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The neglected role of *Enterobius vermicularis* genetic variation in pediatric acute appendicitis

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Abstract

Pediatric acute appendicitis is considered to be one of the most prevalent abdominal emergencies worldwide. While the etiology of appendicitis is believed to be secondary obstruction due to parasitic infection, the role of *Enterobius vermicularis* in acute appendicitis is not widely recognised. This study aimed to establish a correlation between the genetic diversity of *Enterobius vermicularis* parasites isolated from patients diagnosed with acute appendicitis, and the patients' clinical symptoms, ultrasonographic and histopathological findings. This study examined the clinical, radiological, and histopathological findings of patients diagnosed with acute appendicitis over the past decade. The clinical, radiological and histopathological findings of these patients were examined restrospectively. A total of 30 appendectomy specimens identified as *Enterobius vermicularis* by histopathological methods were included in the study and DNA was isolated from these specimens. The cox1 gene region of *Enterobius vermicularis* was analysed using PCR and Evm1/Evm2 primers. *Enterobius vermicularis* genetic diversity was determined by DNA sequencing and phylogenetic analysis. Based on different nucleotide sequencing findings, the parasite was divided into two classes; *Enterobius vermicularis* variant 1 and *Enterobius vermicularis*. The genetic distance value between the *Enterobius vermicularis* isolates was found to be 0.068. The clinical, radiological and histopathological findings of patients with acute appendicitis were examined retrospectively and *Enterobius vermicularis* was identified as the causative agent. The genetic diversity of *Enterobius vermicularis* is important for the prognosis of acute appendicitis in children.

Keywords: Pediatric acute appendicitis, *Enterobius vermicularis*, genetic diversity

Introduction

Pediatric acute appendicitis (PAA) is defined as inflammation of the appendix, and is the most common surgical emergency in children presenting with abdominal pain [1]. Despite the fact that severe abdominal pain, fever and vomiting are among the most prominent clinical symptoms of PAA, a confident preoperative diagnosis remains challenging when appendicitis must be considered in every patient presenting with acute abdominal pain. The signs and symptoms are often non-specific and can mimic many surgical pathologies, thereby adding to the complexity of making the correct diagnosis [2]. It is important to note that there is a strong correlation between physical examination findings, radiological findings, pathological findings and laboratory results in cases of PAA.

In the field of radiological diagnostic techniques, ultrasonography (USG) has been recognised as a valuable tool in diagnosing pediatric appendicitis (PA) for the past three decades. Its use in this context is advantageous due to its non-invasive nature and the absence of ionising radiation, making it a safe and prudent option. The presence of perforated appendicitis, an abscess or a plastron,

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as indicated by ultrasound imaging, together with thickening of the appendix wall and the presence of periappendiceal fluid, helps to confirm the clinical diagnosis of acute appendicitis [3].

All appendectomy specimens must undergo histopathological examination to identify various incidental pathologies, most notably parasitic infections and benign and malignant tumours. These pathologies may necessitate further treatment, particularly in cases where a preliminary diagnosis of PAA has been made. The pathological diagnosis of PAA is confirmed a few days after surgery and does not influence postoperative management [4,5].

PAA is a common pediatric surgical emergency in both developed and developing countries. *Enterobius vermicularis* (*E. vermicularis*), a gastrointestinal parasite, has been reported to cause PAA by obstructing the lumen of the appendix. During ectopic migration, the release and subsequent accumulation of *E. vermicularis* eggs results in obstruction and inflammation of the appendix, leading to PAA [6,7]. *E. vermicularis* has been reported as the causative agent in 1.5% of cases of appendicitis in children aged 7–11 years. Risk factors for the disease include young age, dietary habits, and genetic predisposition. Early diagnosis and surgical intervention are crucial, as untreated cases can lead to perforation, peritonitis or abscess formation [8,9].

In recent years, the development of molecular methods has made it possible to diagnose diseases such as PAA early and monitor the prognosis. Investigating the genetic diversity of *E. vermicularis*, the causative agent of PAA, is important to gain new insights into the disease's etiology. This study aims to investigate the genotypes and intraspecific variation of *E. vermicularis* parasites detected in appendectomy specimens taken from patients diagnosed with PAA based on clinical, radiological, and histopathological examinations over the past decade.

Material and Methods

Patients and Ethical Approval

All procedures in the study were carried out in accordance with the ethical standards of the Aksaray University Faculty of Medicine Clinical Research Ethics Committee (Decision No. 2023/09.02, Date: 11/05/2023), the Declaration of Helsinki and subsequent amendments, and comparable ethical standards.

This retrospective study examined 1.662 patients with suspected acute appendicitis who attended the Pediatric Surgery Polyclinic at Aksaray Training and Research Hospital with suspected acute appendicitis between March 2015 and January 2025. The clinical examinations, radiological findings and histopathological results of these patients were also examined retrospectively. Thirty patients diagnosed with acute appendicitis according to the Alvarado scoring system and found to have *E. vermicularis* based on radiological and histopathological findings were included in the study. Patients without *E. vermicularis* according to their clinical examinations, radiological findings, or histopathological results were excluded from the study. Figure 1 shows the flowchart of the procedures performed to obtain the study results.

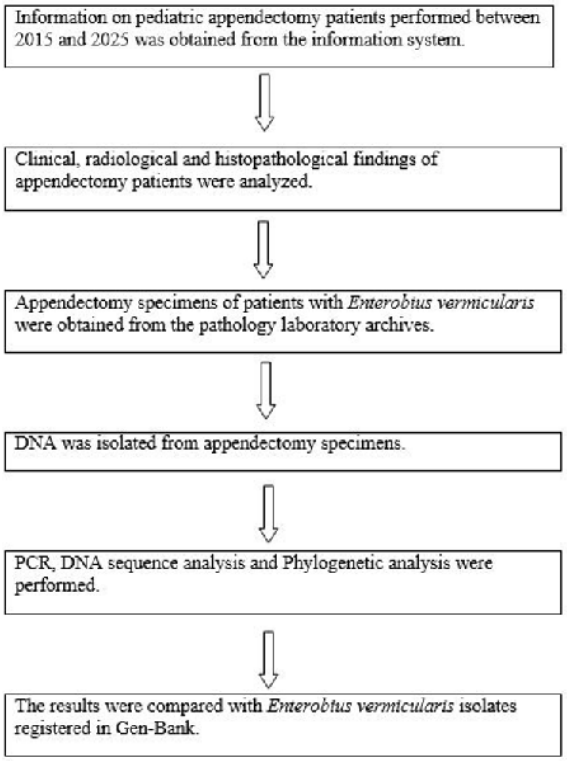

Figure 1. Flow chart showing the procedures in this study

Clinical Examination

The present study comprised a cohort of patients who had applied to the outpatient clinic in question, citing symptoms of nausea and abdominal pain. Over a 10-year period, 1.662 patients underwent surgery following a pre-diagnosis of appendicitis. Histopathological examination results revealed that 70.4% (n=1.170) of cases were acute appendicitis, 20.3% (n=338) were phlegmonous appendicitis and 9.3% (n=154) were perforated appendicitis. The Alvarado score provides a comprehensive evaluation of symptoms, clinical observations and laboratory test results. Symptoms and clinical findings that contribute to the total Alvarado score include the propagation of pre-existing pain, anorexia, and vomiting. Clinical findings include pain in the right lower quadrant, rebound tenderness, and elevated fever. Laboratory findings include an increase in total leukocyte count (WBC) and neutrophil dominance. The evaluation process involves assessing eight parameters, with a maximum total of 10 points allocated for this purpose. A score of 2 or below indicates a low probability of appendicitis (2%). Scores ranging from 3 to 6 indicate an intermediate risk of appendicitis (8–48%), while scores of 7 or above signify a high risk (78–96%). In cases where physical examination findings in pediatric patients were obscured due to non-compliance, imaging modalities were crucial for guidance. An Alvarado score analysis was conducted, incorporating all relevant data. The mean Alvarado score was found to be 8 for 1.219 out of 1.662 patients (66.3%).

Localised peritoneal irritation (McBurney's sign) in the right lower quadrant was observed in all cases. Clinical examination

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revealed that six patients had previously received anti-helminthic drug treatment for *E. vermicularis* infection, which had subsequently recurred.

Radiological Examination

Ultrasound examinations were conducted in all cases using a Toshiba Aplio 500® machine fitted with a 17 MHz high-resolution superficial probe. The inflamed appendix was observed to be located in the anterior region in all cases, either anterior to the psoas muscle or in the retrocecal region.

Histopathological Examination

A series of specimens obtained from appendectomies, accompanied by preliminary diagnoses of primary adrenoleukodystrophy, were examined thoroughly in the hospital's pathology laboratory. The study involved collecting at least three samples from each case. After fixation in 10% formaldehyde and routine tissue processing, 0.4 µm-thick sections were stained with haematoxylin and eosin and examined by a pathologist.

Parasitological Examination

A total of thirty formalin-fixed, paraffin-embedded tissue samples were subjected to a rigorous DNA extraction process. The tissue sections were carefully placed on microscope slides and then incubated at 56°C for 30 minutes. Next, deparaffinisation was initiated using xylene, a solvent employed in this process. The tissue sections were immersed in the solvent for 10 minutes. Each sample was then meticulously removed using a sterile scalpel and transferred to a 1.5 ml microcentrifuge tube. The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used in accordance with the manufacturer's instructions.The partial mitochondrial cytochrome c oxidase 1 (cox1) gene of *E. vermicularis* was amplified by a PCR method using a pair of forward primers Evm1: 5'-TTTTTGGTCATCCTGAGGTTTATATTC-3' and reverse primers Evm2: 5'-CACATTATCCAAAATAGGATTAGCC-3' with an expected product of 390 bp [11]. The total reaction volume was 25 µL containing 2XPCR buffer (Promega), 1.5 mM MgCl2, dNTPs 0.2 mM each, 1 U Taq DNA polymerase, 0.5 pmol of each primer, 5 µL DNA samples and 5 µL sterile water.

Thermal cycling was performed using a Rotor Gene apparatus (Hilden, Germany) according to the following protocol: an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 72°C for 1 minute. This was followed by a final extension step at 72°C for 10 minutes. DNA fragment analysis was conducted using 1.5% agarose gel electrophoresis.

DNA Sequencing and Phylogenetic Analysis

The PCR products were purified for DNA sequencing using a QIAgen Gel Extraction Purification Kit (Qiagen, Hilden, Germany). The purified PCR products were sequenced using the same primers and a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). Analysis of the reaction was conducted in accordance with the instructions provided for the 3730 DNA Analyzer (Applied Biosystems). The obtained sequences were processed using GenBank software and subsequently checked using BLAST analysis software (www.ncbi.nlm.nih.gov/BLAST).

The NJ phylogram was constructed using Kimura's two-parameter model in MEGA 6.06. The tree was scored with 1.000 bootstrap replicates and compared with the nucleotide sequence in the GenBank database using BLAST (blast.ncbi.nlm.nih.gov). The NJ phylogram was constructed using Kimura's two-parameter model implemented in MEGA 6.06. The sequences obtained in the present study have been deposited in GenBank.

Statistical Analysis

In our descriptive, retrospective study, we used frequency distributions and percentages (%) for the variables. No hypothesis testing was employed. IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.) used to summarize the data.

Results

Hallmark characteristics of adult *E. vermicularis* worms were identified in the appendix lumen of all 30 subjects under investigation. The worms were found in the appendix lumen of the appendix, with no correlation to the epithelium, and were accompanied by overt signs of inflammation in the PAA. The patients were aged between three and seventeen years old. The mean age was 10.3 years. Of the 30 patients included in the study, 46.6% were female and 53.4% were male. The study also revealed that six out of 30 cases (20%) of PAA were associated with a history of enterobiasis, which recurred despite the administration of antihelminthics. It was determined that the other patients had never had enterobiasis before.

The presence of parasites was observed in patients with recurrent enterobiasis. Six of the patients included in the study had previously received treatment for enterobiasis and exhibited a history of right lower quadrant pain in conjunction with undifferentiated abdominal pain. The demographic characteristics of the patients included in the study, alongside the characteristics of the *E. vermicularis* parasites isolated from them, are presented in Table 1.

In radiological examination, PAA was diagnosed by ultrasound if the anteroposterior diameter of the appendix was greater than 6 mm from the outside to the inside, if it was not compressed and if there was anechoic fluid or hypoechoic inflammation around the appendix, or if there was wall thickening in the cecum or ileum terminale (Figure 2). A tubular structure measuring less than 6 mm in diameter that was compressed, had a blunt end, and was devoid of peristalsis was interpreted as a normal cecum.

In the histopathological examination revealed a variety of findings, ranging from focal neutrophilic infiltration of the crypt base and intermucosal area of the cecum, to full-thickness neutrophilic infiltration extending to the serosa. Based on these observations, a diagnosis of PAA was made (Figure 3). However, the presence of neutrophil clusters and superficial inflammation in the lumen was not considered to meet the diagnostic criteria for PAA.

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The presence of neutrophilic infiltration outside the serosa was indicative of perianal appendicitis. However, this finding alone was insufficient to establish a diagnosis of PAA. As indicated by the results of the histopathological examination, perforation was observed in the appendectomy specimens of patients with recurrent enterobiasis. Figure 4 shows the identification of *E. vermicularis* eggs in appendectomy specimens.

The sequence variations observed in this study were the result of either the insertion or deletion of nucleotides, primarily within the cox1 nucleotide sequence. Each clinical specimen was identical to the *E. vermicularis* sequence reported in GenBank, and *E. vermicularis* also has a heterotypic structure. The *E. vermicularis* nucleotide sequence showed 100% similarity to the reference strain (GenBank ID: AB221473.1). Figure 5 shows that *E. vermicularis* was classified as *E. vermicularis* and *E. vermicularis* variant 1 based on different nucleotide sequences. The genetic distance value was found to be 0.068 (24/353) among *E. vermicularis* isolates.

The nucleotide changes detected according to the DNA sequencing results are shown in Figure 5. *E. vermicularis* variant 1 was isolated from patients with recurrent enterobiasis, with perforation observed in appendectomy specimens.

Table 1. Demographic characteristics of the patients included in the study and the genetic characteristics of the *E. vermicularis* parasite isolated from the patients

	(n=30) %
Gender	
Female	46.6
Male	53.4
Clinic of enterobiasis	
Recurent enterobiasis	20
No recurrent enterobiasis	80
Genetic variant of *E. vermicularis*	
Variant 1	20
No variant	80
Genetic distance value	0.068

Figure 2. Radiological images of the patients included in the study

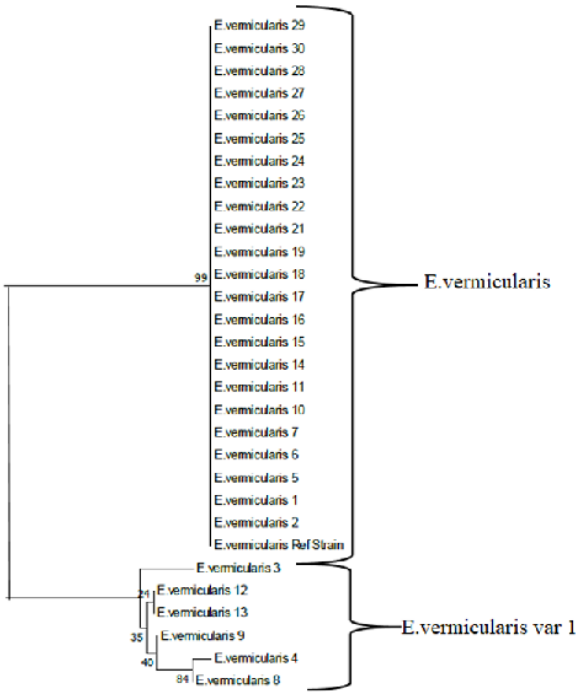

Figure 3. Phylogenic tree of the cox1 region nucleotide sequences of *E. vermicularis* isolates PAA; The tree was constructed using Tamura 3-parameter model in MEGA software version 6.05; Boostrap values shown are based on 100 replicas; Bar represents genetic distance as reflected nucleotide divergence; The genetic diversity was determined to be 0.068

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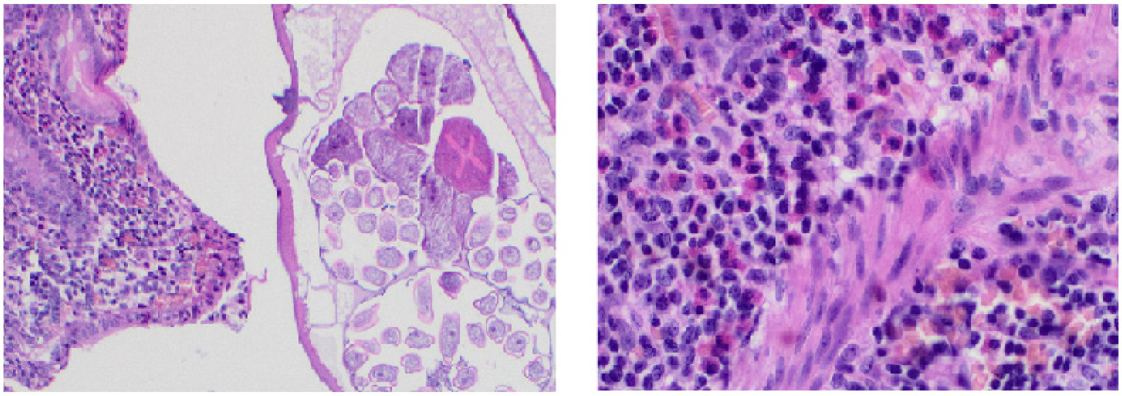

Figure 4. Histopathological images of the patients included in the study

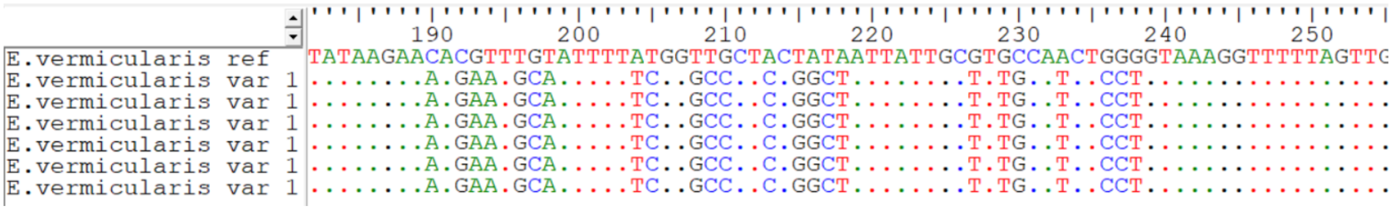

Figure 5. The result of DNA sequencing of *E. vermicularis* isolates and alignment of them *E. vermicularis* ref (AB221473.1); *E. vermicularis* variant 1 was isolated from recurrent enterobiasis

Discussion

It is widely accepted that PAA is the most common cause of peritonitis in children and is therefore regarded as one of the most prevalent causes of abdominal pain in pediatric clinics [10]. In the treatment of PAA, appendectomy is considered the gold standard and is a frequently performed procedure in pediatric surgery [11].

Within relevant scholarly circles, it is widely accepted that careful consideration of the clinical findings is the primary means by which a physician may arrive at a diagnosis of PAA. These findings include periumbilical pain radiating to the right iliac fossa, anorexia, fever, and tenderness in the right iliac fossa. However, the diagnosis of PAA remains challenging, and research into its management has been conducted in different areas and practice models worldwide [12].

Cayrol et al. reported that the clinical findings in children aged 1–16 years old who were diagnosed with acute appendicitis included vomiting, a high fever (temperature≥37.5°C), pain in the right lower quadrant (RLQ), a high white blood cell count (WBC≥10,000/µL) and diffuse abdominal tenderness [13]. Doniger et al. reported the presence of clinical symptoms, including nausea, vomiting, fever, rebound lumbosacral pain (RLQ) and tenderness, in patients diagnosed with PAA [14]. Benabbas et al. conducted a meta-analysis to examine the clinical symptoms exhibited by patients with suspected PAA at a pediatric clinic. The studies were divided into two groups based on their inclusion criteria. The terms "undifferentiated abdominal pain" and "abdominal pain with suspected PAA" denote a range of symptoms. It was reported that, in patients experiencing undifferentiated abdominal pain, a history of pain shifting to the RLQ and the presence of cough/billing pain during physical examination were most strongly associated with PAA [15].

Patients diagnosed with acute appendicitis in the present study exhibited comparable clinical symptoms to those reported in previous research. However, six of the patients had previously been treated for enterobiasis and reported pain shifting to the right lower quadrant alongside undifferentiated abdominal pain.

While the clinical diagnosis of PAA is generally straightforward, there are also pediatric patient groups, especially those exhibiting atypical symptoms, which can complicate the process [16,17]. The existing literature on PAA diagnostic studies has indicated an increase in mortality consequent to perforation. Nevertheless, an increase has been observed in cases of appendectomy performed without adequate pathological or ultrasound confirmation of PAA. It is crucial to recognise this condition in children at an early stage, as it may present as initial PAA symptoms, which can be complicated by appendix perforation. In patients exhibiting PAA, it is crucial to analyse and interpret radiological assessments meticulously, with the aim of minimising any delay in diagnosis that could be caused by erroneous positive or negative diagnoses.

Das et al. have argued that ultrasound examination should be the primary imaging method for patients with suspected PAA, in order to avoid perforation of the appendix or unnecessary appendectomy due to delayed diagnosis [18]. Bekiaridou et al. recommended USG as the primary imaging method for diagnosing acute appendicitis in pediatric patients in their study. This method is characterised by its radiation-free nature, cost-effectiveness, non-invasiveness, high sensitivity and ease of use [19]. In the present study, the performance of PAA was evaluated using USG in pediatric patients with suspected appendicitis.

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Ultrasound was used for this purpose and a preliminary diagnosis was made using this method in all patients. The findings of this study demonstrate the importance of ultrasound in the preliminary diagnosis of PAA.

Appendectomy is widely regarded as the gold standard in the management of PAA. Histopathological examination of specimens from surgical cases is important for detecting cases of unpredictable pathology. Yavuz et al. conducted a study on PAA in pregnant women and reported that histopathological examination confirmed appendicitis in 86.7% of patients who underwent surgery [20]. Gupta et al. investigated the efficacy of the Appendicitis Inflammatory Response score in diagnosing PAA and its correlation with histopathological findings. Appendicitis was diagnosed based on histopathological analysis of tissue samples obtained from 54 patients. A positive correlation was identified between the AIR score and the histopathological findings [21]. The postoperative management of patients who have undergone an appendectomy is generally determined by the surgeon's assessment during the operation.

Şahin et al. examined appendectomy specimens from patients diagnosed with acute appendicitis, using histopathological methods in their study. They found that *E. vermicularis* was present in 2.8% of the specimens included in the study [22]. Agholi et al. conducted a retrospective study of PAA cases and reported that *E. vermicularis* was the most common helminthic agent causing PAA [23]. Mohy et al. demonstrated that the histological spectrum of appendicitis is associated with a multitude of *E. vermicularis* larvae, a lymphoid follicular reactive germinal centre and marked eosinophilic infiltration. They also observed a somatic worm segment in the appendiceal lumen [24]. In the present study, histopathological analysis of appendectomy specimens revealed a significant number of *E. vermicularis* eggs within the lumen. Furthermore, perforation was observed in the specimens of six patients who had previously been treated for enterobiasis.

E. vermicularis can cause diseases, such as PAA, chronic appendicitis, ruptured appendicitis, gangrenous appendicitis, and perforation resulting in peritonitis [25]. There are some case reports in our country and around the world that *E. vermicularis* can cause PAA in humans due to its migratory nature. Sousa et al. investigated the relationship between *E. vermicularis* and PAA between 1997-2019. They reproted taht *E. vermicularis* caused 22 cases of PAA [26].

A plethora of studies on the genetic diversity of *E. vermicularis* have yielded novel information in many areas, including the parasite's geographical distribution, host range, specificity, evolution and drug applications. However, the relationship between the parasite and PAA has not been fully explained in these studies. Kang et al. performed nex-generation sequencing analysis targeting the 18S rRNA gene regions of *E. vermicularis* and detected 0.9% genetic diversity in the parasite [27]. Haghshenas et al. investigated the morphological characteristics and pathological changes of pinworms in old, archived appendectomy specimens. Researchers reported that they found genetic diversity differences of 0–1.9% in *E. vermicularis* isolates from different geographical regions [28].

Piperaki et al. conducted a study in which they examined the genetic characteristics of *E. vermicularis* isolated from humans using a total of seventy seven appendectomy specimens. The researchers performed a PCR analysis targeting the cox 1 gene region of *E. vermicularis*. Thirty-six amplicons were sequenced, resulting in the identification of eleven distinct haplotypes. It was emphasised that these haplotypes had not previously been reported in the region in question. The genetic diversity of *E. vermicularis* was reported to be 7.2% [29]. Shafiei et al. collected 70 appendectomy samples from patients with appendicitis in the North Khorasan region of Iran and Azerbaijan, analysing these samples by targeting the *E. vermicularis* cox1 gene region. They reported that *E. vermicularis* haplotypes detected in appendectomies were divided into three distinct subtypes [30]. In our study, an allelic diversity of 0.068% was detected and it was determined that patients with genetic diversity had previously been treated for enterobiasis and had perforation in their histopathological findings.

E. vermicularis is a parasite found worldwide that infects humans, often causing gastrointestinal symptoms. Although there is a hypothesis that *E. vermicularis* is one of the causative agents of PAA, there is a lack of studies providing robust evidence to support this. The parasite has been reported in the literature as an incidental finding in resected appendix specimens. The results of the clinical, radiological, histopathological and molecular analyses of the PAA patients included in this study provide unequivocal evidence that *E. vermicularis* is one of the causative agents of this disease. The results of the present study demonstrate that *E. vermicularis* is one of the most prevalent infectious agents encountered in the appendix and has the potential to elevate the risk of appendicitis.

Conclusion

Several studies have examined the genetic diversity of *E. vermicularis*, the parasite that causes PAA. However, most existing research in this field focuses on diversity in different geographical areas. This study investigated the relationship between the genetic diversity of *E. vermicularis* and clinical symptoms, as well as radiological and histopathological findings. Genetic diversity was identified in *E. vermicularis* isolates from patients exhibiting diverse clinical symptoms and histopathological findings. These findings have the potential to inform the clinical diagnosis of PAA and contribute to aetiopathological research. However, our study data need to be supported by further studies with larger samples.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

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Ethical Approval

All procedures in the study were carried out in accordance with the ethical standards of the Aksaray University Faculty of Medicine Clinical Research Ethics Committee (Decision No. 2023/09.02, Date: 11/05/2023), the Declaration of Helsinki and subsequent amendments, and comparable ethical standards.

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