

Original Article

Revisiting the viral etiology of aseptic meningitis: A comparative evaluation of PCR and cell culture methods in cerebrospinal fluid specimensEsma Saatci¹, Selma Gokahmetoglu²¹Kayseri City Education and Research Hospital, Department of Microbiology and Clinical Microbiology, Kayseri, Türkiye²Erciyes University, Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Kayseri, Türkiye

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**Abstract**

Aim: Aseptic meningitis is caused by a wide range of infectious agents, and viruses are the leading etiology. Although molecular diagnostic tools have improved considerably, the causative pathogen cannot be identified in a large proportion of cases. In this study, we investigated the presence of Enterovirus, Herpes Simplex Virus (HSV), Varicella-zoster Virus (VZV), and Cytomegalovirus (CMV) in cerebrospinal fluid (CSF) specimens from patients with suspected aseptic meningitis, using both polymerase chain reaction (PCR) and conventional cell culture.

Materials and Methods: We analyzed CSF specimens from 30 patients (15 pediatric, 15 adult; 20 female, 10 male) hospitalized with a prediagnosis of aseptic meningitis at Erciyes University Hospitals between March 2004 and June 2005. HSV-DNA, CMV-DNA, VZV-DNA, and Enterovirus-RNA were tested by real-time PCR. Twenty-three specimens with sufficient volume were also inoculated into Hep-2 and MRC-5 cell cultures for viral isolation. We calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cell culture using PCR as the reference method.

Results: HSV-DNA was positive in 15 of 30 specimens (50%). None of the specimens yielded CMV-DNA, VZV-DNA, or Enterovirus-RNA. Cytopathic effect (CPE) consistent with HSV appeared in 7 of 23 (30.4%) Hep-2 cultures and in only 1 of 23 (4.3%) MRC-5 cultures. Compared with PCR, Hep-2 culture had a sensitivity of 87.5% (95% CI: 52.9 to 97.8%), specificity of 100%, PPV of 100%, and NPV of 93.8%. Five patients (16.7%) died during hospitalization.

Conclusions: HSV was the only viral agent detected in this hospitalized cohort. PCR was faster and more sensitive than cell culture for identifying viral pathogens in CSF. We recommend using both methods together to maximize diagnostic accuracy. The persistent difficulty in identifying the etiology of viral meningitis, even with newer multiplex panels, confirms that comparative diagnostic studies on this topic are still needed.

Keywords: Aseptic meningitis; Herpes simplex virus; Enterovirus; polymerase chain reaction; cell culture; central nervous system infection

INTRODUCTION

Infections of the central nervous system (CNS) continue to cause considerable morbidity and mortality across all age groups, and rapid identification of the causative agent is critical for guiding treatment [1,2]. Aseptic meningitis, defined by lymphocytic pleocytosis in the cerebrospinal fluid (CSF) together with negative routine bacterial cultures, is one of the most common clinical presentations encountered in emergency departments and infectious disease practice [3]. In recent decades, the widespread

implementation of conjugate vaccines against bacterial pathogens has led to a 57.5% decline in bacterial meningitis incidence since 1990 [4]. As a consequence, viral etiologies now account for a growing share of meningitis cases, yet the responsible pathogen remains unidentified in 30 to 50% of suspected viral meningitis episodes, even when modern diagnostic tools are available [3,4].

Enteroviruses are the most frequent agents of viral meningitis, responsible for an estimated 80 to 95% of cases, and they usually cause a mild, self-limited illness [1,5]. In contrast,

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Corresponding Author: Esma Saatci, Kayseri City Education and Research Hospital, Department of Microbiology and Clinical Microbiology, Kayseri, Türkiye
Email: esmasaatci6@gmail.com

Herpes Simplex Virus (HSV) encephalitis can be fatal in 50 to 75% of untreated patients; timely administration of acyclovir reduces this rate to 19 to 38% [6,7]. This difference makes early and accurate detection of HSV in CSF a clinical priority. Varicella-zoster Virus (VZV) and Cytomegalovirus (CMV) cause meningitis less frequently and are seen mainly in immunocompromised individuals [8,9].

The laboratory approach to viral CNS infections has changed considerably over the past two decades. Cell culture, long regarded as the reference standard, has largely been replaced by polymerase chain reaction (PCR)-based methods that offer higher sensitivity and much shorter turnaround times [10-12]. More recently, multiplex syndromic panels such as the BioFire FilmArray Meningitis/Encephalitis panel have made it possible to screen for 14 pathogens in about one hour [13,14]. Nevertheless, false-positive and false-negative results remain a concern with any single method, and direct comparisons between diagnostic approaches continue to provide useful data for clinical decision-making [15].

The present study was originally conducted as the first author's medical specialty thesis at Erciyes University in 2006. CSF

specimens were collected between March 2004 and June 2005 from patients hospitalized with suspected aseptic meningitis. We now present these data with updated statistical analysis and interpretation in the context of current literature. Our aim was to compare PCR and conventional cell culture for the detection of HSV, CMV, VZV, and Enteroviruses in CSF, and to assess the diagnostic agreement between the two methods.

MATERIALS AND METHODS

Study Design, Setting, and Ethics

This retrospective, cross-sectional, single-center study was conducted at Erciyes University Faculty of Medicine, Department of Microbiology Virology Laboratory and Hakan Cetinsaya Experimental and Clinical Research Center (DEKAM), Kayseri, Türkiye. Ethical approval was granted by Erciyes University Faculty Administrative Board (T 04-21 /96681246-051-99 Date: 08.05.2026). As the study utilized archived, de-identified clinical specimens collected during routine diagnostic workup, informed consent was waived. The study was designed and reported in accordance with STROBE guidelines for observational studies (Figure 1).

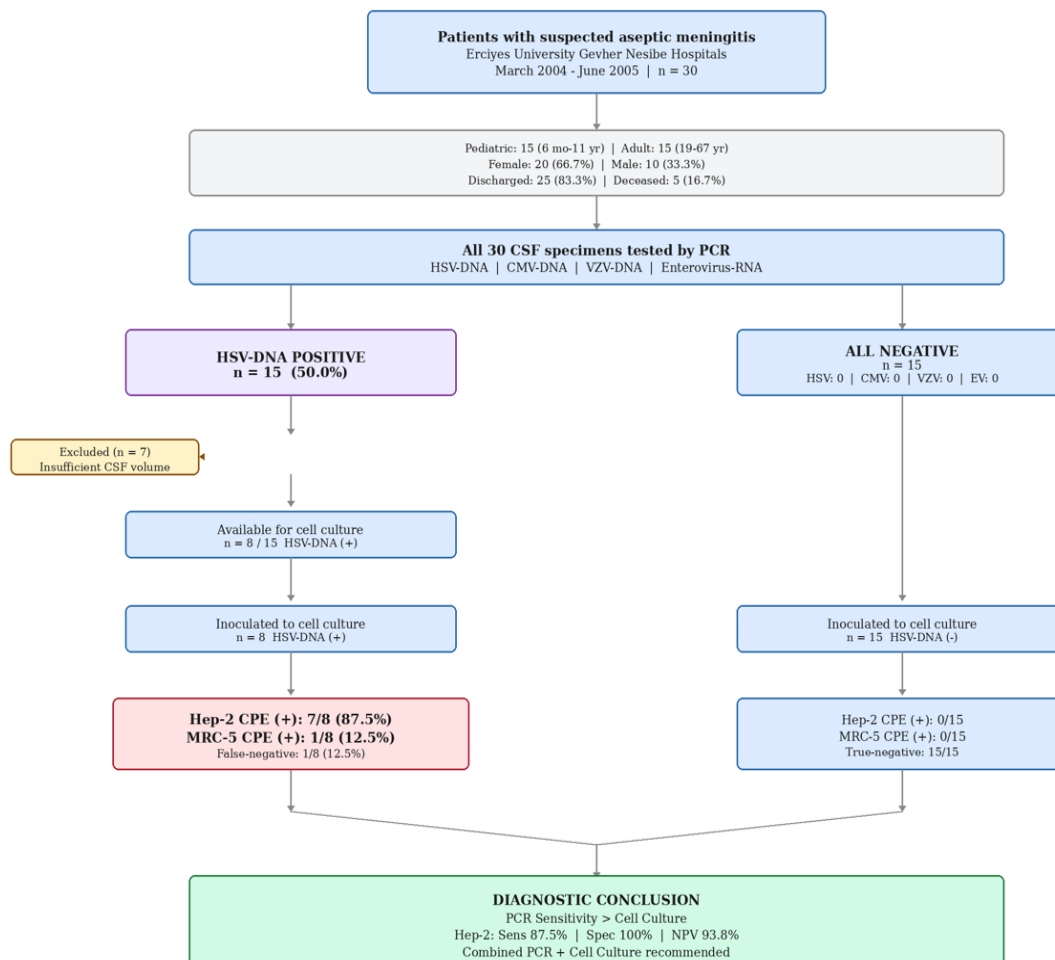


Figure 1. STROBE flow diagram illustrating patient selection, diagnostic methods, and results.

Study Population

Thirty consecutive patients with a clinical prediagnosis of aseptic meningitis, hospitalized at Erciyes University Gevher Nesibe Hospitals between March 2004 and June 2005, were included. CSF specimens were negative for bacteria on Gram staining and culture and CSF with bacterial growth on their cultures were excluded from the study. CSF samples were obtained by lumbar puncture under sterile conditions and stored in duplicate at -70 degrees Celsius for six mounths until the analyses performed. The CSF samples collected over the course of 6 months were analyzed collectively.

Molecular Detection (PCR)

Nucleic acid extraction was performed using PureArt DNA Mini Kit (QIAGEN, Germany). HSV-DNA was detected with HSV QL 1.0 (Fluorion, Iontek, Turkiye); CMV-DNA with CMV QL 1.0 (Fluorion, Iontek, Turkiye); VZV-DNA with VZV DNA RealArt (Artus, Germany); and Enterovirus-RNA with RealArt Enterovirus LC kits (Roche Diagnostics, USA) on a LightCycler platform. Conventional PCR products (HSV, CMV, VZV) were analyzed on 1-2% agarose gels with ethidium bromide staining. Enterovirus real-time RT-PCR utilized fluorimetric detection on the LightCycler instrument.

All tests performed were validated using the positive and negative controls included in the test kit.

Viral Isolation (Cell Culture)

Hep-2 (laryngeal epithelial carcinoma) and MRC-5 (human embryonic lung fibroblast) cell lines were used. Cells were maintained in RPMI 1640 with 10% (growth) or 2% (maintenance) fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 degrees Celsius in 5% CO2. Cultures were examined every other day for CPE: Hep-2 for 14 days and MRC-5 for up to 28 days.

Sterile saline solution was applied to the cell as a negative control and a known HSV-positive patient was also used as a positive control.

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Hep-2 cell culture were calculated using PCR as the reference standard. Exact 95% confidence intervals were computed using the Wilson score method. Descriptive statistics were used for demographic and laboratory data. All analyses were performed using Python (SciPy v1.11, NumPy v1.25).

RESULTS

Patient demographics and clinical departments are presented in Figure 2. Briefly, 15 patients were pediatric (6 months-11 years) and 15 were adults (19-67 years); 20 were female and 10 male. Five patients (16.7%) died during clinical follow-up; 25 were discharged.

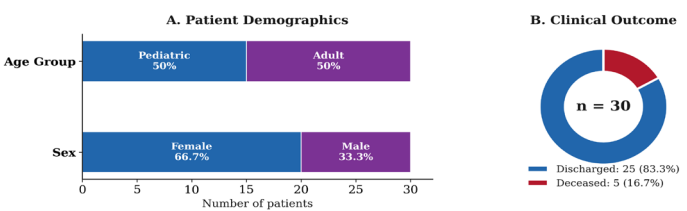


Figure 2. Demographic and clinical characteristics of the study population (n = 30). (A) Patient demographics by age group and sex. (B) Clinical outcome showing discharged vs. deceased patients.

Routine CSF Analysis

CSF samples were inoculate on blood agar, EMB agar, and chocolate agar for bacteriological examination; after incubation for 16 hours, samples showing no bacterial growth were included in the study. All 30 CSF specimens were negative for bacteria on Gram staining and culture. Biochemical parameters were consistent with aseptic meningitis: mononuclear cell predominance, mildly elevated protein, and normal glucose concentrations. Non of the patients had any other underlying medical conditions.

PCR Results

HSV-DNA was detected in 15/30 (50.0%) CSF specimens. CMV-DNA, VZV-DNA, and Enterovirus-RNA were not detected in any sample (Table 1, Figure 3, Figure 4). The distribution was uniform across age groups: 8/15 (53.3%) pediatric and 7/15 (46.7%) adult patients were HSV-DNA positive.

Table 1. PCR detection of viral nucleic acids in CSF specimens (n = 30)

PCR Target	Positive, n (%)	Negative, n (%)
HSV-DNA	15 (50.0)	15 (50.0)
CMV-DNA	0 (0)	30 (100)
VZV-DNA	0 (0)	30 (100)
Enterovirus-RNA	0 (0)	30 (100)

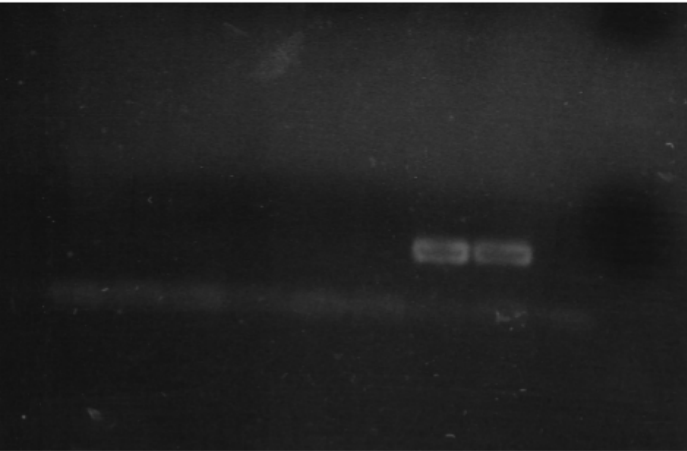


Figure 3. HSV-DNA positivity in a gel electrophoresis system (with positive control).

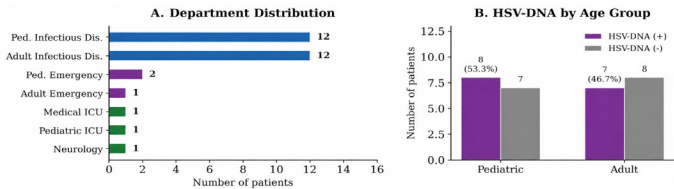


Figure 4. Distribution of patients across hospital departments and HSV-DNA positivity by age group. (A) Department distribution. (B) HSV-DNA detection by pediatric and adult age groups.

Cell Culture Results

Among 23 specimens inoculated into cell culture, In seven patients, CSF volume was insufficient for cell culture inoculation.. CPE (diffuse rounding and clustering of the cells) consistent with HSV was observed in 7/23 (30.4%) Hep-2 cultures beginning on day 2, characterized by cell rounding and progressive intercellular spacing (Figure 5). Only 1/23 (4.3%) MRC-5 cultures showed HSV-compatible CPE, attributable to delayed inoculation from cell supply difficulties (Figure 6). No CPE consistent with CMV, VZV, or Enterovirus was observed in any culture.

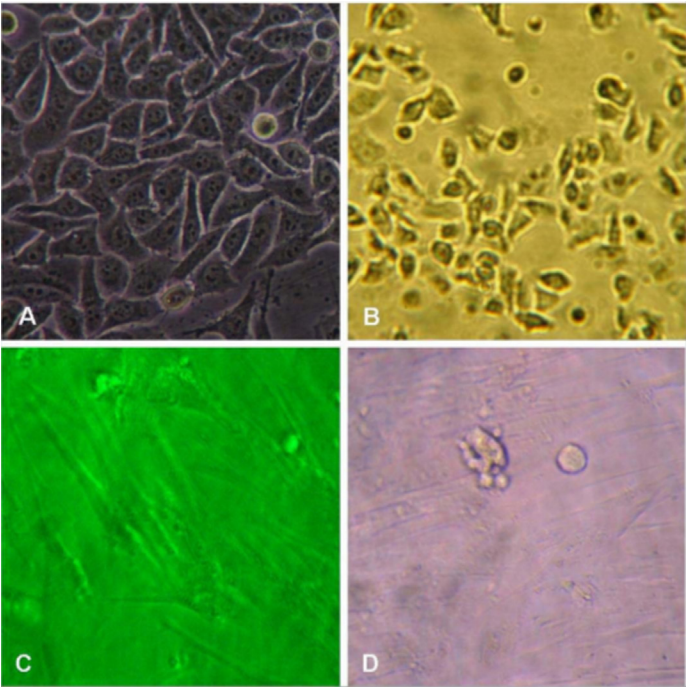


Figure 5. Microscopic images of cell cultures. (A) Normal Hep-2 cell monolayer. (B) Cytopathic effect (CPE) consistent with HSV in Hep-2 cells, showing cell rounding and increased intercellular spacing (day 2 post-inoculation). (C) Normal MRC-5 cell monolayer. (D) CPE consistent with HSV in MRC-5 cells.

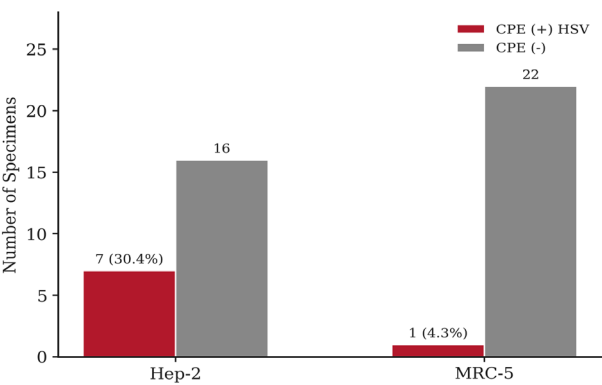


Figure 6. Cytopathic effect (CPE) results by cell line (n = 23 inoculated specimens). Hep-2 demonstrated markedly higher sensitivity than MRC-5 for HSV detection.

Diagnostic Performance: PCR versus Cell Culture

Among 23 specimens tested by both methods, the 2x2 contingency analysis (Figure 7) yielded the following performance metrics for Hep-2 cell culture using PCR as the reference: sensitivity 87.5% (7/8; 95% CI: 52.9-97.8%), specificity 100% (15/15; 95% CI: 79.6-100%), PPV 100% (7/7), and NPV 93.8% (15/16; 95% CI: 71.7-98.9%).

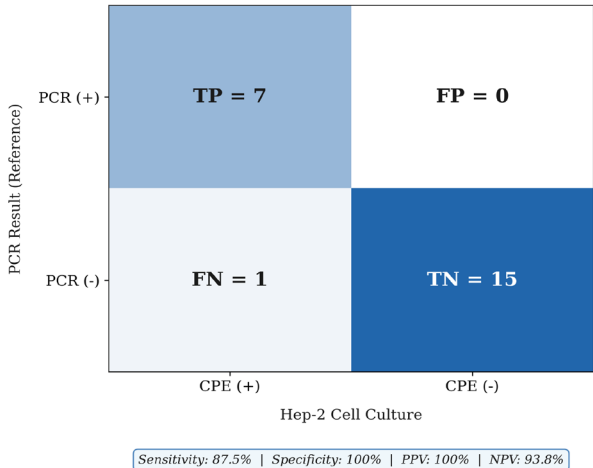


Figure 7. Diagnostic concordance between PCR and Hep-2 cell culture for HSV detection (n = 23). Heatmap showing 2x2 contingency matrix with calculated performance metrics.

One PCR-positive specimen was culture-negative (false-negative rate: 12.5%), possibly reflecting low viral titer or antibody neutralization in CSF. MRC-5 sensitivity was only 12.5% (1/8) (Table 2).

Table 2. Diagnostic performance of cell culture for HSV detection using PCR as the reference standard (n = 23)

Metric	Sensitivity	Specificity	PPV	NPV	Accuracy
Hep-2	87.5%	100%	100%	93.8%	95.7%
MRC-5	12.5%	100%	100%	68.2%	69.6%
95% CI (Hep-2)	52.9-97.8	79.6-100	-	71.7-98.9	-

Abbreviations: PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval (Wilson method)

Clinical Outcomes

Five of 30 patients (16.7%) died during clinical follow-up. All five deceased patients had been hospitalized with severe CNS infection presentations and had tested positive for HSV-DNA. The mortality rate underscores the potential lethality of aseptic meningitis, particularly when caused by HSV.

DISCUSSION

In this study(In a study conducted between March 2004 and June 2005), we compared PCR and cell culture for the detection of four viral agents in the CSF of patients hospitalized with suspected aseptic meningitis. HSV was the only pathogen identified, with a positivity rate of 50%, and PCR proved more sensitive than cell culture for its detection. Although the specimens were collected approximately two decades ago, the central question addressed here, whether combining molecular and culture-based methods adds diagnostic value, has not lost its practical relevance.

The HSV-DNA positivity rate of 50% in our series is considerably higher than what has been reported elsewhere. Read and Kurtz [10] found HSV in 3.4% of 1,683 CSF samples in the United Kingdom, while Hukkanen and Vuorinen [8] reported 4.6% among 922 specimens in Finland. Markoulatos et al. [11] detected HSV in 4.7% of 86 patients in Greece, and Casas et al. [16] in 1.9% of 156 patients in Spain (Figure 8). A more recent study from Saudi Arabia using the FilmArray panel identified HSV in 4.2% of 240 cases [17]. The high rate we observed most likely reflects the composition of our study group: all patients were hospitalized with clinical presentations severe enough to require inpatient care and lumbar puncture. Patients with milder symptoms, who are more likely to have enteroviral meningitis, were often managed in the emergency department and discharged without CSF sampling. This selection toward sicker patients naturally increased the proportion of HSV-positive cases.

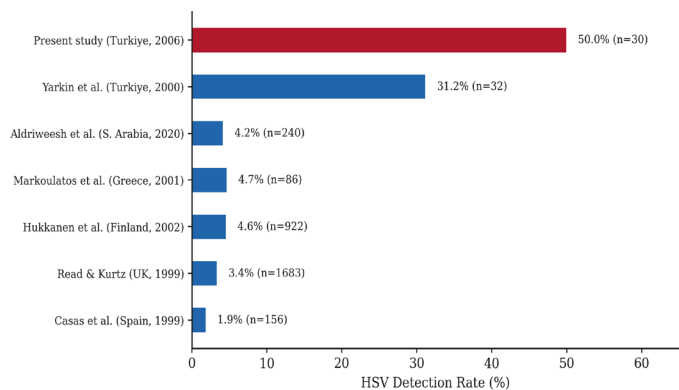


Figure 8. Comparison of HSV detection rates in CSF across published studies. The elevated rate in the present study reflects the selection of hospitalized patients with severe presentations.

We did not detect Enterovirus in any of the 30 specimens, a finding that clearly differs from the well-established pattern in

which Enteroviruses account for the majority of viral meningitis cases [1,5]. The most plausible explanation is the selection process described above. Enteroviral meningitis typically runs a benign course, and affected patients were rarely hospitalized during the study period. Our observation is consistent with the data of Rai et al. [18], who detected Enterovirus in 68% of CSF specimens when outpatient populations were included. This discrepancy carries a practical message: the spectrum of viral etiologies observed in any study is strongly influenced by the clinical setting in which patients are recruited. A considerable number of enteroviral meningitis cases likely go undiagnosed in daily practice, sometimes resulting in unnecessary antibiotic courses [19].

When we compared the two diagnostic methods directly, Hep-2 cell culture showed a sensitivity of 87.5% and specificity of 100% against PCR, with an overall accuracy of 95.7%. These figures are in line with previous reports. Madhavan et al. [20] found HSV by PCR in 35% of CSF specimens but could not isolate the virus in any culture, and Lakeman and Whitley [21] demonstrated 98% agreement between CSF PCR results and brain biopsy cultures in confirmed HSV encephalitis. Hukkanen and Vuorinen [8] similarly reported that culture isolated only Enteroviruses but missed HSV, VZV, and CMV entirely. In our series, one PCR-positive specimen failed to produce CPE in Hep-2 culture, possibly because intrathecal antibodies neutralized the virus or because the viral titer was too low to initiate productive infection after prolonged storage at minus 70 degrees Celsius [20,21]. The much lower sensitivity of MRC-5 culture (12.5%) was related to a delay in cell inoculation caused by difficulties in procuring the cell line, highlighting a well-known practical limitation of culture-based diagnostics.

Five of our 30 patients (16.7%) died during hospitalization. This mortality rate is much higher than the 0.61% observed in a large Japanese nationwide study of 29,486 viral meningitis hospitalizations [4] and reflects the severity of our patient group. The fact that seemingly benign presentations can progress to life-threatening illness within hours, particularly in HSV encephalitis when treatment is delayed, has implications that extend beyond infectious diseases [6,7]. Viral meningitis patients also pose a transmission risk that should not be overlooked: Enteroviruses can be shed in feces for weeks, and HSV may spread through close contact during asymptomatic periods. This is relevant for healthcare workers, family members, and other patients sharing the hospital environment. In the context of forensic medicine, identifying the causative virus in fatal CNS infections is often necessary to establish the cause and manner of death [22]. Our data suggest that postmortem CSF PCR should be part of the standard workup in suspected viral encephalitis fatalities, because cell culture may miss the pathogen if viral viability has been lost. When diagnostic delay is investigated in medicolegal cases, the superiority of PCR documented here and elsewhere supports the position that molecular testing represents the current standard of care.

These findings have relevance for clinicians across several disciplines. Emergency physicians should be aware that patients discharged without lumbar puncture may have undiagnosed viral CNS infections. Pediatricians should keep in mind that neonatal enteroviral meningoencephalitis, though uncommon, carries significant morbidity and mortality [5]. Neurologists evaluating patients with recurrent aseptic meningitis should consider HSV-2 as a possible cause; recent case reports have shown that Mollaret meningitis can go unrecognized for years until CSF PCR is performed [23,24]. For forensic specialists, the limited sensitivity of cell culture for HSV in CSF further supports the use of molecular methods in postmortem investigations.

Since the time of our study, the introduction of multiplex syndromic panels has changed the diagnostic approach to CNS infections considerably. The BioFire FilmArray Meningitis/Encephalitis panel, cleared by the FDA in 2015, can detect 14 pathogens simultaneously in about one hour [13]. A comprehensive review published in 2026 showed greater than 90% agreement between this panel and conventional reference methods [14], and the QIAstat-Dx panel yielded positive percent agreement between 90.6% and 100% across seven clinical evaluations [15]. A Turkish multicenter study recently confirmed the practical utility of the Bio-Speedy MX-17 panel [19]. These newer platforms, however, are not free of problems: false-positive results for bacterial targets and occasional false-negative results for viruses have been reported, and their cost limits widespread use in resource-constrained settings [13-15]. A systematic review by Farias et al. [25] also showed that viral meningitis epidemiology is not static; Enterovirus circulation was temporarily suppressed during the COVID-19 pandemic and rebounded afterward, illustrating the ongoing relevance of this clinical entity. Even with older, single-target PCR assays, the principle that molecular detection is superior to culture holds true, and the data presented here serve as a useful benchmark for evaluating newer technologies.

Limitations

Our study has a number of limitations. The sample included only 30 patients, which limited statistical power and resulted in wide confidence intervals (for example, sensitivity 95% CI: 52.9 to 97.8%). The single-center design means that results may not be generalizable to other settings. Because all patients were hospitalized, milder cases of viral meningitis, most of which are caused by Enteroviruses, were underrepresented. The diagnostic kits used in 2004 and 2005 are now outdated, and current real-time PCR platforms offer better sensitivity and the ability to test for multiple targets at once. We did not perform HSV typing (HSV-1 vs. HSV-2), which would have added valuable clinical information. The delayed inoculation of MRC-5 cultures due to cell supply problems weakened the comparison between the two cell lines. Finally, quantitative viral load data were not available, so we could not assess whether culture positivity was related to viral burden.

Notwithstanding these limitations, the study contributes original comparative data from a region that is underrepresented in the viral meningitis literature and provides a methodological link between older and newer diagnostic approaches.

CONCLUSION

In this retrospective single-center study from central Türkiye, HSV was the only viral pathogen detected in CSF of patients hospitalized with suspected aseptic meningitis. CMV, VZV, and Enteroviruses were not found. PCR proved faster and more sensitive than conventional cell culture. The mortality of 16.7% in our cohort serves as a reminder that aseptic meningitis is not always a benign condition. Using PCR and cell culture together remains a sound approach for maximizing the diagnostic yield. The fact that a large proportion of viral meningitis cases still cannot be attributed to a specific pathogen, even with contemporary multiplex panels, underlines the continued need for comparative diagnostic research in this field.

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.

Ethical Approval

Erciyes University Faculty Administrative Board (T 04-21 /96681246-051-99, Date: 08.05.2026).

Patient Consent

Informed consent was waived for this retrospective study using archived, de-identified specimens.

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