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ORIGINAL ARTICLE

Before the COVID-19 Era, Were Coronaviruses Involved in Cases of Encephalitis and Meningoencephalitis? A Cohort Study

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ABSTRACT

Over the past three decades, a clear neuroinvasive and neurotropic potential has been demonstrated for human coronaviruses (HCoVs), involving both primary and secondary central nervous system (CNS) involvement. The classical coronaviruses (229E, OC43, and NL63) are primarily responsible for upper and lower respiratory tract infections but have also been implicated in gastrointestinal and neurological diseases. **Objective:** To demonstrate the involvement of coronaviruses in encephalitis and meningoencephalitis prior to the SARS-CoV-2 pandemic, and to compare these findings with the neurological manifestations observed during COVID-19. **Materials and Methods:** To determine the etiological spectrum of encephalitis in Batna, Algeria, we conducted a prospective study using a broad diagnostic approach from April 2012 to August 2015. We adopted a case definition consistent with those used in previous studies, encompassing acute cerebral dysfunction with signs of inflammation, as well as cases presenting with meningeal inflammation and an associated encephalitic component (meningo-encephalitis). Patients were recruited from three departments — intensive care, infectious diseases, and pediatrics — and data were collected using a standardized case report form. The study aimed to identify etiological agents through a standardized diagnostic procedure and to describe the epidemiological, clinical, and biological characteristics, as well as outcomes associated with the different causes. **Results:** Using a comprehensive diagnostic approach, over 30 pathogens were tested in blood and cerebrospinal fluid (CSF). Among the 141 patients with acute infectious encephalitis, a viral etiology was established in 73 (51.8%), including 18 cases involving coronaviruses, of whom 4 had no coinfection. The median age was 20 years (range: 0–45), with immunosuppression present in 16.7% of cases. Sudden onset was observed in 38.9% of patients, neck stiffness in 38.9%, and a Glasgow Coma Scale score below 8 in 22.2%. Respiratory and hepatic involvement were each observed in 27.8% of patients. Pleocytosis was noted in 77.8% of cases, with hyperalbuminorachia in 55.6%. Respiratory viral coinfection was detected in 11.1%. The median duration of hospitalization was 19.5 days (range: 1–874), with a mortality rate of 5.6%. **Conclusion:** The neurological involvement of coronaviruses remains to be fully elucidated. Nonetheless, coronaviriform particles have been observed in brain tissue sections, and coronaviruses have been isolated from patients with multiple sclerosis (MS). Moreover, anti-coronavirus antibodies have frequently been detected at high titers in the CSF of MS patients.

Keywords: Encephalitis, Meningoencephalitis, Respiratory Involvement, Coinfections.

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1. INTRODUCTION

Encephalitis and meningoencephalitis are non-purulent inflammations of the central nervous system (CNS), caused in 80% of cases by viruses, followed by intracellular pathogens. These conditions can be life-threatening [1]. Coronaviruses are ubiquitous pathogens first discovered in the 1960s. Several strains, including HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1, have been identified as causative agents of upper respiratory tract infections in humans [2,3]. Under certain conditions, they may cause severe pneumonia and extend to involve other organs, notably the gastrointestinal tract and the CNS [2–6].

Over the past 20 years, novel strains of coronaviruses have emerged as causes of acute respiratory distress syndrome (ARDS): SARS-CoV-1, responsible for a global outbreak of atypical pneumonia affecting approximately 8,000 people (10% mortality) between November 2002 and July 2003 [2]; and MERS-CoV, responsible for severe pneumonia in 2,500 individuals with a 34% case fatality rate, according to WHO [1,3]. Since 2019, SARS-CoV-2 has emerged as a systemic virus responsible for a major global pandemic of acute respiratory failure, with severe systemic forms observed in vulnerable populations such as the elderly and pregnant women [3], and more than six million deaths worldwide as of November 2022 [7].

For over four decades, a neuroinvasive potential has been identified in strains such as HCoV-OC43, HCoV-229E, SARS-CoV-1, and SARS-CoV-2 (associated with MS and ADEM) [1,2,8]. These viruses have been shown to cause neurological disease via neuronal degeneration in animal models, with over 95% mortality [2,5]. Two main pathways have been suggested: retrograde neuronal dissemination via the olfactory nerve [5,6], and hematogenous spread following viremia [2,9].

To our knowledge, few studies have evaluated the involvement of coronaviruses in encephalitis and meningoencephalitis prior to the COVID-19 era. Besides our study conducted between 2012 and 2015, a study published on May 2, 2018, in Beijing, China, reported that anti-CoV IgM antibodies were detected in 22 out of 183 children (12.02%) hospitalized for acute encephalitis and in 26 out of 236 children (11.02%) hospitalized for acute encephalitis with associated respiratory tract infection between May 2014 and April 2015 [8,10]. Today, encephalitis has emerged as one of the most lethal manifestations of COVID-19 in both pediatric and adult populations [11].

Our objective is to demonstrate the involvement of coronaviruses in encephalitis and meningoencephalitis in Batna between 2012 and 2015, before the advent of SARS-CoV-2, and to compare our findings with those observed during SARS-CoV-2 infection.

2. MATERIALS AND METHODS

This was a longitudinal prospective descriptive study conducted in Batna, Algeria, involving patients aged 28 days and older, hospitalized in the departments of infectious diseases (EPH), pediatrics (CHU), and medical intensive care (CHU) for encephalitis or meningoencephalitis, who were HIV-negative, during the period from April 1, 2012, to August 31, 2015. Patients were included according to the criteria and definitions set by the SPILF recommendations [12]: hospitalized patients diagnosed with encephalitis or meningoencephalitis, aged over 28 days, presenting with altered consciousness, seizures, central or peripheral neurological signs, autonomic disturbances, with fever ≥38°C, and CSF abnormalities, defined as either a white cell count ≥4 cells/mm³ or protein concentration ≥0.4 g/L; or patients over 28 days of age hospitalized for neurological symptoms and/or radiological signs of febrile encephalitis with normal CSF, in view of the frequency of post-infectious encephalitis. Patients were excluded if they met the following criteria: HIV-positive status, brain abscess, or non-infectious CNS disorders.

Epidemiological, clinical, biological, and radiological parameters were collected on a standardized case investigation form completed upon patient admission. The form recorded sociodemographic characteristics, suggestive clinical presentation, geographic origin, season of onset, vaccination status, history of animal contact or exposure to vectors, travel history, immunosuppression, personal medical history, signs suggestive of tuberculosis, mode of onset, general and functional signs, physical signs, neurological and extra-neurological manifestations.

Biological samples were collected at admission according to standard procedures for suspected encephalitis or meningoencephalitis. Additionally, for the purpose of complementary microbiological investigations, samples were collected and immediately frozen at −80°C for later analysis: EDTA blood (5 mL) or dry blood tube (5 to 10 mL), pharyngeal swab, and CSF (1 tube of 15 drops). These were supplemented by blood samples (EDTA or dry tubes, 5 mL) collected at discharge.

Complementary analyses (PCR, viral culture, and serological tests) were performed at the Institut des Agents Infectieux (IAI) of the University Hospital of Lyon. Samples were extracted using the Nuclisens Easymag system (BioMérieux). Following extraction, various real-time PCR and RT-PCR assays were conducted to detect viral and bacterial pathogens in blood, CSF, and pharyngeal swab specimens.

Viral isolation techniques were applied to pharyngeal swabs preserved at −80°C, diluted 1:10, and inoculated onto monkey kidney cell cultures. These culture-based data complemented those from the microbiology laboratory at Batna Hospital, where cell cultures were used to detect pyogenic bacteria and *Mycobacterium tuberculosis* in the CSF and other samples.

The Ct threshold for the PCR test that was the lowest indicated a high viral load. For CMV, EBV, HHV6, which are recurrent viruses, Ct value was compared between blood and CSF. Serological diagnosis was performed for certain pathogens using the two serum samples collected and, in some cases, CSF.

Annex 1 shows the etiological investigation algorithm and diagnostic strategy followed during the etiological investigation in our study. The etiological investigation followed SPILF recommendations for the classification of encephalitis and meningoencephalitis cases [12]. This classification was designed to assess the level of evidence linking microbiological results from the analyzed samples to the observed encephalitic presentation. Based on the results, each case was categorized according to the level of diagnostic certainty (confirmed, probable, or possible case). A single patient could be assigned more than one etiology: Confirmed cases are those with positive CSF microbiological results (PCR, direct exam, culture) or positive CSF counter immunoelectrophoresis. Probable cases are those with positive diagnostic results in a biological fluid other than the CSF or a CSF serologic test (seroconversion or high IGM titer). Possible cases are patients with high serum IgM titer or symptoms or signs compatible with a certain infection associated with an epidemiological context in whom diagnostic tests with a known low sensitivity were negative. Unknown etiology cases are patients in whom results of all tests were negative.

Ethical guidelines, including medical confidentiality and obtaining patients' consent for prescribed therapeutic measures, were strictly adhered to throughout the study. Verbal consent for the lumbar puncture procedure was obtained from the patients or their families.

3. RESULTS

In Batna, a wide spectrum of pathogens was found to be responsible for cases of acute encephalitis and/or meningoencephalitis. During our study period, from April 1, 2012, to August 31, 2015, we identified 141 patients with encephalitis and/or meningoencephalitis who were HIV-negative and who met the inclusion and exclusion criteria defined in our protocol. The number of pathogens tested per patient varied from 1 to 31. In 13 patients, only one diagnostic test was performed due to immediate positivity, whereas for others, a full panel of tests was necessary. On average, fewer tests were required for patients in whom an etiology was identified compared to those without a confirmed etiology (15.01 vs. 20.17 tests per patient).

A viral etiology was established in 73 of the 141 patients (51.8%), among whom 18 patients (12.8%) were found to have coronavirus infection (figure 1).

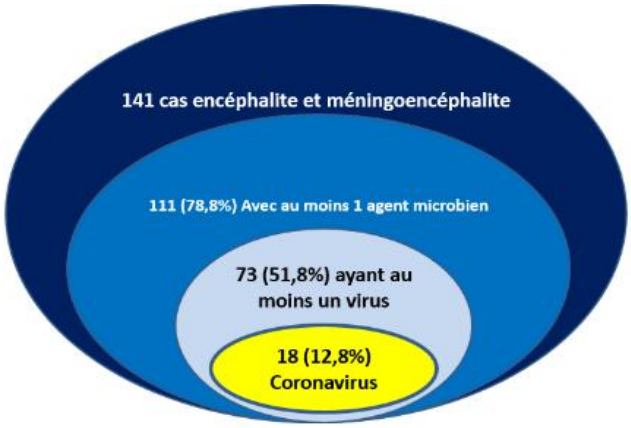

Figure 1. Classification of Encephalitis by Causative Agent.

Prior to the SARS-CoV-2 pandemic, we were surprised by the relatively high number of coronavirus cases (n=18) in nasopharyngeal swabs . Among these, 4 were mono-viral infections associated with encephalitis, and 14 were coinfections. Four of these coinfected cases involved more than two pathogens: (1) tuberculosis-CMV, (2) EBV-chlamydiae, (3) tuberculosis-chlamydiae, and (4) Streptococcus pneumoniae-mycoplasma. Two cases involved more than three pathogens: (1) EBV-tuberculosis-herpes and (2) adenovirus-parvovirus-Lyme. The serotypes OC43, 229E, NL63, HKU1, and MERS-CoV could not be identified due to an insufficient quantity of biological fluid tested.

The median age was 20 years (range: 0–45), with the most affected age group being 16–64 years (55.6%), followed by the 28 days to 5 years group (27.8%). A male predominance was observed, with a sex ratio of 2. Most patients (13/18, 72.2%) were admitted during winter or spring. More than half (55.6%) resided in urban areas, while 8 patients (44.4%) had lived in a village within the previous 3 months. Animal contact was reported in 3 patients (16.7%). Immunosuppression was present in 3 patients (16.7%).

The onset was abrupt in 7 cases (38.9%). Fever ≥38.5°C was present in 12 cases (66.7%), and neck stiffness was observed in 7 patients (38.9%). Consciousness disturbances were documented in 11 patients (61.1%), including 4 with a Glasgow Coma Score <8 (22.2%). Cranial nerve involvement was noted in 27.8% of patients. Aphasia, amnesia, myelitis, and psychiatric symptoms were each observed in 11.1% of cases. In addition to these neurological signs, respiratory and hepatic involvement were documented in 5 patients each (27.8%). None of the patients had cardiovascular symptoms (table 1).

Table 1. Epidemiological, Clinical, Biological, and Radiological Characteristics of the 18 Cases of Coronavirus-Associated Meningoencephalitis.

Clinical Features	n (%)
Median age	20 years
Male-to-female ratio	2
Abrupt onset	7 (38.9%)
Fever ≥38.5°C	12 (66.7%)
Respiratory involvement	5 (27.8%)
Neck stiffness	7 (38.9%)
Glasgow score <8	4 (22.2%)
Behavioral disturbances	10 (55.6%)
Seizures	7 (38.9%)
Focal neurological signs	4 (22.2%)
Psychiatric disturbances	2 (11.1%)
Pleocytosis	14 (77.8%)
Hypoglycorrhachia	6 (33.3%)
Hyperproteinorachia	10 (55.6%)
Abnormal neuroimaging	10 (55.6%)
Abnormal EEG	9 (50.0%)
ICU admission	3 (16.7%)
Neurological sequelae	5 (27.8%)

Pleocytosis was observed in 14 patients (77.8%), lymphocytic in 6 (33.3%)—including 2 with monomicrobial infection—polynuclear in 3 (16.7%)—including 1 with monomicrobial infection—and mixed in 5 (27.8%)—with 1 monomicrobial case. Hypoglycorrhachia was present in 6 cases (33.3%), of which 3 were coinfected with tuberculosis, Mycoplasma, or Chlamydiae. Hyperalbuminorachia was noted in 10 cases (55.6%), including 3 with coinfections involving the same pathogens.

Inflammatory syndrome was characterized by: elevated C-reactive protein >50 mg/L in 4 patients (22.2%), positive procalcitonin in 13 patients (72.2%), and hyperleukocytosis in 7 patients (38.9%). Neuroimaging was abnormal in 10 patients (55.6%), with varied lesion topographies. Electroencephalograms (EEGs) were recorded for 12 patients. EEG findings compatible with encephalitis were observed in 50% of cases. Temporal lobe abnormalities were seen in 5 patients (27.8%) (figure 2).

The median duration of hospitalization was 19.5 days (range: 1–874). In-hospital complications included: hemodynamic instability in 2 patients (11.1%), status epilepticus in 3 patients (16.7%), glasgow score <8 in 6 patients (33.3%), and ICU transfer in 3 patients (16.7%). Neurological sequelae—including hydrocephalus, behavioral disorders, memory impairment, intellectual deficits, and psychomotor delay—were observed in 5 patients (27.8%). One death was recorded (5.6%).

4. DISCUSSION

Even before the emergence of the COVID-19 pandemic, during the period 2012–2015, we were surprised by the significant involvement of coronaviruses in the diagnosis of encephalitis and meningoencephalitis: 18 cases (12.76%), including 4 cases without coinfection. Our findings were soon supported by a study conducted during the same period and published in 2016 in Beijing, China.

In this study, among 183 and 236 children hospitalized for acute encephalitis (either isolated or associated with respiratory tract infection), anti-CoV IgM antibodies were detected in 22/183 (12.02%) and 26/236 (11.02%) children, respectively, between May 2014 and April 2015 [8]. Similarly, Morfopoulou et al. described a case of acute encephalitis in an 11-month-old immunocompromised child, in whom coronavirus was identified in brain biopsies [10].

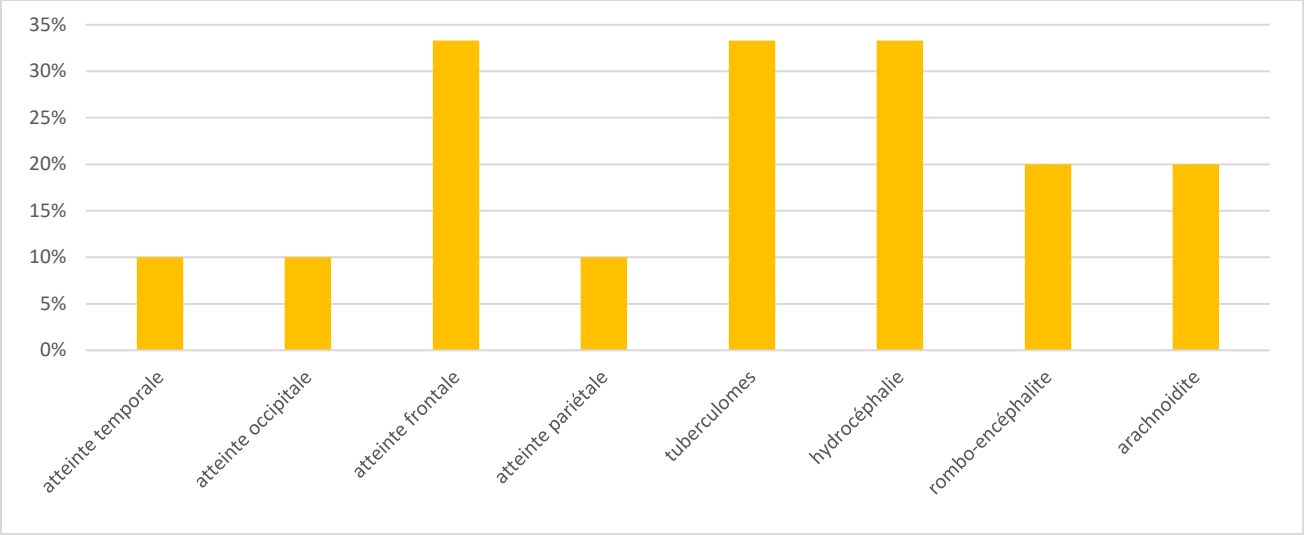

Figure 2. Topographic Distribution of Neurological Lesions on CT.

Before the emergence of SARS-CoV-2, no coronaviruses had ever been isolated from CSF. In February 2020, Takeshi et al. were the first to detect viral RNA particles in the CSF of a patient with COVID-19 who presented with signs of acute encephalitis [14]. Since then, several studies have reported similar findings, including Isabel S. [13] and Md Asiful Islam [11], who described cases of acute encephalitis in 138 and 91 patients, respectively.

In our study, a high frequency of cases among young individuals was observed. The median age was 20 years (range: 0–45), with 27.8% of patients belonging to the 28-day to 5-year age group. This result is supported by Li Y, who reported a median age of 3 years [8]. The literature has consistently noted that classical coronaviruses are more commonly detected in pediatric populations [3]. We also observed a male predominance with a sex ratio of 2, while Li Y reported a higher sex ratio of 4.5 [8]. In contrast, COVID-19-related encephalitis affected older individuals, with Isabel S. reporting a mean age of 59.4 years and an equal sex distribution [13], and Md Asiful Islam reporting a mean age of 55 years with a male predominance (sex ratio 1.1) [11].

Risk factors for severe coronavirus infection before the COVID-19 era included environmental and genetic factors and immune-mediated processes [1]. During the COVID-19 pandemic, encephalitic involvement was more often associated with comorbidities such as hypertension, diabetes, and renal failure [13]. In our cohort, 44.4% of patients resided in rural areas compared to 77.3% in the Li Y study [8]. Immunosuppression was present in 2 patients (11.1%) in our cohort versus 68.1% in the cohort reported by Md Asiful Islam [11], and similar to the immunocompromised case described by Morfopoulou [10].

Infectious encephalitis typically presents with sudden onset, fever, sometimes meningeal signs, and variable neurological manifestations. A fever >38.5°C was observed in 12 patients (66.7%) in our series, consistent with results from Isabel S. (63.6%) [13], Li Y (81.8%) [8], Morfopoulou [10], and Takeshi [14]. Headaches and neck stiffness were reported in 12 (66.7%) and 7 (38.9%) cases, respectively, in our study—compared to 45.5% and 31.8% in the Li Y study [8], 27.3% neck stiffness in Isabel S. [13], and only 20.5% headache in Md Asiful Islam [11]. The most common neurological signs reported in the literature were impaired consciousness, confusion, and seizures—observed by Morfopoulou [10], Isabel S. [13], Takeshi [14], and Md Asiful Islam [11].

In our cohort, a high proportion of focal neurological deficits (50%) was observed. Additional neurological signs may have been related to coinfections, including: psychiatric symptoms (11.1%) in cases with tuberculosis, HSV, and EBV, aphasia (11.1%) in patients with HSV, EBV, or tuberculosis, cerebellitis (16.7%) in patients with EBV, HSV, Mycoplasma, or tuberculosis, and myelitis (11.1%) in patients with EBV or influenza B.

Hepatic involvement may have resulted either from coronavirus infection or from coinfection with viruses such as EBV or influenza B, or tuberculosis. Respiratory involvement, frequently observed during coronavirus infection, may have been exacerbated by coinfection. Md Asiful Islam found that neurological symptoms appeared before respiratory symptoms in 78% of patients [11]. In our population, pleocytosis was observed in 14 cases (77.8%), compared to 45.5% in Li Y [8] and Takeshi [14], whereas CSF was normal in the Morfopoulou case [10]. We noted a predominance of lymphocytic pleocytosis (33.3%). The mixed reaction pattern (27.8%) may have been due to coinfection with tuberculosis, Mycoplasma, Chlamydiae, EBV, or CMV.

Hypoglycorrhachia was observed in 6 patients (33.3%), 3 of whom (16.7%) had potential bacterial coinfections (tuberculosis, Mycoplasma, or Chlamydiae), compared to 18.1% reported by Li Y [8]. Hyperproteinorachia was seen in 10 patients (55.6%), compared to 36.4% in Li Y [8]. Hyponatremia was found in 4 patients (22.2%), potentially explained by coinfection with tuberculosis, Listeria, or influenza B.

Neuroimaging was pathological in 55.6% of our cases, compared to 50% in Li Y [8] and 64% in Md Asiful Islam [11]. Arachnoiditis, tuberculomas, and hydrocephalus may have been due to coinfection (e.g., tuberculosis or EBV). EEG findings were compatible with encephalitis in 50% of patients, with temporal lobe abnormalities present in 5 cases (27.8%). These findings are consistent with those reported by Md Asiful Islam (75%) [11]. Only one death was recorded in our cohort, compared to 13.4% mortality in Isabel S. [13] and 20% mortality and 37.8% neurological sequelae in Md Asiful Islam [11].

It was essential for this study to rely on existing infrastructures, as the additional analyses performed at the Lyon laboratory ultimately served only to better document, from a microbiological perspective, the cases observed in Batna after the fact. Furthermore, the lack of certain examinations in hospital settings (such as imaging or EEG) hindered rapid etiological diagnosis and, consequently, optimal patient management.

To our knowledge, this is the first prospective study of this scale conducted in Algeria and Africa, and its sample size is comparable to those undertaken for similar objectives in the United States, the United Kingdom, or France. From an etiological standpoint, our approach revealed a high number of possible coinfections. Some of these merely reflect the reactivation of endogenous viruses, for which determining their role in the infectious process proved challenging. The observed differences also highlight the specific characteristics of Algerian epidemiology and thus underscore the necessity for an approach tailored to the country's situation, both in terms of the incidence of various pathogens and the optimized patient management that should follow.

5. CONCLUSION

Given their known neuroinvasive potential, it is therefore plausible to consider that a respiratory virus circulating globally may establish and persist within the human central nervous system and potentially contribute to the development or exacerbation of neurological diseases such as multiple sclerosis, Alzheimer's disease, or recurrent encephalitis. In-depth studies aimed at better understanding both the adaptive mechanisms of neuroinvasive human coronaviruses and their potential association with various neurological consequences are now more than ever warranted and necessary.

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Competing interests: The authors declare that they have no competing interest.

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Annex 1. **Etiological Investigation Algorithm and Diagnostic Strategy.**

The etiological investigation was conducted in accordance with the recommendations of the *Société de Pathologie Infectieuse de Langue Française* (SPILF) for the management of patients with encephalitis (150). The recommended diagnostic procedure (150) is structured into three sequential levels, based on the frequency of infectious agents implicated in encephalitis and the need for early initiation of targeted therapy for specific pathogens. This stepwise strategy is grounded in epidemiological data describing the most common causes of infectious encephalitis in France.

Level 1: Frequent and Urgent Infections

- Standard bacteriological examination of cerebrospinal fluid (CSF)
- Herpes simplex virus type 1 and 2 (HSV-1/HSV-2); if initial testing is negative, repeat testing on day 4 after symptom onset
- Varicella-zoster virus (VZV)
- *Mycoplasma pneumoniae*

Level 2: If Level 1 Investigations Are Negative

- HSV-1/HSV-2 (testing at day 0 and day 4) on CSF
- Enteroviruses
- Cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesvirus 6 (HHV-6), adenovirus
- *Chlamydia* spp., *Borrelia* spp., *Coxiella burnetii*, *Bartonella* spp., *Listeria monocytogenes*
- Tick-borne encephalitis virus (TBEV)
- *Mycobacterium tuberculosis*

Level 3: If Levels 1 and 2 Investigations Are Negative

- *Rickettsia* spp., *Ehrlichia* spp., *Tropheryma whipplei*
- Influenza A and B viruses, parainfluenza viruses, measles virus, mumps virus, rubella virus
- West Nile virus (and/or other locally circulating arboviruses)
- Lymphocytic choriomeningitis virus, JC virus, parechovirus

Special Considerations

Additional investigations were performed in specific clinical contexts, particularly in cases involving recent travel, according to the geographical area visited (e.g., chikungunya virus, Japanese encephalitis virus, dengue virus, West Nile virus, coronaviruses). Testing for Nipah or Hendra virus was considered in cases of travel to endemic regions. Rabies virus infection was investigated in cases of dog bites associated with neurological symptoms such as agitation, aerophobia, or hydrophobia.

These diagnoses were established using appropriate microbiological techniques. To ensure methodological consistency, diagnostic testing followed established technical recommendations, which were strictly applied in the present study.