study. An overall direct economic loss of 5,904.031€ was incurred during the period in this study from a totality of 12,321.2 kg of liver condemned. The indirect economic loss is due to carcass weight reduction as a result of fasciolosis infection was 44,746,454€. Therefore, total annual economic loss due to fasciolosis is the summation of the losses from organ condemnation (direct loss) and carcass weight reduction (indirect loss), with a total of 50,650,484€.

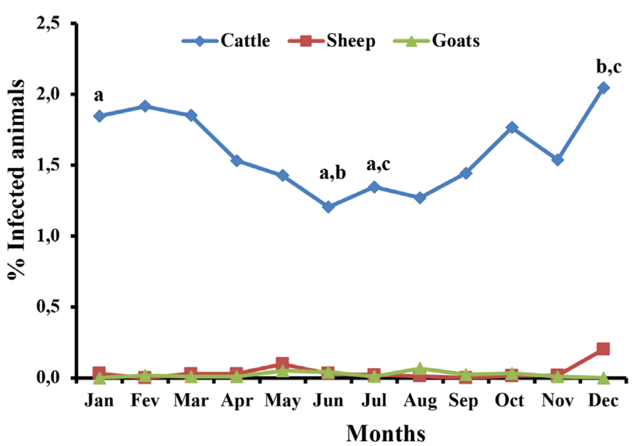

Figure 4. Monthly variations in proportions of infected animals for fasciolosis in slaughterhouse during the year 2017-2023 in Bejaia province.
[a, b, c]: Values with different superscripts in the same species between months are significantly different ($p \leq 0.05$)

Table 3. Prevalence of ruminant fasciolosis on the basis of different seasons in slaughterhouse during the year 2017-2023 of Bejaia province

Species	Season	Number slaughtered animals	Number of infected animals by *Fasciola* (%)
Cattle	Winter	29371	549 (1.92^a)
	Spring	37787	524 (1.47^b)
	Summer	34259	461 (1.46^b)
	Autumn	30544	545 (1.78^b)
Sheep	Winter	18843	4 (0.03)
	Spring	31264	17 (0.06)
	Summer	22901	3 (0.01)
	Autumn	18650	14 (0.09)
Goats	Winter	33060	5 (0.02)
	Spring	35360	12 (0.03)
	Summer	27677	9 (0.02)
	Autumn	35468	5 (0.01)

[a, b]: Values with different superscripts in the same species between seasons are significantly different ($p \leq 0.05$), Seasons: Winter (January-March), spring (April-June), summer (July-September), autumn (October-December)

Table 4. Multivariate logistic regression analysis of *Fasciola* spp. infection with the associated risk factors among ruminants in Bejaia province

Variable	Fasciolosis status (infected/non-infected)	Odds ratio	CI (95%)	p-value	Interpretation
Species					
Cattle	2079/129882	1.00 (Ref.)			Most affected species
Sheep	38/91620	0.03	<0.01-0.05	<0.001	Very low risk
Goats	29/131536	0.02	<0.01-0.04	<0.001	Very low risk
Season					
Summer		1.00 (Ref.)	-	-	Lowest risk season
Autumn		1.45	1.25-1.68	<0.001	Significantly increased risk
Winter		1.32	1.15-1.52	<0.001	Moderately increased risk
Spring		1.01	NS	NS	No significant difference
Year (vs. 2020)					
2017		0.56	0.48-0.65	<0.001	Lower risk vs. 2020
2018		0.45	0.38-0.54	<0.001	Very low risk
2019		0.70	0.60-0.82	<0.001	Moderate risk
2020		1.00 (Ref.)	-	-	Reference (peak)
2021		0.78	0.68-0.89	<0.001	Decline after peak
2022		0.76	0.66-0.87	<0.001	Stable post-peak
2023		0.53	0.45-0.63	<0.001	Marked decrease
Metrological variables					
Temperature (+ 1 °C)		0.91	-	0.03	Risk decreases with heat
Rainfall (+10 mm)		1.08	-	0.01	Risk increases with rainfall
Relative humidity		Slight trend	NS	>0.05	Non-significant effect

NS: Non significatif (p>0.05), OR: Odds ratio, CI: Confidence interval, Ref.: Reference method

Table 5. Annual variation of confiscated livers (kilogram) due to *Fasciola* infection (2017-2023) and its corresponding direct and indirect economic loss (Euro) in slaughterhouses of Bejaia province

Species	Season	Liver condemnation (kg)	Direct economic losses due to liver condemnation (Euro)	Indirect economic losses due to ruminant fasciolosis (Euro)
Cattle	**2017**	2052	738,485	6,400,416
	2018	1367	490,630	4,086,720
	2019	1473	562.860	5,035,296
	2020	1903	721,600	6,974,592
	2021	1816	518,408	6,728,640
	2022	2386	646,020	8,492,544
	2023	1256	621,006	5,396,328
Sheep	**2017**	4	170,101	148,156
	2018	4	162,110	140,944
	2019	1	37,974	35,303
	2020	8	255,887	244,083
	2021	10.5	165,982	269,920
	2022	9.5	539,125	584,492
	2023	7	179,971	175,473

Table 5. Continued

Species	Season	Liver condemnation (kg)	Direct economic losses due to liver condemnation (Euro)	Indirect economic losses due to ruminant fasciolosis (Euro)
Goats	**2017**	12	34,725	16,716
	2018	3.2	9,424	4,435
	2019	1.6	4,920	2,512
	2020	2.4	6,831	3,721
	2021	3	4,710	3,553
	2022	2	3,262	2,610
	2023	0	0	0
All		**12321.2**	**5,904,031**	**44,746,454**

Direct economic losses due to liver condemnation: LC = CSR * LCo * P
Where: LC = losses due to liver condemnation; CSR = average number of animals slaughtered at abattoir during the study period; LCo = average cost of one liver in Bejaia province, Algeria; P = prevalence of the fasciolosis at the study abattoir.
Indirect economic losses due to ruminant fasciolosis: CW = CSR * CL * MC * P * (n) kg
Where: CW = losses due to carcass weight reduction; CSR = average number of animals slaughtered at abattoirs during the study period; MC = average price for 1 kg of meat in Bejaia province, Algeria; P = prevalence of fasciolosis in the study abattoir; (n) kg = average carcass weight of animal; CL = carcass weight loss (10%) in individual animal due to fasciolosis

DISCUSSION

Fasciolosis is the most common helminthes infection that affects ruminants with economic losses in livestock productivity considerable. Moreover, World Health Organization reported that 2.4 million people are infected with fasciolosis and a huge risk of infection to humans (23). Sanitary surveillance by veterinary inspectors plays an important role in the parasitic infection control of livestock. The slaughterhouse is considered a crucial epidemiological point for the data collecting of fasciolosis infection and their tracking in ruminants. The authors are convinced that it is very necessary to continue the epidemiological investigations on fasciolosis infection in slaughterhouses in Bejaia province (Algeria) (12). Moreover, to the best of our knowledge, the economic loss data regarding fasciolosis in slaughterhouses have not been reported. In Algeria, veterinary health authorities have implemented awareness campaigns to control the spread of parasitose in ruminants (e.g., fasciolosis), such as regular deworming of livestock, in order to reduce the risk of human infection. However, the results obtained in the study indicate that fasciolosis is still widespread in livestock farms, and is an economically important disease in the study area (Bejaia province, Algeria).

The present study revealed that the rate of cattle and goats slaughtering was higher than for sheep (37.2%, 37%, and 25.8%, respectively). It should be noted that the demand for red meat in the province of Bejaia is mainly focused on beef, particularly in mountainous regions; whereas the consumption of mutton is primary for the religious festival of Eid Al-Adha. Based on detailed post-mortem inspection, the overall prevalence of fasciolosis in cattle, sheep and goats recorded in the current study (1.58%, 0.04%, and 0.02%, respectively) is widely different compared to several investigations. The fasciolosis prevalence of bovine slaughtered in the present study is slightly lower compared to the previous report (12), which reported respective prevalence of 2.83%, 0.13%, and 0.12% in abattoirs of Bejaia province during the period 2009-2016. It could be due to increased anthelmintic use, changes in climate (rainfall and humidity rates), or improved farmer awareness in the Bejaia province. The rate of fasciolosis calculated in our study was very low to those cattle (8%), sheep (0.54%) and goats (0.26%) recorded previously in the Jijel area (24). In this study, the difference of fasciolosis rate observed in slaughtered cattle, sheep and goats might be attributed to grazing area (humid and marshy area). In order to lower the rate of exposure to fluke metacercariae, it is also recommended to avoid from grazing livestock on marsh forage. In addition, the selective behavior of goats during grazing may limit their consumption of infested plants by *Fasciola* sp., i.e., goats browse the leaves and branches of bushes, shrubs, and trees (25). Also, this bovine fasciolosis rate at slaughter is similar compared to investigation in Saharan Region of Ouargla (1.7%) (16). The low rate observed in our study can be explained that the local farmers used anthelmintics routinely for prophylaxis of animal fasciolosis rather than for treatment. Likewise, when compared with the data recorded in different areas of Algeria, the prevalence of cattle fasciolosis recorded in this study was lower than 6.9% and 5.9% prevalence reported in Northern Algeria by Mezali et al. (26) and Chougar et al. (27), respectively. On the other hand, higher prevalence rates of bovine fasciolosis in slaughterhouse have been documented by Ouchene-Khelifi et al. (16) in El-Tarf and Meguini et al. (15) in Souk Ahras (26.7% and 12.3%, respectively). The disparities in prevalence in the different regions of Algeria could be influenced mainly by the climatological factors such as temperature, rainfall and humidity. In worldwide, the prevalence of *Fasciola* sp. infection in cattle is high compared to investigation in semi-arid region of Egypt (11.1%) (28) and (30.9%) (29), Ghana (10.27%) (30), Mexico (24.9%) (31), Ireland (23.68%) (32), Pakistan (42.8%) (33) and Malaysia (36.9%) (34). In another survey, the prevalence of *Fasciola* sp. in Slaughtered ruminants at Muyinga Slaughterhouse (Burundi) was 13.04% (95% CI: 5.10%-20.99%) in cattle, 3.16% (95% CI: 0.67%-5.64%) in goats and 0% in sheep (35). However, the rate of organ condemnation due to fasciolosis was 2.028% in the cattle slaughterhouse in Bavaria, southeast Germany (36). The variation in prevalence in different countries might be due to use of anthelminthic drugs properly, awareness among the farmers, proper control measures (37). Fasciolosis infection seems to be spreading throughout in animal farms of the world; this could be explained by climate factors favorable developing the intermediate host, such as temperature and humidity, as well as access to pasture, the livestock production system, and length of the grazing season (26,31). In addition, the variation in previous investigations might be attributed to age and immune status of animals (38).

Fasciolosis infection in slaughtered cattle showed annual fluctuations throughout the study period, with a detection high rate of 2.39%. The reason for this significant increase in the prevalence of fasciolosis in cattle could be due to the economic crisis, i.e., coronavirus period, which has affected the world, especially the agricultural sector. Consequently, appropriate prophylactic or therapeutic control measures, such as the use of anthelmintic drugs, are neglected. The overall monthly prevalence rate of the fasciolosis in cattle showed instability in levels over the seven years period of the study. However, rates of fasciolosis infection decreased significantly from April to August. This could be due to the to better management and husbandry practices against this parasitose, which corresponds to the grazing period (39).

In this retrospective survey, ruminant fasciolosis persisted throughout the year; these findings were in concordance with other previous reports by Chaouadi et al. (40). The rate of fasciolosis infection observed in slaughtered cattle showed significant autumn and winter slightly high prevalence. The seasonal prevalence of *Fasciola* sp. infection was significantly different ($p<0.001$) in the high throughput abattoirs of South Africa, i.e., 12.8%, 10.8%, 6.5%, and 7.8% during summer, autumn, winter and spring, respectively (41). This could be attributed to a variety of climatic conditions in this study region. It is noted that autumn and winter temperatures (17-24 °C), relative humidity (69-72%), and rainfall (81-95 mm) recorded during 7-year are sufficient for the development of *Fasciola* sp. larval stages and the growth of snails. However, because fluke metacercariae may persist longer in previously flooded places and on vegetation, dry pastures are not entirely safe (42,43). Moreover, it has been shown that rainfall and temperatures above 10 °C affect the hatching of *Fasciola* sp. eggs and the growth of snails (44). For this reason, it would be more judicious deworming livestock twice a year, especially before and after autumn season, as a method of controlling liver flukes (45). These findings were in concordance with previous report of Al-Jibouri et al. (46), who found that the prevalence of fasciolosis in cattle was associated with lower temperature and higher relative humidity. Indeed, Utrera-Quintana et al. (31) indicated that season was significantly associated with an increased risk of parasitic infection. Furthermore, Ahmed et al. (47) found that long rainy seasons in Ethiopia have a significant role in *Fasciola* sp. infection because of predisposing factors for snails to complete their life cycles, therefore facilitating the survival of parasites. In addition, Kuerpick et al. (48) has been reported seasonal prevalence, which the prevalence of fasciolosis is related heavy rainfall, and invasion of sheep in endemic fluke areas during seasonal grazing.

Numerous authors reported that fasciolosis infection is an important disease from an economic point of view, as it causes huge financial losses to butchers and farmers. In this study, the total economic loss caused by ruminant fasciolosis in slaughterhouses of Bejaia province was calculated based on liver seizure for abnormalities caused by *Fasciola* sp. All fasciolosis-related liver defects were considered unfit for human consumption at the slaughterhouse and were condemned. Our study revealed abattoir direct and indirect economic losses of 5.9 and 44.7 million € during the study period because of liver condemnation due to *Fasciola* sp. infection, respectively. This huge economic loss might be attributed to the fact that a 7-year survey and three animal species were considered in this study. In our study, it has also been estimated that annual economic losses due to fasciolosis in slaughterhouses of Bejaia province during a 7-years period are more than €50 million. This magnitude of economic losses indicates that ruminant fasciolosis poses an important and serious risk to the livestock sector in Algeria, especially in Bejaia province. Likewise, the losses as a result of liver condemnation valued in the Uganda abattoir are estimated at 92 US$ millions annually (49). Similar observations have been reported by Odeniran et al. (50), who revealed that a total of US$ 77,940,024/year was estimated as overall economic losses to small ruminant fasciolosis. In another study, the average cost of liver condemnation has been reported as US$ 0.2 million annually in Saudi Arabia (51). Also, Swai and Ulicky (52) reported that economic loss due to liver condemnation was approximately 1,780 US$ during 13 months in Tanzania. The variations in the amount of financial losses could be attributed to the difference in the length of carrying out the study, the rates of rejection of organs, and the number of animals slaughtered. Furthermore, our findings and previous studies suggest that fasciolosis in ruminants has a significant economic impact on the global agricultural sector. Therefore, in order to minimize these economic losses, it is essential to redouble efforts by improving control strategies to fight fasciolosis infection.

CONCLUSION

This is the first retrospective survey (2017-2023) on ruminant fasciolosis and estimation of financial loss due to *Fasciola* sp. infection in slaughterhouses in the Bejaia province. Our study revealed that the prevalence of cattle fasciolosis was generally high compared to sheep and goats in abattoir of Bejaia province (Algeria) associated with significant financial losses for butchers and livestock farmers in the study area. It is therefore necessary to reproduce this type of study in other regions of Algeria in order to generate data and calculate global economic losses because of *Fasciola* spp. infection. The management practices of animals, especially grazing habits and access to freshwater habitats could decrease Fasciola infections. Thus, molluscicide control and rotation of grazing areas are the basic control strategies. Preventive and therapeutic measures need to be further implemented in the field, including grazing management, reducing the number of intermediate hosts, as well as diagnosing and treating animals with anthelmintics. Furthermore, livestock farmers should be made aware of the importance of this parasitose in order to reduce economic losses.

*Ethics

Ethics Committee Approval: Ethical review and approval were waived for this study because the data in this study were collected from Provincial Veterinary Inspection of Bejaia (Algeria).

Informed Consent: This study was conducted solely using data collect from provincial Veterinary Inspection, no human participants subjects were involved. Informed consent was not required.

Footnotes

*Authorship Contributions

Concept: E-H.B., A.A., Design: E-H.B., A.A., Data Collection or Processing: K.R., K.M., Analysis or Interpretation: M.I., K.R., K.M., N.B.D., A.A., Literature Search: E-H.B., O.B., M.I., A.A., Writing: E-H.B., A.A.

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

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Assessment of the Knowledge Levels of University Students in Health Sciences Regarding Parasitic Diseases

Sağlık Alanındaki Üniversite Öğrencilerinin Paraziter Hastalıklar ile İlgili Bilgi Düzeylerinin Değerlendirilmesi

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ABSTRACT

Objective: A comprehensive understanding of parasitic diseases, which affect both human and animal health, is crucial for university students in health sciences as they prepare for their future roles as healthcare professionals. This study aims to assess the knowledge levels of university students in health sciences regarding parasitic diseases.
Methods: This descriptive study was conducted with 498 students from the faculty of health sciences and the vocational school of health services at a state university in the Marmara Region. Data were collected using a questionnaire that included socio-demographic information and questions assessing students' knowledge of parasitic diseases. Statistical analyses were performed using SPSS 26.0®, with a significance level set at $p \leq 0.05$.
Results: The participants had a mean age of 20.84±2.63 years, and 82.3% were female. A total of 80.9% of the students lived with their parents, and 73.7% reported a moderate family income. Nearly half (44.2%) were first-year students, 30.1% were enrolled in the department of physiotherapy and rehabilitation, and 68.1% resided in state dormitories. Most students (92.4%) recognized the importance of hand hygiene in preventing parasitic diseases, and 71.3% acknowledged that the location of household toilets could contribute to the occurrence of such diseases. However, 70.1% reported not receiving specific training or certification on hygiene. Female students demonstrated the highest awareness of hand hygiene benefits in preventing parasitic infections, while nursing students had the most extensive hygiene training (course/certificate) ($p<0.05$).
Conclusion: Our findings indicate that a significant proportion of students lack specialized courses or certifications on hygiene practices and have limited knowledge of parasitic diseases. Therefore, integrating scientific meetings and training sessions on parasitic diseases and preventive measures into the health sciences curriculum is essential.
Keywords: University students, health sciences, infection, parasitic diseases, hygiene

ÖZ

Amaç: Hem insan hem de hayvan sağlığını etkileyen paraziter hastalıkların kapsamlı bir şekilde anlaşılması, gelecekte sağlık profesyoneli olacak sağlık bilimleri öğrencileri için büyük önem taşımaktadır. Bu çalışma, sağlık bilimleri öğrencilerinin paraziter hastalıklar konusundaki bilgi düzeylerini değerlendirmeyi amaçlamaktadır.
Yöntemler: Bu tanımlayıcı çalışma, Marmara Bölgesi'nde bulunan bir devlet üniversitesinin sağlık bilimleri fakültesi ve sağlık hizmetleri meslek yüksekokulundan 498 öğrenci ile gerçekleştirilmiştir. Veriler, öğrencilerin sosyo-demografik bilgilerini ve paraziter hastalıklarla ilgili bilgi düzeylerini değerlendiren bir anket kullanılarak toplanmıştır. İstatistiksel analizler SPSS 26.0® programı ile yapılmış olup, anlamlılık düzeyi $p \leq 0,05$ olarak kabul edilmiştir.
Bulgular: Katılımcıların yaş ortalaması 20,84±2,63 yıl olup, %82,3'ü kadındır. Öğrencilerin %80,9'u ailesiyle yaşamakta ve %73,7'si ailesinin gelir düzeyini orta seviyede olarak tanımlamaktadır. Katılımcıların neredeyse yarısı (%44,2) birinci sınıf öğrencisi olup, %30,1'i Fizyoterapi ve Rehabilitasyon Bölümünde öğrenim görmektedir ve %68,1'i devlet yurtlarında kalmaktadır.

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Türkiye PARAZİTOLOJİ Dergisi

Öğrencilerin büyük bir çoğunluğu (%92,4), el hijyeninin paraziter hastalıklara karşı korunmada önemli olduğunu belirtirken, %71,3'ü evdeki tuvaletin konumunun paraziter hastalıkların oluşumuna katkıda bulunabileceğini kabul etmiştir. Ancak, öğrencilerin %70,1'i hijyen konusunda özel bir eğitim veya sertifika almadığını bildirmiştir. Kadın öğrenciler, el hijyeninin paraziter enfeksiyonları önlemedeki faydaları konusunda en yüksek farkındalığa sahipken, hemşirelik bölümü öğrencileri hijyen konusunda en fazla eğitimi (ders/sertifika) almıştır (p<0,05).

Sonuç: Bulgularımız, öğrencilerin önemli bir kısmının hijyen uygulamaları konusunda özel bir ders veya sertifika almadığını ve paraziter hastalıklar konusunda sınırlı bilgiye sahip olduklarını göstermektedir. Bu nedenle, sağlık bilimleri müfredatına paraziter hastalıklar ve korunma yöntemleri ile ilgili bilimsel toplantılar ve eğitim programlarının entegre edilmesi gerekmektedir.

Anahtar Kelimeler: Üniversite öğrencileri, sağlık bilimleri, enfeksiyon, paraziter hastalıklar, hijyen

INTRODUCTION

Evaluating the knowledge levels of university students regarding parasitic diseases is of significant importance for increasing their awareness and developing educational approaches on this topic. Furthermore, it is crucial for students aspiring to become healthcare professionals to have a comprehensive understanding of parasitic diseases affecting both humans and animals, as this is vital for public health. Parasitic infections remain a global health issue, affecting millions of people worldwide, particularly in developing and underdeveloped countries. Over 300 identified helminth species and more than 70 protozoan species directly impact humans. In tropical and subtropical regions, parasitic diseases with zoonotic characteristics often transmitted from animals to humans are common (1). Additionally, such diseases are more prevalent in areas with inadequate infrastructure, low socio-economic and educational levels, contaminated water and food, poor adherence to hygiene practices, respiratory infections, blood transfusions, and organ transplants (1-3).

The prevalence of these diseases varies depending on the region, local conditions, and the implementation of public health protective measures. Knowledge about these diseases and their transmission pathways to humans and animals is critical for the prevention and control of infections (2,3). The competence of students who will be employed in the healthcare sector regarding parasitic diseases is particularly important for raising societal awareness and actively participating in the fight against these diseases. Raising awareness about the prevention and reduction of parasitic disease transmission can be achieved through health education (4).

This study aims to assess the knowledge levels of university students in the health field regarding parasitic diseases.

METHODS

Type of Study

This study was conducted as a descriptive research study.

Population and Sample

The population of this descriptive study consisted of students enrolled in the faculty of health sciences (departments of nursing, health management, and physiotherapy and rehabilitation) and the vocational school of health services (programs of first and emergency aid, medical laboratory techniques, physiotherapy, health institutions management, and health tourism management) at a university. Since the study aimed to include the entire population, no sampling method was applied. The research was completed with 498 students who met the inclusion criteria and voluntarily agreed to participate (n=498).

Inclusion Criteria

Students were eligible to participate if they met the following criteria:

-Enrollment in the faculty of health sciences or the vocational school of health services.

-Voluntary participation in the study.

-Proficiency in Turkish.

-Not currently employed in any healthcare field.

Data Collection Tools

Data were collected using a structured questionnaire designed to assess students' personal characteristics and knowledge levels regarding parasitic diseases and their transmission routes. Students' responses were scored (1 point for each correct answer). The questionnaire consisted of 30 questions evaluating various aspects of parasitic diseases, including transmission routes, risk factors, the effectiveness of vaccines in prevention, hand hygiene, water disinfection, and the importance of balanced nutrition.

Implementation of Data Collection Tools

Data collection took place during the spring semester of the 2023-2024 academic year. The questionnaire was administered online, and the link was shared with student groups in their respective classes. Participation was voluntary, and students completed the self-reported questionnaire at their convenience.

Statistical Analysis

The research data were analyzed using the SPSS 26.0 software package. Descriptive statistics, including frequencies, percentages, arithmetic means, and standard deviations, were used for data analysis. Students' knowledge scores were categorized into two clusters using K-Means Clustering Analysis, and cluster characteristics were assessed through receiver operating characteristic (ROC) analysis. Scores ranged from 0 to 30, with a score of ≥24.5 considered indicative of sufficient knowledge of parasitic diseases based on ROC analysis. The chi-square test was used for the analysis of categorical data, and a p-value <0.05 was considered statistically significant in all analyses.

Ethical Considerations

Ethical approval for the study was obtained from The Ethics Committee of Sakarya University of Applied Sciences (E-26428519-050.99-123583-43/06-2024) along with institutional permission from the university where the research was conducted. Informed consent was obtained from all students who participated in the study.

RESULTS

Most participants were female, and a large proportion were enrolled in the physiotherapy and nursing departments (Table 1).

An examination of the sources from which students obtained information about parasitic diseases revealed that 28.1% reported health-related courses at school, 8.4% newspapers and magazines, 10.0% television, 11.0% friend groups, 38.0% the internet, 11.8% healthcare professionals, and 6.8% family members.

As shown in Table 2, knowledge adequacy levels differed significantly across certain characteristics. Nursing students had the highest proportion of adequate knowledge compared to other departments (p=0.001). Higher adequacy was also observed among students who reported reading about parasitic diseases (46.1% compared to 32.9%, p=0.002), had taken health-related courses at school (48.6% compared to 33.8%, p=0.002), received hygiene training (55.0% compared to 30.7%, p=0.001), and refrained from sharing personal items (51.5% compared to 35.8%, p=0.010). No significant

Table 1. Distribution of students' socio-demographic and descriptive characteristics

Characteristic	Mean ± SD	
Age (mean ± SD)	20.84±2.63	
	Number	**%**
Gender		
Female	410	82.3
Male	88	17.7
Department/program		
Nursing	117	23.5
Physiotherapy and rehabilitation	150	30.1
Health management	71	14.3
First aid and emergency care	40	8.0
Health institutions management	25	5.0
Medical laboratory techniques	45	9.0
Health tourism management	32	6.4
Physiotherapy	18	3.6
Income status		
Income less than expenses	14	2.8
Income equal to expenses	367	73.7
Income more than expenses	117	23.5
Family structure		
Nuclear family	403	80.9
Extended family	95	19.1
Living arrangements		
With family	104	20.9
In a state dormitory	339	68.1
In a student house	35	7.0
In a private dormitory	20	4.0
Number of household members		
1-4 people	238	47.8
5-8 people	247	49.6
9-12 people	13	2.6
Mother's education level		
Illiterate	28	5.6
Primary school	217	43.6
Middle school	104	20.9
High school	98	19.7
Bachelor's degree	28	5.6
Associate degree	16	3.2
Postgraduate degree	7	1.4

Table 1. Continued

Characteristic	**Mean ± SD**	
Father's education level		
Illiterate	5	1.0
Primary school	132	26.5
Middle school	108	21.7
High school	171	34.3
Bachelor's degree	52	10.4
Associate degree	17	3.4
Postgraduate degree	13	2.6
Total	**498**	**100**

SD: Standard deviation

Table 2. Distribution of students' knowledge levels on parasitic diseases based on various characteristics

Characteristic	**Knowledge level**						**Statistical analysis**
	Successful		**Unsuccessful**		**Total**		
	n	**%**	**n**	**%**	**n**	**%**	
Gender							
Female	159	38.8	251	61.2	410	100	p=0.243 X^2=0.468
Male	30	34.1	58	65.9	88	100	
Department							
Nursing	62	53.0	55	47.0	117	100	**p=0.001** X^2=31.455
Physiotherapy and rehabilitation	52	34.7	98	65.3	150	100	
Health management	22	31.0	49	69.0	71	100	
First aid and emergency care	18	45.0	22	55.0	40	100	
Health institutions management	3	12.0	22	88.0	25	100	
Medical laboratory techniques	22	48.9	23	51.1	45	100	
Health tourism management	8	25.0	24	75.0	32	100	
Physiotherapy	2	11.1	16	88.9	18	100	
Living arrangements							
With family	39	37.5	65	62.5	104	100	p=0.285 X^2=3.789
In a state dormitory	134	39.5	205	60.5	339	100	
In a student house	8	22.9	27	77.1	35	100	
In a private dormitory	8	40.0	12	60.0	20	100	
Reading information on parasitic diseases							
Yes	88	46.1	103	53.9	191	100	**p=0.002** X^2=8.678
No	101	32.9	206	67.1	307	100	
Health-related courses at school							
Yes	68	48.6	72	51.4	140	100	**p=0.002** X^2=9.327
No	121	33.8	237	66.2	358	100	
Receiving hygiene training (course/certificate)							
Yes	82	55.0	67	45.0	149	100	**p=0.001** X^2=26.345
No	107	30.7	242	69.3	349	100	
Sharing personal items							
Yes	35	51.5	33	48.5	68	100	**p=0.010** X^2=6.112
No	154	35.8	276	64.2	430	100	
Total	189	38.0	309	62.0	498	100	

associations were found with gender (p=0.243) or living arrangements (p=0.285).

DISCUSSION

This study is significant in assessing the knowledge levels of university students in the health field regarding parasitic infections (e.g., their importance and transmission routes) and in increasing their awareness of infection control measures while implementing more innovative educational approaches.

Nursing students were found to have higher knowledge levels about parasitic diseases compared to students from other departments ($p<0.05$) (Table 2). This may be attributed to the greater emphasis on Parasitology courses in their curriculum. A previous study highlighted that specific teaching methods could significantly enhance students' understanding of parasitic diseases (5). Another study in the literature reported that students generally had insufficient knowledge of parasitic diseases, with significant differences in knowledge levels between academic programs, particularly in terms of prevention and treatment (6).

A study conducted in Morocco revealed that university students had a moderate understanding of leishmaniasis vectors but lacked adequate preventive practices (7). Raising awareness about parasitic diseases and implementing innovative educational programs are critical to addressing knowledge gaps and improving students' ability to recognize and prevent these diseases (6,8).

A study in São Paulo, Brazil, demonstrated that didactic interventions, such as using booklets on soil-transmitted helminths, significantly improved students' knowledge, attitudes, and practices regarding parasitic infections (9). Furthermore, research on healthcare professionals' knowledge about mandatory disease diagnoses revealed that only a small percentage had adequate knowledge (10). In the present study, students who had previously read about parasitic diseases and received hygiene education exhibited higher levels of knowledge adequacy, as expected ($p<0.05$) (Table 2). In line with previous research indicating that students who received formal education on parasitic diseases within health-related courses demonstrated significantly higher levels of knowledge and preventive practices (6,11), our findings support the integration of parasitology content into university curricula as an effective approach to addressing knowledge gaps and promoting infection control behaviors. Nevertheless, a study conducted in Xexéu-PE found that, despite awareness of parasitic diseases, many students lacked basic hygiene practices, such as washing hands before meals (12). Several studies have consistently highlighted deficiencies in students' understanding of parasitic diseases, emphasizing the need for enhanced scientific education interventions (6,13,14).

The Turkish Ministry of Health's 2019-2023 strategic program for infection prevention included the development of a "national infection prevention and control curriculum". This strategy emphasized collaboration with local academic institutions to develop and adapt curricula based on national needs and resources. The program also called for the integration of infection prevention and control education into continuous medical, nursing, and allied health professional training, along with periodic curriculum updates in collaboration with the Council of Higher Education, the Medical Specialization Board, and the Ministry of National Education (15).

This study also found that students who avoided sharing personal items had higher knowledge adequacy regarding parasitic diseases ($p<0.05$) (Table 2). While the direct relationship between sharing personal items and knowledge of parasitic diseases has not been widely explored in the literature, many studies collectively emphasize students' general knowledge and awareness of parasitic diseases. This suggests that behaviors such as avoiding sharing personal items may indirectly reflect a better understanding of transmission routes and preventive measures (16). Students who refrained from sharing personal items likely demonstrated greater awareness of parasitic disease prevention due to their understanding of these concepts.

Although students were generally aware of parasitic diseases, gaps in knowledge regarding transmission and prevention remain, along with certain misconceptions. Addressing these gaps and ensuring the practical application of knowledge necessitate a reformulation of teaching and learning processes (17).

CONCLUSION

Our study revealed that a significant number of students had not read about parasitic diseases and had not received a dedicated course or certification on hygiene. According to the findings, students from the Medical Laboratory Techniques program demonstrated greater knowledge about parasitic diseases. Additionally, students who read about parasitic diseases, received hygiene training, and avoided sharing personal items were found to have a higher level of knowledge regarding parasitic diseases.

The results of this study underscore the importance of education in enhancing students' knowledge levels about parasitic diseases and influencing their behaviors. While knowledge levels may vary, consistent educational efforts can bridge the gap between awareness and practical application, leading to improved health outcomes. Integrating hygiene education with parasitic disease awareness is crucial to promoting better health outcomes among students. In this context, it is essential to comprehensively integrate these topics into curricula across all levels of education and to organize training sessions and seminars. Such initiatives not only educate students but also highlight the need for comprehensive educational strategies that motivate behavioral changes.

However, it is important to acknowledge that knowledge alone does not necessarily lead to behavioral change. It is equally critical to train more healthcare professionals with expertise in accurately diagnosing and treating parasitic diseases, managing infection control, and preventing further spread. Ensuring the widespread employment of such experts in all regions will play a key role in improving public health outcomes.

*Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Sakarya University of Applied Sciences's Ethics Committee (E-26428519-050.99-123583-43/06-2024) along with institutional permission from the university where the research was conducted.

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Footnotes

This study was presented as an oral presentation at the 3rd International Eurasian Health Sciences Congress on August 28-29, 2024.

*Authorship Contributions

Concept: C.B.O., Design: C.B.O., Data Collection or Processing: C.B.O., F.C., Analysis or Interpretation: C.B.O., F.C., Literature Search: C.B.O., F.C., Writing: C.B.O., F.C.

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Tissue-level Consequences of Segmental Abnormalities in *Hirudo verbana*: A Morphological and Histological Approach

Hirudo verbana'da Segmental Anormalliklerin Doku Düzeyindeki Sonuçları: Morfolojik ve Histolojik Bir Yaklaşım

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ABSTRACT

Objective: This study aimed to investigate the histological and morphological differences between healthy and deformed specimens of the medicinal leech *Hirudo verbana*, with particular focus on tissue integrity and cellular organization.
Methods: We conducted comparative histological analysis using haematoxylin and eosin staining on tissue sections obtained from both healthy and deformed leeches. The evaluation included examination of epithelial layer integrity, muscle tissue organization, secretory cell distribution, and pigment accumulation patterns.
Results: Healthy specimens demonstrated well-preserved epithelial layers with regularly arranged circular and longitudinal muscle fibers. The secretory cells (T1, T2A, and T2B types) maintained normal distribution and activity, while melanin pigment showed limited and organized deposition in connective tissues. In contrast, deformed leeches exhibited significant structural abnormalities including disrupted epithelial layers, disorganized muscle architecture (particularly in circular muscles), and reduced T2B secretory cell populations. Notable accumulation of mononuclear immune cells, accompanied by increased melanin deposition, was observed in association with inflammatory foci. Morphological abnormalities included body segmentation defects, tissue atrophy, functional impairment of suckers, and compromised mobility.
Conclusion: Our findings demonstrate that deformation in *Hirudo verbana* leads to substantial histological and morphological alterations affecting epithelial integrity, muscular organization, secretory functions, and pigmentation patterns. These changes may significantly impact the leeches' biological functionality and therapeutic potential. Further investigation using immunohistochemical techniques and molecular analyses are warranted to elucidate the underlying mechanisms of these pathological changes.
Keywords: *Hirudo verbana*, histopathology, deformation, medicinal leech

ÖZ

Amaç: Bu çalışmada, tıbbi sülük türü *Hirudo verbana*'nın sağlıklı ve deforme olmuş örnekleri arasındaki histolojik ve morfolojik farklılıkların, özellikle doku bütünlüğü ve hücresel organizasyon üzerindeki etkilerinin araştırılması amaçlanmıştır.
Yöntemler: Hem sağlıklı hem de deforme olmuş sülüklerden elde edilen doku kesitlerinde hematoksilin ve eozin boyama kullanarak karşılaştırmalı histolojik analiz gerçekleştirdik. Değerlendirme, epitel tabaka bütünlüğünün, kas dokusu organizasyonunun, salgı hücresi dağılımının ve pigment birikim modellerinin incelenmesini içeriyordu.
Bulgular: Sağlıklı örnekler, düzenli olarak düzenlenmiş dairesel ve uzunlamasına kas liflerine sahip iyi korunmuş epitel katmanları gösterdi. Salgı hücreleri (T1, T2A ve T2B tipleri) normal dağılım ve aktiviteyi korurken, melanin pigmenti bağ dokularında sınırlı ve organize birikme gösterdi. Buna karşılık, deforme olmuş sülükler, bozulmuş epitel katmanları, düzensiz kas mimarisi (özellikle dairesel kaslarda) ve azalmış T2B salgı hücresi popülasyonları gibi önemli yapısal anormallikler gözlendi. Enflamatuvar odaklarla ilişkili olarak, melanin birikiminin artmasıyla birlikte mononükleer bağışıklık hücrelerinin belirgin birikimi gözlendi. Morfolojik

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anormallikler arasında vücut segmentasyon kusurları, doku atrofisi, vantuzların işlevsel bozukluğu ve hareket kabiliyetinin azalması yer aldı.
Sonuç: Bulgularımız *Hirudo verbana*'daki deformasyonun epitel bütünlüğünü, kas organizasyonunu, salgılama işlevlerini ve pigmentasyon desenlerini etkileyen önemli histolojik ve morfolojik değişikliklere yol açtığını göstermektedir. Bu değişiklikler sülüklerin biyolojik işlevselliğini ve terapötik potansiyelini önemli ölçüde etkileyebilir. Bu patolojik değişikliklerin altında yatan mekanizmaları açıklamak için immünohistokimyasal teknikler ve moleküler analiz kullanılarak daha fazla araştırma yapılması gerekmektedir.
Anahtar Kelimeler: *Hirudo verbana*, histopatoloji, deformasyon, tıbbi sülük

INTRODUCTION

Freshwater leeches are segmented, hermaphroditic worms that feed on blood. Their bodies are highly segmented, including brain components, with each segment containing distinct organs such as ganglia and testes. Two suckers are responsible for crawling and attachment, while the anterior region contains three jaws equipped with numerous teeth. Leeches typically bite warm areas of the host and extract blood through rhythmic contractions. They use sharp, saw-like teeth in their jaws to make incisions in the host's skin, facilitating blood extraction. Simultaneously, they secrete saliva containing anticoagulants, such as hirudin, to prevent clotting and ensure uninterrupted feeding (1-3). Feeding usually lasts 15-40 minutes, during which a single leech can consume 10-15 mL of blood. Digestion is facilitated by symbiotic microorganisms such as *Aeromonas hydrophila* and *Pseudomonas hirudinia*, along with various digestive enzymes (4-6). Furthermore, it is important to consider the physiological characteristics of leeches after feeding, particularly their ability to store blood in their stomachs for long periods without spoilage, as highlighted in the literature (6).

Two thousand years ago, in ancient Egypt, leeches were first used for medical purposes. In recent years, leech therapy, known as hirudotherapy, has regained popularity (7). In 1884, Haycraft discovered a substance in leech saliva that prevents blood clotting, which was later identified as hirudin, an effective anticoagulant (8). During the 1980s, medical leech therapy became increasingly important in plastic and reconstructive surgery, particularly in reattachment of severed limbs and flap surgeries, as a supportive method to enhance blood circulation. Recognizing its medical significance, the United States Food and Drug Administration approved the sale and use of leeches in plastic and microsurgery in 2004 (9). Currently, bioactive substances obtained from medicinal leeches have shown therapeutic potential in diverse clinical settings, including antihypertensive effects, anticoagulant activity, anti-inflammatory properties, and enhanced wound healing following surgical interventions (10).

With the increasing use of leeches for medical purposes in the treatment of various diseases, uncontrolled harvesting of medicinal leeches from natural habitats has become a growing concern. Several factors negatively impact leech populations, including the decline of wetland habitats, the global reduction of amphibian species (e.g., frogs), the abandonment of traditional grazing practices in favor of stable-based livestock farming, drainage of wetlands for agriculture, and various other agricultural activities (11-13). As a result of these challenges, the decline in leech populations and the rising demand for safe, sterile, and traceable medicinal leeches have made it necessary to establish open or closed-system leech breeding facilities for sustainable production.

Morphological abnormalities in medical leeches may arise due to physical trauma experienced during their growth and feeding process after emerging from the cocoon or during their use for therapeutic purposes, as well as the inability to fully replicate their natural habitat. These abnormalities result in irreversible segmentation damage, leading to the detachment of the distal part of the body, which subsequently causes infection and ultimately the death of the leech. This pathological condition is highly undesirable for medical leeches, which are both labor-intensive to cultivate and have significant economic value. In this study, we aimed to histologically investigate the underlying causes of these morphological deformities, which are a major concern in the global medical leech farming industry, leading to mortality and economic losses.

METHODS

Acquisition of Deformed Medicinal Leeches and Experimental Groups

Medicinal leeches (*Hirudo verbana*) that developed morphological and neurological deformities in their body segments due to various factors during cultivation at the Kırşehir University Leech Production and Research Unit were included in the study. Seven doctor-sized and medium-sized leeches exhibiting body constrictions were assigned to the "Group 2-deformed leeches" group. All leeches had been fed last one month prior to the experiment.

According to the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees, an Ethics Committee Approval Certificate is required for vertebrate animals. Since medicinal leeches are invertebrates, they are not included in the definition of "experimental animals." Therefore, no ethics committee approval was required. Informed consent could not be obtained because the study did not involve human participants, patient-derived samples, or any direct or indirect intervention in human subjects.

Group 1 "C" (Control): Morphologically normal doctor-sized healthy leeches (DSHL) and medium-SHL (MSHL) (n=7).

Group 2 "Deformed Leeches": Doctor-sized leeches with body deformities (DSDL) and medium-sized leeches with body deformities (MSDL) (n=7) (Figure 1).

Their larger size allows them to consume more blood (approximately 10-15 mL), making them suitable for long-term treatments.

Doctor-sized leeches (8-15 cm in length, 4-6 g in weight) and medium-sized leeches (6-10 cm in length, 2-4 g in weight) were used in this study. The DSDL are capable of consuming approximately 10-15 mL of blood, whereas medium-sized leeches can consume 5-10 mL. Both groups possess anticoagulant and bioactive secretory functions, which are important for therapeutic applications (14).

Anesthesia and Fixation of Medicinal Leeches

The anesthesia process was initiated by placing the medicinal leeches in a 10% ethanol solution. The ethanol concentration was gradually increased until the leeches ceased movement and response. Once relaxed in 70% alcohol, the leeches were fully anesthetized within approximately 15-30 minutes. Following anesthesia, the leeches were gently passed between the fingers to remove excess mucus and blood residues (15). Finally, in order to preserve tissue integrity and prepare the samples for subsequent analyses, all leech specimens were fixed in 10% formalin for 1 hour and then anatomically divided into anterior, middle, and posterior segments.

Histopathological Studies

The body parts of leeches from each experimental group were fixed in 10% formalin for 72 hours. Following fixation, the samples were washed under running tap water, passed through a graded ethanol series (50%, 70%, 80%, 96%, and 100%), subsequently, the tissue samples were embedded in paraffin blocks and labeled accordingly. Serial sections of 5 µm thickness were taken from the paraffin blocks of healthy leeches, matching the regional numbers of the deformation areas identified in the deformed specimens.

The sections were incubated at 56 °C overnight, then deparaffinized using xylene and rehydrated through a graded ethanol series before being washed in water. To examine the general histological structure, the sections were stained with hematoxylin and eosin. After staining, they were dehydrated again through an increasing ethanol series, cleared in xylene, and mounted with Entellan before being covered with a coverslip. Finally, the prepared slides were analyzed under a light microscope (Nikon Eclipse Si, Tokyo, Japan). The histopathological evaluation was primarily performed by the researcher. To ensure methodological reliability, all slides were independently re-evaluated in a blinded manner by a veterinary pathologist, with the results showing consistent agreement between both evaluators.

Statistical Analysis

No statistical analysis was required in the study.

RESULTS

Comparative analysis between healthy and deformed leeches across both size groups revealed pronounced morphological and physiological abnormalities in the deformed specimens. Irregular body segmentation, tissue loss in the distal region, atrophy, and in some cases, body ruptures were detected (Figure 1). Certain regions exhibited hardening, irregular muscle contractions, and uncoordinated crawling movements, along with reduced reflex responses. Due to movement restrictions, a decrease in body flexibility and locomotor capacity was noted. Additionally, loss of tissue pigmentation, weakening of the sucker function, and mucus accumulation in the segmented areas were observed. Furthermore, tissue damage, vascularization disorders, and lesion formation were identified in the affected regions.

In the DSHL, histological examinations revealed that the epithelial layer maintained regular outer contours with preserved tissue integrity. Both the circular muscle (CM) and longitudinal muscle (LM) layers exhibited organized structures, while blood vessels (BVs) were clearly defined and structurally intact. Evaluation of secretory cells indicated that T1, T2A, and T2B cells responsible for active secretion were healthy and equally distributed. Additionally, pigment (P) accumulation was observed in an organized manner beneath the muscle layers. In contrast, the DSDL exhibited epithelial layer irregularities, with disrupted outer contours and epithelial folding due to connective tissue proliferation. There was a loss of structural integrity and disorganization in the CM and LM layers. BV appeared deformed, with mononuclear cell accumulation detected. Regarding secretory cells, a notable decrease in T2B cells was observed, and in certain areas, melanin pigment deposits (melanin Ps) replaced secretory cells. Furthermore, distinct inflammatory cell clusters were detected beneath the epithelium, indicating infection sites (Figure 2).

In the MSHL group, the epithelial layer was found to be intact and well-structured. The CM and LM layers were well-organized, and

Figure 1. Macroscopic images of deformed leeches. (Left) Doctor-sized deformed leech, (right) medium-sized deformed leech. Scale bar: Left=2 cm; right=1.5 cm

the muscle structures maintained their integrity. BV exhibited normal morphology. Secretory cells with active secretion functions (T1, T2A, and T2B) were equally distributed and appeared healthy. P accumulation followed a normal distribution. In the MSDL group, the epithelial layer was disrupted and irregular. The CM and LM layers showed disorganization and loss of structural integrity. BV displayed morphological irregularities and deformation. A notable reduction in T2B secretory cells was observed, with some areas showing replacement of these cells by P accumulation. Additionally, inflammatory cell clusters were identified in the subepithelial tissue regions, indicating tissue damage and immune response activation (Figure 3).

DISCUSSION

This study provides a comparison of the morphological and histological characteristics of healthy and deformed leeches belonging to the species *Hirudo verbana*. Through microscopic evaluations, notable structural differences were identified between the groups, particularly in the epithelial organization, muscular architecture, vascular morphology, secretory cell distribution, and P accumulation. The presence of inflammatory foci and marked tissue disruption in deformed specimens further highlights the pathological consequences of deformation. These findings suggest that physical deformities significantly compromise the biological integrity of medicinal leeches, potentially impairing their physiological functions and therapeutic efficacy. Evaluating these alterations is essential not only for understanding the mechanisms of deformation but also for improving cultivation conditions and ensuring the quality and safety of leeches used in medical applications.

Medicinal leeches are primarily hematophagous parasites that feed on the blood of their hosts. In healthy leeches, botryoidal cells are arranged in clusters, while in malformed leeches, botryoidal tissue transforms from a clustered arrangement into a typical hollow (tubular) structure. In excessively fed leeches, cracks can occur on the intestinal surface, and tissue destruction in these regions triggers an immunological response. These findings provide a possible explanation for the development of post-feeding morphological deformities and highlight the physiological mechanisms that may underlie tissue damage in leech farming systems (7). In this study, epithelial disruptions were markedly more pronounced in the deformed leech groups, indicating a loss of structural integrity in the outermost tissue barrier. This impairment is particularly critical, as the epithelium plays a vital role in protecting internal tissues from external insults, including microbial pathogens. Hildebrandt and Lemke (16) emphasized that epithelial damage in *Hirudo medicinalis* could compromise the barrier function, predisposing the organism to infections and triggering localized immune responses. Consistent with this, our study observed the presence of inflammatory cell clusters and infection foci beneath the damaged epithelial regions in deformed leeches, supporting the notion of immune activation and tissue defense mechanisms. A different study showed that exposure to various concentrations of cadmium caused altered epithelial cell boundaries, vacuolar degeneration in the epidermis, and

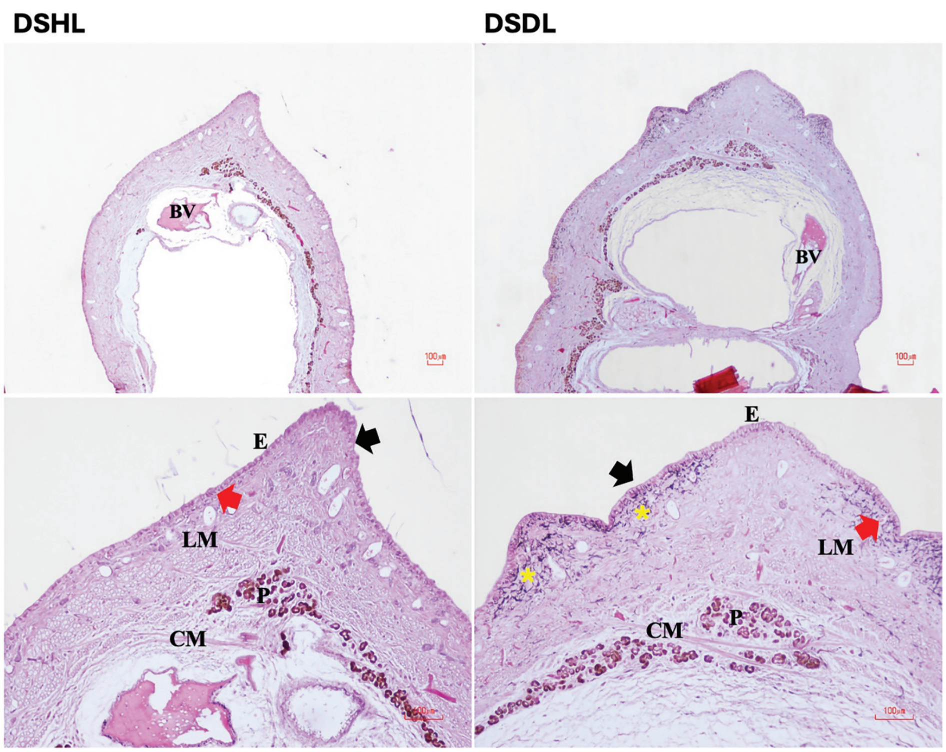

Figure 2. Histological sections of doctor-sized healthy (DSHL) and deformed (DSDL) leeches stained with hematoxylin and eosin. Black arrow: Cuticle, E: Epithelium, CM: Circular muscle, LM: Longitudinal muscle, BV: Blood vessel. Secretory cells with active secretion function are identified as T1 (red arrow). T2A and T2B secretory cells are distributed throughout the subepithelial connective tissue. P: Melanin pigment deposits in the submucosal layer. Infection sites characterized by inflammatory cell infiltration and increased melanin pigment accumulation in the subepithelial region; Yellow stars: Infection sites characterized by inflammatory cell infiltration and increased melanin pigment accumulation in the subepithelial region. Scale bar: 100 µm

structural damage in the cuticle in a freshwater leech species (17). In our study, the increased P accumulation observed beneath the damaged epithelial regions was primarily associated with melanin-like structures. Such pigmentation has previously been linked to stress responses and innate immune activity in invertebrates, where melanin synthesis plays a role in defense mechanisms, particularly under inflammatory conditions (18), which are known to be associated with oxidative stress-induced tissue damage in leeches (17). Importantly, melanin deposition in invertebrates has also been implicated in innate immune defense. It has been demonstrated that phenoloxidase-mediated melanogenesis plays a critical role in encapsulating and neutralizing pathogens, suggesting a functional link between P accumulation and immune activation. As proposed by Salzet (19), melanin and melanin-like compounds may serve not only as by-products of immune activation but also as active agents within antimicrobial defense systems. In this context, the increased P accumulation observed in deformed leeches may reflect a compensatory response aimed at limiting tissue damage and microbial invasion through localized immune activation. These findings reinforce the hypothesis that morphological deformation in medicinal leeches affects not only structural integrity but also elicits physiological stress responses that could potentially compromise their therapeutic reliability.

In leeches, muscle fibers are separated from the inner digestive tube by a layer of loose connective tissue (20). The tubular body wall of the leech consists of three distinct muscle layers of varying thickness: circular, longitudinal, and oblique muscle fibers. These three layers, along with dorsoventral muscles, are primarily responsible for maintaining posture and facilitating various movements such as swimming, crawling, and directional orientation (21). In the literature, disorganization of the muscle layers has been prominently observed in deformed leeches, emphasizing the critical role of muscular architecture in essential biological functions such as locomotion and feeding. Perkins et al. (22) highlighted that structural impairments in the musculature may significantly compromise the leech's physiological performance, including its ability to attach, move, and feed efficiently. Consistent with these findings, this study demonstrated that deformation-related disruptions in the circular and LM layers were evident in the affected specimens, potentially impairing their biological activity.

In this study, histological examination revealed a regular distribution of T1, T2A, and T2B secretory cells in healthy leeches. In contrast, a marked reduction in T2B cells was observed in the deformed groups. According to the literature, T2B cells play an important role in the secretion of biologically active molecules in medicinal leech species such as *Hirudo medicinalis* (10,12,23). Therefore, the decrease in T2B cells may be associated with reduced biological functionality and diminished therapeutic potential. On the other hand, a different study conducted on *Limnatis nilotica* reported that exposure to cadmium resulted in morphological changes in type 1 secretory cells and vacuolar degeneration in the epidermis, while type 2 secretory cells exhibited a numerical increase without morphological alterations. This finding contrasts with our results regarding the reduction in T2B cells, and the discrepancy may be attributed to species-specific

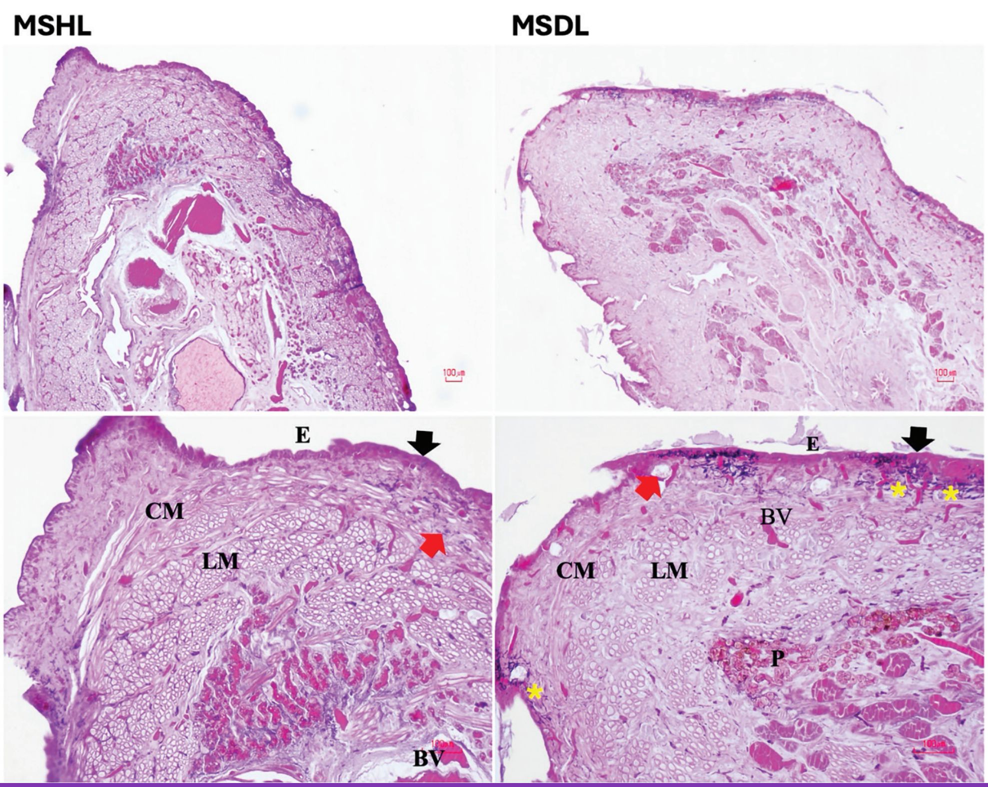

Figure 3. Hematoxylin-eosin stained tissue sections from the medium-sized healthy leech (MSHL) and medium-sized deformed leech (MSDL) groups. Black arrow: Cuticle, E: Epithelium, CM: Circular muscle, LM: Longitudinal muscle, BV: Blood vessel, secretory cells with active secretory function are designated as T1 (red arrow); T2A and T2B secretory cells are distributed throughout the subepithelial connective tissue. P: Melanin pigment deposits in the submucosal layer. Yellow stars: Infection sites characterized by inflammatory cell infiltration and increased melanin pigment accumulation in the subepithelial region. Scale bar: 100 µm

differences and the distinct nature of the stressors involved. In our study, the deformation is likely linked to biomechanical stress or infection rather than a chemical agent such as cadmium. These observations suggest that various stress factors may elicit cell-type-specific responses depending on the species and tissue type involved (17).

In the deformed groups, infectious foci were also found to be more prevalent. The accumulation of mononuclear cells beneath the epithelium, along with the observed decrease in T2B secretory cells and replacement of these cells by melanin Ps, suggests the activation of a local immune response. Additionally, the presence of distinct inflammatory cell clusters beneath the epithelium indicates localized tissue damage and immune reaction in the affected areas. A more comprehensive investigation of these infections can be achieved through techniques such as immunohistochemistry, which would allow for the characterization of specific cell types and the cytokines they secrete in the infected regions thereby providing deeper insight into the extent and nature of the inflammatory response (24). The inflammatory responses and cellular alterations accompanying tissue damage observed in the study by de Eguileor et al. (25) are consistent with previously described wound healing processes in annelids. In the literature, it has been reported that leeches utilize macrophage-like cells, natural killer-like cells, and granulocytes located in the connective tissue and vascular system to mediate tissue repair. However, this cellular defense mechanism appears to be less rapid and efficient compared to other annelid groups such as oligochaetes, polychaetes, and sipunculids. This difference has been attributed to the reduced coelomic cavity and the limited number of coelomocytes in leeches (26,27). While oligochaetes and polychaetes are capable of initiating relatively rapid tissue repair through the migration of immune cells from the coelomic fluid, leeches exhibit reduced regenerative capacity and increased vulnerability to extensive tissue damage. These findings are particularly significant in explaining the persistence of inflammatory foci and irreversible tissue deformities observed in our study.

CONCLUSION

This study demonstrated that morphological deformities observed in *Hirudo verbana* medicinal leeches can affect several histological parameters including epithelial integrity, muscle organization, secretory cell distribution, and immune responses potentially impairing their physiological functions. The observed reduction in T2B secretory cells, along with the accumulation of melanin Ps in certain regions, suggests a disruption in normal secretory activity. Additionally, increased inflammatory foci and the accumulation of mononuclear immune cells beneath the epithelium indicate a localized immune response, linking deformation to tissue damage and the activation of defense mechanisms. These findings imply that deformation may negatively impact the therapeutic effectiveness of leeches. Further molecular-level studies are needed to better understand the underlying mechanisms. In this context, optimizing leech breeding conditions and improving quality control processes remain essential for ensuring their reliability in medical applications.

*Ethics

Ethics Committee Approval: According to the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees, an Ethics Committee Approval Certificate is required for vertebrate animals. Since medicinal leeches are invertebrates, ethics commitee approval was not required according to national regulations.

Informed Consent: Informed consent could not be obtained because the study did not involve human participants, patient-derived samples, or any direct or indirect intervention in human subjects.

Footnotes

*Authorship Contributions

Concept: H.T.Y., E.K., A.B., M.Ç., Design: H.T.Y., E.K., A.B., M.Ç., Data Collection or Processing: H.T.Y., E.K., M.K., N.Ç., Analysis or Interpretation: A.B., Literature Search: H.T.Y., E.K., M.K., N.Ç., Writing: H.T.Y., E.K., M.Ç.

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

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The Hidden Danger in the Eyelids: Diagnosis and Treatment of Bilateral Phthiriasis Palpebrarum - A Rare Pediatric Case Report

Göz Kapaklarındaki Gizli Tehlike: Bilateral Phthiriasis Palpebrarum Tanısı ve Tedavisi - Nadir Bir Pediyatrik Olgu Raporu

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ABSTRACT

Phthiriasis palpebrarum is a rare infestation of the eyelids caused by *Phthirus pubis*. This condition is particularly noteworthy as it is a rare cause of red eyes in children and can often be confused with other forms of blepharoconjunctivitis. In this study, we examined an 11-year-old male patient who was brought to our clinic by his family and presented with redness and itching in both eyes that had persisted for a month. Biomicroscopic examination revealed two live lice with mobile legs and a semi-transparent appearance on the eyelashes of both upper eyelids, together with a large number of eggs, particularly concentrated on the right eye. No foreign body was found in the eye. After the necessary protective measures had been taken, the lice and their eggs were carefully removed from the eyelashes using forceps. The parasitological examination of eyelash samples confirmed the presence of adult lice and eggs of *Pthirus pubis*. After a suitable treatment plan was developed and detailed hygiene recommendations were thoroughly explained, the patient was discharged. During the follow-up examination conducted one week later, no lice or eggs were detected on the eyelashes, and the blepharoconjunctivitis had regressed.
Keywords: Blepharitis, phthiriasis palpebrarum, *Pthirus pubis*

ÖZ

Phthiriasis palpebrarum, *Phthirus pubis*'in etkeni olduğu nadir görülen bir göz kapağı enfestasyonudur. Genellikle diğer blefarokonjonktivit nedenleri ile karıştırılabilen bu durum, özellikle pediatrik kırmızı göz olgularının nadir bir sebebi olarak dikkat çeker. Bu çalışmada, her iki gözde bir aydır devam eden kızarıklık ve kaşıntı şikayetleriyle ailesi tarafından kliniğimize getirilen on bir yaşındaki bir erkek hastayı değerlendirmeyi ve bu olguya ışık tutmayı amaçladık. Biyomikroskopik incelemede, her iki üst göz kapağının kirpiklerinde hareketli ayaklara sahip, yarı şeffaf görünümde iki canlı bit ve özellikle sağda yoğunlaşmış çok sayıda yumurta tespit edildi. Göz içinde ise herhangi bir yabancı cisme rastlanmadı. Gerekli koruyucu önlemler alındıktan sonra bitler ve yumurtaları tutundukları kirpiklerden dikkatli bir biçimde penset yardımıyla uzaklaştırıldı. Kirpiklerden alınan örneklerin parazitolojik incelemesinde erişkin *Pthirus pubis* ve yumurtaları olduğu saptandı. Uygun tedavi planı oluşturulup gerekli hijyen önerileri detaylı bir şekilde anlatıldıktan sonra hasta taburcu edildi. Bir hafta sonra yapılan kontrol muayenesinde, kirpiklerde herhangi bir bit veya yumurtaya rastlanmadı ve blefarokonjonktivitin gerilediği gözlendi.
Anahtar Kelimeler: Blefarit, phthiriasis palpebrarum, *Pthirus pubis*

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INTRODUCTION

Pediculosis pubis is mainly transmitted through sexual contact or close physical interaction. It usually occurs in the pubic region, although it can also affect the scalp, armpits, eyebrows or eyelashes (phthiriasis palpebrarum). It is considered a rare cause of blepharoconjunctivitis (1). Pubic lice are spread through sexual contact in adults and adolescents. They can be transmitted from contaminated hands to the eyelashes. In children, transmission occurs through close contact, sleeping in the same bed with infected parents or sharing objects, and sexual abuse (2). The associated blepharoconjunctivitis may be due to a hypersensitivity reaction to the parasite and can therefore be misdiagnosed as allergic blepharoconjunctivitis (3). Although infestation occurs in all age groups, especially in poor hygienic conditions and in chronic, treatment-resistant blepharitis, this diagnosis should be kept in mind, especially in pediatric cases (4). Such infestations can be easily overlooked or evaluated as simple blepharitis if only the ocular surface is considered during biomicroscopic examination and the examination of the eyelids is ignored (5). In this study, we report a case of isolated bilateral palpebral involvement presenting as blepharoconjunctivitis in an 11-year-old boy.

CASE REPORT

An 11-year-old male patient presented to our clinic complaining of redness and itching in both eyes that had persisted for one month. According to his family, he had previously seen a doctor, but his symptoms persisted despite treatment. Corrected distance visual acuity was 20/20 in both eyes. Biomicroscopic examination revealed two semi-transparent live lice with mobile legs on the eyelashes of both upper eyelids, along with numerous oval whitish eggs, particularly concentrated on the right side (Figure 1). The conjunctiva appeared hyperemic, with follicular structures observed beneath the eyelids (Figure 2). Aside from this, the anterior and posterior segment examinations were deemed normal. No foreign body was found in the eye. Topical pilocarpine hydrochloride (pilosed 2%, Bilim İlaç) and cyclopentolate hydrochloride (sikloplejin 1%, Abdi İbrahim İlaç) drops were applied to the roots of the eyelashes. After local anesthesia with proparacaine hydrochloride (alcaine 0.5%, Alcon), the immobilized lice and their eggs were carefully removed mechanically with forceps from the eyelashes to which they were attached, observing the necessary protective measures, and collected in a container for parasitological examination (Figure 3). The procedure was terminated after ensuring that all lice and eggs had been removed. To avoid possible contamination, a biomicroscope was preferred, which was not actively used in the procedure, and the biomicroscope was cleaned with bleach after the procedure. Parasitological examination of the samples taken from the eyelashes revealed adult lice and eggs of *Pthirus pubis* (Figures 4-6). As a treatment, it was recommended to apply tobramycin eye ointment (tobrased 0.3%, Bilim İlaç) together with liquid vaseline (petrolatum compound) to the eyelashes three times a day, then comb the eyelashes and clean them with tea tree oil. Although there were no complaints in the family members, no parasites were found during examinations for asymptomatic infestation. It was explained that the use of shared objects should be avoided, that clothes and bedding should be washed at high temperatures and then ironed with high steam pressure and that maximum hygiene should be observed. The family's socio-economic status was good and they were educated people. When the cause of the infestation was investigated, no suspicion of sexual abuse arose, and there were no individuals in the patient's surroundings with similar symptoms. It was observed that both the family and the patient maintained good personal hygiene. A dermatological

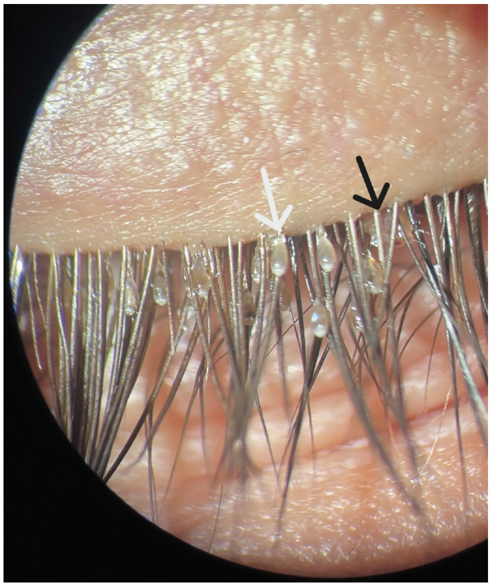

Figure 1. White arrow: Oval, greyish-white egg of a louse hanging from an eyelash. Black arrow: Live, translucent adult louse firmly attached to an eyelash (the photos were taken with a biomicroscope at X16 magnification)

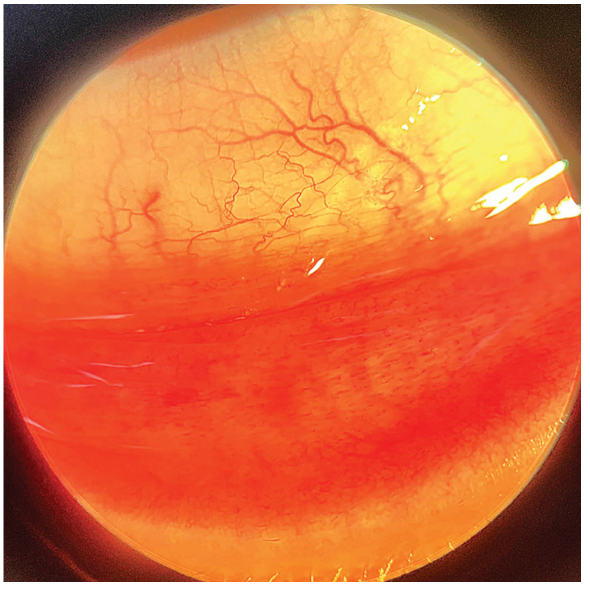

Figure 2. The conjunctiva appeared hyperemic and follicular structures were observed under the eyelids (the photos were taken with a biomicroscope at X16 magnification)

Figure 3. After mechanical cleaning with forceps, eggs and lice were collected for parasitological examination

Figure 5. Light microscopic examination of a young egg of *Phthirus pubis* at ×10 magnification. The egg measured approximately 1 mm in length and 0.4 mm in width (scale bar: 1 mm)

Figure 4. Light microscopic examination of an adult *Phthirus pubis* at ×4 magnification. The specimen measured approximately 1.4 mm in length and 0.8 mm in width (scale bar: 1 mm)

Figure 6. Light microscopic examination of a mature and hatching lice egg of *Phthirus pubis* at X10 magnification. The egg measured approximately 1.2 mm in length and 0.4 mm in width (scale bar: 1 mm)

examination was carried out due to the suspicion of a possible lice infestation on other parts of the body. However, it was found that no infestation with *P. pubis* was found on other parts of the body during the examination. At the follow-up examination, which was performed 1 week later, it was found that the patient's complaints of irritation and redness had disappeared. On biomicroscopic examination, it was found that the lice and eggs in the eyelashes had completely disappeared and the blepharoconjunctivitis had regressed. The case and the family were informed about personal hygiene and possible transmission routes of the infestation. In addition, written informed consent was obtained.

DISCUSSION

Human lice are a public health problem affecting millions of people worldwide, especially in developing countries. Lice have been known for over 10,000 years, and the oldest human lice eggs were found on a hair shaft in an archaeological study (6). Human lice typically occur in the form of head lice in 19% of school children and in the form of pubic lice in 2% of the adult population (7).

Human blood-sucking lice belong to two families: *Pediculidae* and *Pthiridae*, with the corresponding genera being *Pediculus* and *Pthirus*, respectively. *Pediculus humanus capitis* denotes the head louse, while *P. pubis* refers to the pubic louse, also known as the crab louse. *P. pubis* prefers to cling to thick, stiff hair such as pubic hair, eyelashes and eyebrows, but can also infest other parts of the body (8).

P. pubis is about 0.8-1.2 mm long and has three pairs of legs. The first pair of legs is short and weak, while the other two pairs end in crab-like claws that can cling to pubic or other body hair (Figure 4). *P. pubis* lays 50 eggs during its lifetime (Figures 5, 6) and adult lice die at temperatures above 40 degrees. The eggs can survive 15-60 minutes in a 60-degree wash (9).

In adults, infestation with eye lice is usually caused by the transfer of pubic lice from the pubic area to the eyelid area by scratching and rubbing the eyes; in children, infestation with eye lice is caused by close contact with adults and the use of shared objects. It should also be borne in mind that children may have been sexually abused because they do not yet have terminal body hair. Although phthiriasis palpebrarum is most commonly reported in school-age children and adults, it has been documented even in the neonatal period. However, in Türkiye, reliable epidemiological data on the annual incidence of phthiriasis palpebrarum are lacking, and the literature consists mainly of isolated case reports. In pediatric cases, eyelash involvement requires particular caution. Although transmission may occur through non-sexual routes, the possibility of sexual abuse must always be assessed within the scope of forensic medicine and social services. According to current legislation in Türkiye, physicians have a mandatory reporting obligation in such cases (10,11). Adult patients should also be screened for diseases transmitted through sexual contact. In view of the fact that the disease can be transmitted through sexual contact, it is recommended that partners be examined and treatment initiated if necessary. To prevent recurrence of the disease, the patient should be advised on personal hygiene and cleaning of shared objects. Contaminated cosmetics should not be reused and items such as clothing, towels and sheets should be washed at a high temperature (60 °C) for 30-60 minutes and then ironed at a high temperature (11).

In the treatment of phthiriasis palpebrarum, it is usually sufficient to remove the lice and eggs mechanically with tweezers or to cut off the eyelashes and then apply petrolatum ointment to the eyelids two or three times a day for 10 days. Petrolatum ointment kills the lice, prevents the lice from attaching to the eyelashes and prevents them from hatching (12). Other effective topical treatments include 0.3% tobramycin eye ointment, 0.5% moxifloxacin eye ointment, physostigmine ointment, 1% malathion shampoo, 1% yellow mercuric oxide eye ointment, parasympathomimetics such as 4% pilocarpine gel, topical botulinum toxin and 20% fluorescein drops. Topical antiparasitics such as natural pyrethrin, 1% permethrin, 0.2% phenothrin may also be prescribed. In cases that are not treated for a long period of time, secondary bacterial infections may occur. In such a case, treatment should be supplemented with topical antibiotic drops and ointments. In cases where itching and irritation are severe, topical treatment with antihistamines may be recommended to relieve symptoms (13,14). Tea tree oil, known for its broad spectrum antimicrobial and anti-inflammatory properties, has also been shown to be effective in treatment (15). Inactivation of lice and eggs by argon laser phototherapy and mechanical removal has also been shown to be effective (16). Although there are various alternatives and newly proposed methods in the treatment of phthiriasis palpebrarum, the treatment process is difficult and no clear consensus has been reached.

CONCLUSION

In persistent and unresponsive cases of blepharoconjunctivitis, phthiriasis palpebrarum should be considered a potential diagnosis. Eggs and lice can be detected through a careful eyelid examination using a biomicroscope with X40 magnification. While various treatment approaches exist, mechanical cleaning and the application of liquid petroleum jelly are often sufficient. Special attention should be given to the possibility of sexual abuse, especially in pediatric cases. If suspicion arises, a multidisciplinary approach involving pediatrics for potential child abuse and dermatology for sexually transmitted diseases is essential. Phthiriasis palpebrarum can be seen not only in individuals from low socioeconomic backgrounds but also in the general population.

*Ethics

Informed Consent: The case and the family were informed about personal hygiene and possible transmission routes of the infestation. In addition, written informed consent was obtained.

Footnotes

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*Authorship Contributions

Concept: Y.S.A., R.U., Design: Y.S.A., R.U., Ş.S.Y., Data Collection or Processing: Ş.S.Y., Analysis or Interpretation: Y.S.A., R.U., H.D.İ., O.Ö., Ş.S.Y., Literature Search: Y.S.A., R.U., O.Ö., Writing: Y.S.A.

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Case Report
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Risk of Oral Myiasis in Intensive Care Unit: A Case Report Caused by *Calliphoridae*

Yoğun Bakım Ünitesinde Oral Miyaz Riski: Calliphoridae Kaynaklı Bir Olgu Sunumu

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ABSTRACT

Myiasis is a rare ectoparasitic infestation characterized by the invasion of living tissue by fly larvae of the order Diptera. While larvae typically target wounded skin, ocular, nasopharyngeal, and gastrointestinal regions, oral myiasis is a less frequently reported but clinically significant site of infestation. Herein, we report a case of oral myiasis in a 68-year-old male patient who was intubated and managed in the intensive care unit following a high-altitude fall resulting in a Type III odontoid fracture. A tracheostomy was subsequently performed and during routine replacement of a nasogastric tube, multiple motile larvae were observed in the oral cavity. The larvae were mechanically removed, and the affected area was thoroughly irrigated with a sodium bicarbonate solution. Parasitological examination, including both macroscopic and microscopic analysis, identified the larvae as third-instar forms of the *Calliphoridae* family. Despite ongoing intensive supportive care, the patient developed sepsis and multiorgan failure, culminating in cardiopulmonary arrest and death. This case suggests that oral myiasis may occur even under strict adherence to standard infection control measures. It highlights the necessity for individualized risk assessment and the implementation of advanced, targeted prevention strategies in vulnerable intensive care unit populations, considering the complex interplay of host, environmental, and systemic factors.
Keywords: Oral myiasis, intensive care, *Calliphoridae*, parasitic infestation

ÖZ

Miyaz, Diptera takımına ait sinek larvalarının canlı dokuya invazyonu ile karakterize nadir bir ektoparaziter enfestasyondur. Larvalar genellikle yaralı deri, oküler, nazofarengeal ve gastrointestinal bölgeleri hedef alırken, oral miyaz daha az sıklıkla bildirilen ancak klinik açıdan önemli bir tutulum bölgesidir. Bu olgu sunumunda, yüksekten düşme sonrası entübe edilerek yoğun bakımda takip edilen ve Tip III odontoid fraktürü nedeniyle trakeostomize edilen 68 yaşındaki bir erkek hastada gelişen oral miyaz tablosu sunulmuştur. Nazogastrik sonda değişimi sırasında oral kavitede çok sayıda hareketli larva tespit edilmiş, fiziksel olarak uzaklaştırılan larvaların bulunduğu bölge sodyum bikarbonat ile irrigasyon yoluyla temizlenmiştir. Parazitolojik incelemeler, larvaların *Calliphoridae* ailesine ait üçüncü dönem larvalar olduğunu doğrulamıştır. Hastada, takip sürecinde sepsis ve çoklu organ yetmezliği gelişmiş; tüm yoğun girişimlere rağmen kardiyopulmoner arrest sonrası eksitus gerçekleşmiştir. Bu olgu, standart enfeksiyon kontrol önlemlerine tam olarak uyulmasına rağmen miyazın, konak faktörleri ve çevresel koşulların etkileşimiyle gelişebileceğini ortaya koymakta; yoğun bakım hastalarında bireyselleştirilmiş risk analizi ve gelişmiş önleme stratejilerinin önemini vurgulamaktadır.
Anahtar Kelimeler: Oral miyaz, yoğun bakım, *Calliphoridae*, paraziter enfestasyon

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Myiasis, derived from the Latin words "*myia*" (fly) and "*iasis*" (disease), refers to a rare ectoparasitic infestation characterized by the invasion and development of fly larvae from the order Diptera within the tissues of humans or animals (1). Clinically, it results from the colonization of viable or necrotic tissue by larvae and can lead to significant complications if not diagnosed and treated promptly. The clinical manifestations vary depending on the anatomical site of infestation, the species of the larvae, and the immunological status of the host (2). Although more frequently reported in tropical and subtropical regions, isolated cases of myiasis have been documented in other geographical areas when favorable environmental conditions are present. Warm and humid climates promote increased fly activity, facilitating oviposition or larval deposition onto open wounds, mucosal surfaces, or natural body orifices of susceptible hosts (3). Predisposing factors include poor hygiene, low socioeconomic status, advanced age, chronic illnesses, immunosuppression, substance use, prolonged immobilization, and long-term intubation (4).

Myiasis is classified anatomically as wound, furuncular, cavitary, or hematophagous types, and ecologically as obligatory, facultative (primary, secondary, tertiary), or accidental (1,3). Globally, 37 fly species from 10 different families within the Diptera order have been implicated in human myiasis, with species from the *Calliphoridae* (12 species) and *Sarcophagidae* (9 species) families being the most common. While oral myiasis has been reported in the literature, it remains a relatively infrequent manifestation compared to cutaneous, ocular, nasopharyngeal, and gastrointestinal forms (4). It typically occurs in individuals with poor oral hygiene, necrotic periodontal tissues, immunosuppressive states, or reduced consciousness (5). The larvae thrive in moist, oxygen-poor environments; periodontal pockets provide an ideal habitat for larval development. Their photophobic behavior further encourages deeper tissue migration as they progress to the pupal stage (6).

In this report, we present a case of oral myiasis in a patient who developed the condition following prolonged intubation due to trauma-induced loss of consciousness. The aim of this study is to discuss the diagnostic approach, clinical management, and therapeutic interventions in the context of current literature, while emphasizing the clinical relevance of this uncommon but potentially serious infestation.

CASE REPORT

A 68-year-old male patient was admitted to the Anesthesiology Intensive Care Unit of Manisa Celal Bayar University Hafsa Sultan Hospital following a high-altitude fall, resulting in severe cervical trauma, impaired consciousness, and acute respiratory failure. He was intubated upon arrival and underwent cervical computed tomography, which revealed a Type III odontoid fracture at the C2 vertebra. Tracheostomy was performed early during mechanical ventilation, and multidisciplinary critical care management was initiated. During the intensive care unit stay, the patient received invasive mechanical ventilation and enteral nutrition via a nasogastric tube. Nursing care, oral hygiene, and antimicrobial therapy were administered according to current evidence-based protocols. On day 12 of hospitalization, larvae were observed in the oral cavity during routine nasogastric tube replacement. Detailed clinical examination confirmed that the infestation was confined to the oral mucosa, with no evidence of larval presence around the tracheostomy site or within the trachea. Mechanical debridement was performed, followed by irrigation of the affected area with a sodium bicarbonate solution. The removed larvae were preserved in 70% ethanol and referred to the parasitology laboratory for identification. Specimens measuring 7-8 mm with typical segmental morphology were observed during macroscopic evaluation. Stereomicroscopy and light microscopy demonstrated 12 segmented bodies, prominent paired oral hooks, well-developed oral sclerite structures, and complete peritreme closure with distinct button and slit formations on posterior spiracles. Based on morphological features and taxonomic analysis, the larvae were conclusively identified as third instar (L3) *Calliphoridae* species belonging to the order Diptera (Figure 1). Despite supportive measures, the patient developed progressive cardiovascular and respiratory failure and did not respond to cardiopulmonary resuscitation, resulting in death five days later.

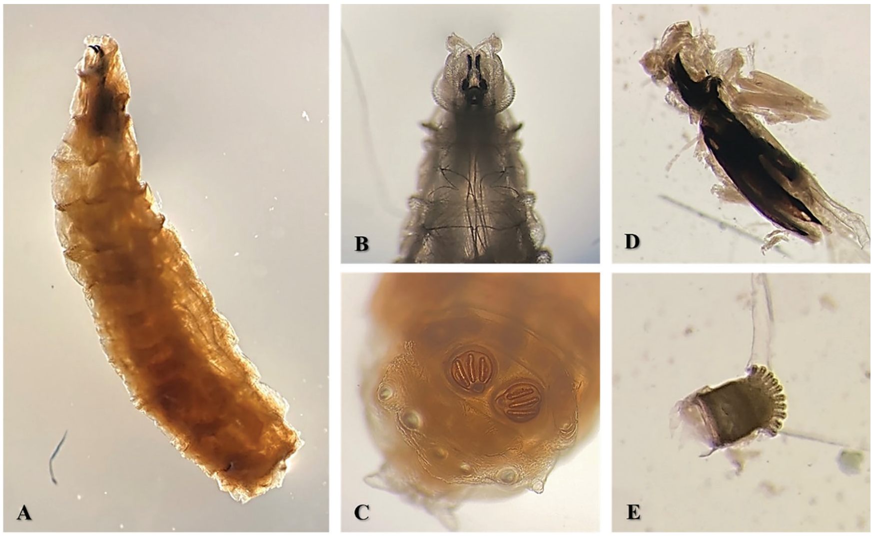

Figure 1. Microscopic characterization of a third-instar larva belonging to the *Calliphoridae* family. A: Total view of the 3rd stage larvae (4x), B: Anterior end demonstrating the cephalopharyngeal skeleton and anterior spiracle (40x), C: Posterior end showing a trifurcated posterior spiracle (40x), D: Cephalopharyngeal skeleton (40x), E: Anterior spiracle (40x)

Since the patient was lost when myiasis was identified, the patient's approval could not be obtained.

DISCUSSION

Oral myiasis is an uncommon form of parasitic infestation, defined by the colonization and development of dipteran larvae within the oral cavity. Although documented in the literature, it occurs less frequently than cutaneous, ocular, or other anatomical variants (7,8). The oral environment typically serves as a natural barrier to larval development due to continuous mechanical cleansing, salivary flow, and antimicrobial components. However, factors such as immunosuppression, prolonged intubation, poor oral hygiene, necrotic tissue, and loss of consciousness can compromise these defenses and create a favorable environment for infestation (9). Clinically described for the first time in 1909, oral myiasis may present with symptoms such as oral pain, swelling, fetid odor, and a sensation of larval movement (10). In intensive care patients, the inability to maintain oral hygiene and the presence of necrotic tissue increase the risk of fly oviposition. Under suitable temperature and humidity conditions, deposited eggs hatch rapidly, and the emerging larvae invade the oral mucosa. Once penetrated into the tissue, larvae orient their posterior spiracles toward the surface for respiration while embedding their anterior segments into deeper tissues. This configuration allows them to be protected from environmental factors and continue their development. Through this invasive process, larvae typically reach the prepupal stage within 8 to 12 days (11). During their development in human tissue, larvae progress through three instar stages (L1 to L3), each characterized by distinct morphological features. Although myiasis more commonly involves anatomical sites such as the nasal cavity, eyes, ears, and lungs, oral involvement remains relatively uncommon. This infrequency is likely attributable to the oral cavity's limited exposure to external environmental factors and its effective local defense mechanisms. Nevertheless, when these barriers are compromised, the oral cavity may become a potential reservoir for larval development (12).

Reported cases of oral myiasis worldwide vary depending on the geographic distribution of the causative fly species, environmental conditions, and individual risk factors. Species such as *Oestrus ovis* (13), *Musca domestica* (14), *Chrysomya bezziana* (15) and *Lucilia sericata* (16) have been isolated in different regions and are associated with host-specific conditions including poor oral hygiene, history of trauma, immobility, and intensive care hospitalization. Similarly, cases reported from Türkiye indicate that oral myiasis is often linked to predisposing factors such as prolonged intubation, inadequate oral hygiene, and immunosuppression. In this context, *Lucilia sericata* (17), *Sarcophaga* spp. (18), *Wohlfahrtia magnifica* (19), *Hypoderma bovis* (20) and larvae from the *Calliphoridae* family (21,22) have been isolated in various clinical scenarios, including intubated patients, individuals with impaired consciousness, and residents of rural areas. These findings clearly demonstrate that the development of oral myiasis is influenced not only by the presence of specific vector species but also by the host's general health status, immune condition, and living environment.

In our case, the patient's prolonged immobility due to impaired consciousness in a rural setting, along with direct exposure to environmental factors, created a favorable condition for flies of the *Calliphoridae* family to deposit eggs within the oral cavity. The persistently open mouth position reduced salivary secretion, and consequent deterioration in oral hygiene provided a microenvironment conducive to larval colonization. This scenario significantly increases the risk of infestation, particularly in elderly, immunosuppressed, or unconscious individuals. The submucosal localization of the larvae within the oral mucosa may lead to the absence of typical symptoms in the early stages of infestation, potentially delaying diagnosis. Unlike superficial involvement, larval invasion into deeper tissues complicates the identification of pathognomonic signs during routine oral examinations, often resulting in a diagnosis at more advanced stages. Similarly, the literature indicates that larvae in the L1 and L2 stages tend to progress asymptomatically, with clinical manifestations usually emerging upon transition to the L3 stage (10). These findings suggest that relying solely on clinical symptoms may be insufficient for diagnosing oral myiasis. A comprehensive assessment that includes a detailed patient history, environmental exposure, and understanding of larval developmental stages is essential for timely and accurate diagnosis.

CONCLUSION

In conclusion, the occurrence of oral myiasis in the present case, despite strict adherence to standard infection control measures within an intensive care unit, suggests that such infestations cannot be entirely prevented through systemic measures alone. Impaired consciousness, prolonged mouth opening, and compromised mucosal barrier integrity, combined with exposure to environmental vectors, created favorable conditions for infestation. This underscores the need for individualized and proactive surveillance strategies in high-risk patients, supplementing existing clinical protocols. Structured follow-up systems, including regular oral examinations and targeted larval screening, may facilitate earlier diagnosis and improve treatment outcomes. Moreover, documenting such infrequent yet clinically significant cases is crucial for raising awareness and guiding both diagnostic and preventive approaches in comparable clinical contexts.

* Ethics

Informed Consent: Since the patient was lost when myiasis was identified, the patient's approval could not be obtained.

Footnotes

This study was presented as an electronic poster at the 40th International Turkish Microbiology Congress, held on November 16-20, 2022, in Antalya, Türkiye.

*Authorship Contributions

Surgical and Medical Practices: A.Z.A., Ö.H., D.T.A., Concept: A.Y., T.A., D.T.A., Ü.Z.O., Design: A.Y., T.A., D.T.A., Ü.Z.O., Data Collection or Processing: A.Y., T.A., A.Z.A., Ö.H., Analysis or Interpretation: D.T.A., Ü.Z.O., Literature Search: A.Y., T.A., Writing: A.Y., T.A., D.T.A., Ü.Z.O.

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