Review

Epidemiology and diagnosis of viral and bacterial central nervous system infections in Sri Lanka: A Narrative review

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Abstract

Bacterial central nervous system (CNS) infections are life-threatening diseases with high mortality and long-term neurological sequels, while viral CNS infections are mild and self-limited. In Sri Lanka, 1000-1500 and 150-250 patients with meningitis and encephalitis respectively are reported annually to the Epidemiology Unit. *Haemophilus influenzae*, Group B *Streptococcus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Neisseria meningitidis*, Group D *Streptococcus*, *Leptospira borgpetersenii* serovar Tarasssovi, *Listeria monocytogenes*, *Streptococcus bovis* biotype 2, enterovirus, Herpes Simplex Virus type 1 and type 2, Varicella zoster virus and cytomegalovirus have been reported up to 2022 in patients with meningitis while dengue virus, Japanese encephalitis virus, Varicella zoster virus, West Nile virus, Human Bocavirus Type 1, 2 and 3, human adenovirus type 41, Echovirus type 9 and 25, Herpes Simplex Virus type 1 and *Mycoplasma pneumoniae* have been reported in patients with encephalitis and meningoencephalitis. However less than 10% of cases have a definitive aetiological diagnosis with conventional bacterial culture methods routinely used to diagnose bacterial CNS infections, while molecular assays are used only for selected common viral pathogens in government hospitals in Sri Lanka. Use of antibiotics prior to cerebrospinal fluid collection, delay in sample collection, and low volume of samples have been identified as factors for low sensitivity of test results. Scientists, microbiologists and virologists are responsible for developing new test methods to identify current and previously unidentified causative microorganisms to extend the spectrum of neurotropic organisms in Sri Lanka to enhance the aetiological diagnosis.

Keywords*: Central nervous system infections, Sri Lanka, Diagnosis, Epidemiology*

Introduction

Central nervous system (CNS) infections include meningitis, encephalitis, meningoencephalitis, and brain abscesses. These CNS infections are caused by viruses, bacteria, fungi, and parasites, with more than 30% of CNS infections remaining without an

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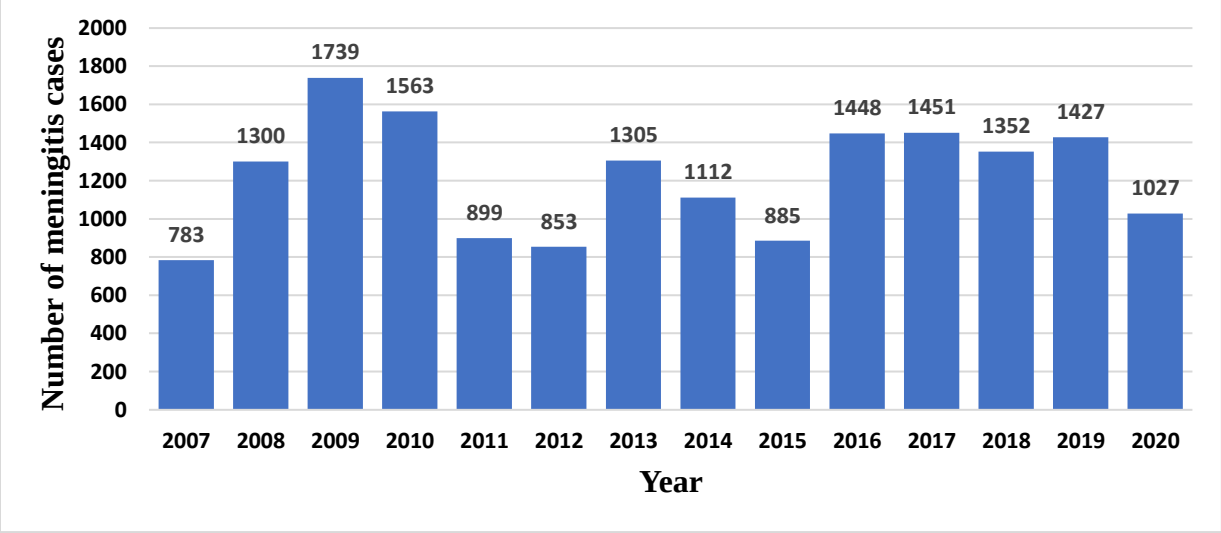
aetiological diagnosis.[1] Bacterial CNS infections are life-threatening diseases with high mortality and long-term neurological sequelae, while viral CNS infections are mild and self-limited. The greatest challenge with CNS infections is the aetiological diagnosis, due to the indistinguishable clinical presentation with varying combinations of fever, headache, altered level of consciousness, seizures, and focal neurological deficits. The spectrum of aetiological agents of CNS infections varies according to the geographical area, population's immunity, season and affected age groups. This review summarises the epidemiology and diagnosis of CNS infections in Sri Lanka.

Aetiology and epidemiology

Meningitis

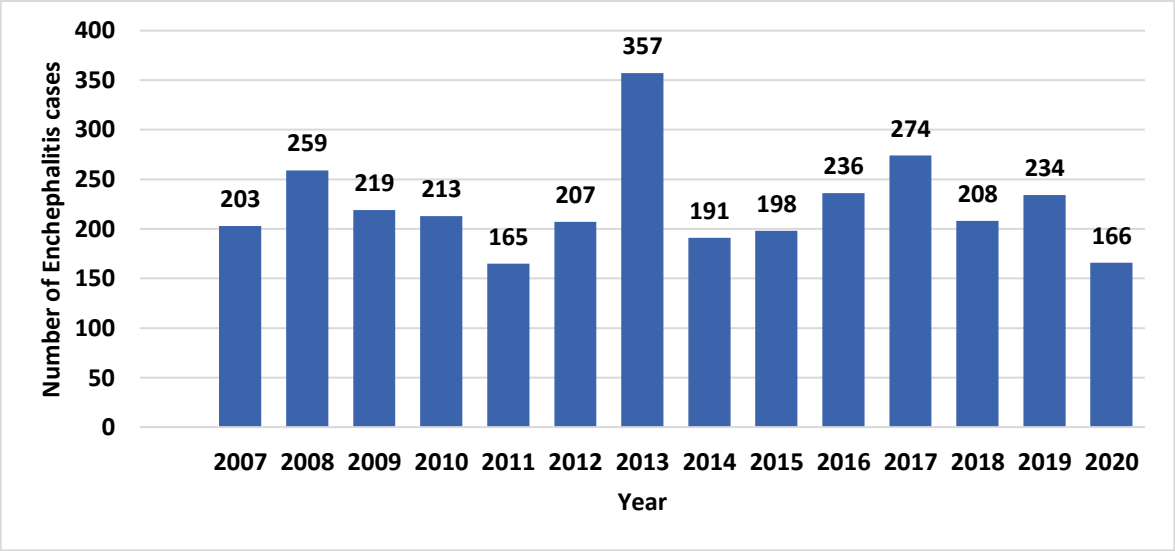
Meningitis is inflammation of the meninges. Globally, 89,909 deaths in infants aged 0 days to 11 months, 56,412 deaths in children aged 1–4 years, and 318,400 deaths in all ages were reported due to infectious meningitis in 2016.[2] In addition, though viral meningitis is more common, it is not associated with significant mortality and morbidity compared to bacterial meningitis.

Bacterial meningitis has been a notifiable disease in Sri Lanka since 2005. Based on the surveillance case definition of meningitis, patients with suspected meningitis should have fever with acute onset with one or more signs of meningeal irritation/inflammation such as neck stiffness, irritability, poor sucking (in infants), seizures, bulging fontanelles (in infants), altered consciousness and other signs of meningeal irritation/inflammation.[3] The annual distribution of meningitis and encephalitis cases from 2007 to 2020 are shown in Figures 1 and 2.



(Data taken from weekly bulletin, Epidemiology Unit, Sri Lanka)[4]
Notification criteria were clinically suspected or/and clinically and laboratory-confirmed cases.

Figure 1: Annual meningitis cases in Sri Lanka from 2007-2020



(Data taken from weekly bulletins, Epidemiology Unit, Sri Lanka)[4]
Notification criteria were clinically suspected or/and clinically and laboratory-confirmed cases

Figure 2: Annual encephalitis cases in Sri Lanka from 2007-2020

Haemophilus influenzae type b vaccine (Hib vaccine) was introduced to the EPI programme in Sri Lanka in 2008. Before the introduction of the Hib vaccine to the national immunisation programme, a prospective study was conducted in the Colombo district with children aged <5 years old admitted to Lady Ridgeway Hospital, Colombo South Teaching Hospital, Sri Jayawardenapura General Hospital, Homagama Base Hospital, and Avissawella Base Hospital. Based on WHO generic protocol, children aged <5 years with clinically suspected bacterial meningitis were included in the study. Culture and latex agglutination assays for antigen detection were used to confirm the aetiology and *H. influenzae* typing carried out on all *H. influenzae* isolates. In this study, 50% of meningitis in children <5years old were due to *H. influenzae* type b, which was one of the highest rates in the Asian region, with 20.1 cases per 100,000 children.[5] In addition, 60% of meningitis in infants <12 months were caused by *H. influenzae* type b. Group B *Streptococcus* and *Streptococcus pneumoniae* were identified as the 2nd and 3rd common causes of confirmed bacterial meningitis accounting for 23% and 13% of bacterial meningitis cases respectively. Other than these 3 organisms, 8 patients with *Neisseria meningitidis*, 5 with *Escherichia coli* and 2 with Group D *Streptococcus* were reported in the study.

A study was conducted in Lady Ridgeway Hospital, Colombo to identify invasive *H. influenzae* infections in children <5 years after introduction of the Hib vaccine. Culture and *H. influenzae* antigen assays in CSF were used to diagnose *H. influenzae* infection. As a result of Hib vaccination, reported cases were shown to have gradually decreased from 2008 to 2011. More than 98% of patients with *H. influenzae* type b infection in this study group were not fully immunised with Hib vaccine.[6] However, in 2014-15, Jayaweera *et al.* diagnosed *H. influenzae* meningitis in 2 children <12 years in the Anuradhapura district who had been immunised with Hib vaccine.[7] The authors suggested this could be due to either *H. influenzae* other than type b or lack of efficacy of the vaccine. However, no serotyping or molecular assays were performed to determine serotype of the isolates.
S. pneumoniae meningitis incidence rates in children <5-years were 8 cases per 100 000 children in 2004 and 13 cases per 100 000 children in 2005-2009.[5,8] The increased incidence

rate in 2005-2009 was probably due to exclusion of data from private sector laboratories in the 2004 study.[8]

In surveillance of the etiology of meningitis in the Anuradhapura district from 2014-2015,[7] bacterial meningitis in children aged 1 month to 12 years was associated with *E. coli, S. pneumoniae, H. influenzae, Mycobacterium tuberculosis, Listeria monocytogenes* and *S. agalactiae.* Cytomegalovirus, enterovirus, and Herpes Simplex Virus type 1 (HSV-1) were identified as causes of viral meningitis. The highest number of bacterial meningitis cases was reported with *E. coli* K1 with a >50% incidence rate, and enterovirus was the commonest cause of viral meningitis in the study area.

Prior to 2013, a prospective observational study was conducted over two years in patients aged >12 years with clinically suspected CNS infection, with 215 patients included in the study.[9] *S. pneumoniae* was confirmed as the causative agent in one patient with bacterial meningitis by blood culture. None of the remaining 214 patients had a definitive causative diagnosis.

A laboratory-based descriptive study was conducted at Teaching Hospital Peradeniya from December 2016 to March 2017 in patients aged >1 year with clinically suspected bacterial CNS infection.[10] Of 80 samples tested for *H. influenzae*, *S. pneumoniae* and *N. meningitidis* using multiplex PCR assay, five samples were positive for *H. influenzae,* and 3 were positive for *S. pneumoniae.* The five patients who were diagnosed with *H. influenzae* meningitis were adults.

Leptospira borgpetersenii serovar Tarasssovi was reported from 2 patients with aseptic meningitis at Colombo South Teaching Hospital, Kalubowila.[11] Six patients with CNS infections caused by *Leptospira* species were reported from Colombo South Teaching hospital in 2008.[12] All these patients had died, and common CNS clinical manifestations were features of encephalitis and generalised tonic-clonic seizures.[12]

Streptococcus bovis is a rare organism associated with meningitis globally. *S. bovis* biotype 2 was isolated from a neonate with Down syndrome who presented with neonatal meningitis in the Kelaniya area.[13]

Two patients with viral meningitis caused by VZV and HSV-2 were reported in a study conducted at National Hospital Kandy in 2017-2019.[14]

Encephalitis

Encephalitis denotes inflammation of the brain parenchyma and can often present as meningoencephalitis by extension of the inflammation into the meninges. A viral aetiology is the commonest cause of infectious encephalitis. Symptoms can include headache, fever, meningeal signs, seizures, stupor, disorientation, coma, tremors, paresis (generalised), hypertonia, and loss of coordination.

Encephalitis due to a viral aetiology was confirmed in only 27.3% of patients with suspected encephalitis during 2012-2014 in Colombo.[15] In this study, dengue (40.7%), Japanese encephalitis (JE: 25.9%), Varicella zoster virus (VZV: 11.1%) and West Nile virus (WNV: 11.1%) were identified as the aetiology of viral encephalitis. This was the first time WNV

CNS infections were reported in Sri Lanka. WNV IgM was detected in serum samples from 3 patients. CSF for WNW IgM was checked in only one patient and was positive.[15,16] Two samples had a definitive diagnosis with plaque reduction neutralisation test (PRNT) for WNV.

Human Bocavirus Type 1, 2 and 3 were identified in patients with encephalitis in Sri Lanka for the first time in a study conducted at Colombo North Teaching Hospital, Ragama.[17] CSF samples were tested for pathogens associated with encephalitis and diarrheagenic viruses using PCR. Of 191 patients with clinically suspected encephalitis, dengue type 2 (1/191; 0.5%), human adenovirus type 41 (7/191; 4%), human echovirus type 9 or 25 (2/191; 1%) and human bocavirus (5/191; 3%) were identified. None of the human bocavirus DNA positive CSF samples were positive for human bocavirus type 1–4 specific IgM or IgG. The age of patients with an identified aetiology ranged from 13 months to 55 years. Surprisingly, HSV, the most common cause of adult sporadic viral encephalitis in developed countries was not reported in this study.[15,17]

Another study showed that of 381 CSF samples from patients with clinically suspected JE acute encephalitis, only 23 (6%) were positive for JE IgM. Of these 23 patients, only 11 (48%) cases were confirmed as JE encephalitis by excluding dengue using a dengue IgM capture ELISA and dengue real-time PCR assay, while 4 patients (17%) were confirmed as dengue encephalitis by detection of dengue RNA in CSF samples.[18] The remaining 8 CSF samples were positive for both JE and dengue IgM. A 14-year-old boy with dengue encephalitis was reported from Jaffna in 2016.[19] Seven patients admitted to Teaching Hospital Kandy from March 2017 to January 2018 had dengue encephalitis.[20]

The neurotropic nature of dengue virus serotype 2 and 4 and the ability of the neurotropic dengue virus to cross the blood-brain barrier had been shown with molecular analysis.[17,21,22] The number of reported symptomatic dengue cases, the severity of the disease and the seroprevalence rate have significantly increased over the years in Sri Lanka.[20,23]

Echovirus type 9 has been identified in nine patients out of 17 paediatric patients in Colombo with clinically suspected meningoencephalitis.[24] This Sri Lankan echovirus type 9 strain was genetically closely related to the strain isolated in France and Korea. These 17 patients were aged between 1 month and 10 years.

VZV encephalitis prevalence in a study group of clinically suspected patients from Western, Southern and Central Provinces of Sri Lanka in 2015-2016 was 9% with an 8.3% prevalence in adults and 9.7% prevalence in children.[25]

During 2017-2019, 142 CSF samples from children <14 years old and 210 CSF samples from adults (>14 years old) with clinically suspected CNS infections in Central, North Central and Eastern Provinces of Sri Lanka were analysed to determine the positivity rate of HSV and VZV CNS infections. A 2-year-old child had encephalitis with HSV1, and 4 children and 1 adult had VZV encephalitis.[14] All 5 patients with VZV encephalitis were clinically confirmed as primary infections, and the positivity rate of VZV encephalitis among patients was 1.42%.

Mycoplasma pneumoniae has been identified in five children with meningoencephalitis and three children aged 2-12 years with encephalitis from November to May 2001 at Teaching Hospital Karapitiya.[26]. The particle agglutination test was used to detect the antibody titre in serum samples for *M. pneumoniae*.

Diagnosis

Clinical Presentation

Early clinical diagnosis is quite challenging with the non-specific signs and symptoms of CNS infections. Although fever, neck stiffness, and altered level of consciousness are considered the classic symptom triad of CNS infections, only a minority of patients present with this classical triad. At least one symptom of this classic triad is seen in 99-100% of patients with meningitis.[27] Meningitis can usually be excluded in the absence of at least one of these symptoms.[28] Other associated signs and symptoms are headache, vomiting and nausea, seizures, and rash. Other than these symptoms, classical signs of physical examinations to diagnose meningitis include Brudzinski's and Kernig's signs.[29]

The triad of classical symptoms was seen in only 46.1% of 108 children aged 1 month to 12 years with meningitis in Anuradhapura.[7] However, fever was seen in all the patients aged 1 month to 10 years with meningoencephalitis who were Echovirus positive, of whom 66.7% had a headache, 77.8 % had vomiting, and neck stiffness was seen in 66.7%.[24] Three patients who were positive for WNV had fever and an altered level of consciousness. Neck stiffness and increased tone were seen in two of the WNV positive patients.[16] Although extrapyramidal features and flaccid paralysis are common in WNV neuroinvasive diseases, patients in Colombo who were WNV positive did not develop these distinguishable clinical features.[16]

A prospective observational study conducted at Colombo North Teaching Hospital, Ragama, showed definite microbiological confirmation in only one of 215 patients with clinically suspected CNS infection.[9] Of the 215 patients, 0.7% had an alternative laboratory diagnosis, and diagnosis was uncertain in 26%.

Laboratory Diagnostic methods and issues in laboratory diagnosis

As clinical features have little value in confirming CNS infections due to their non-specific nature, laboratory diagnosis is essential for confirmation of the aetiology of CNS infections. Cerebral spinal fluid (CSF) analysis is the common laboratory diagnostic method for patients clinically suspected of having meningitis. Gram stain of CSF, cytology and biochemistry results provide an initial clue for predicting the aetiology, and standard markers are available to distinguish viral, bacterial and fungal causes (Table 1). However, these standards do not always give a reliable picture due to low sensitivity. As shown by Ranawake (2015), a minority of patients (10%) with bacterial meningitis are negative for CSF cytology indicative of bacterial meningitis.[28] Balachandra *et al.* reported a patient with suspected viral aetiology from whom *H. influenzae* was isolated, and 2 patients with normal CSF findings in whom *S. pneumoniae* was isolated.[10] Hence, the bacterial and viral meningitis case classification as described in Surveillance Case Definitions for Notifiable Diseases in Sri Lanka (given below) may not identify all patients with bacterial/viral CNS infections.

Case classification as follows as in Surveillance Case Definitions for Notifiable Diseases in Sri Lanka[3]	
Suspected:	A case compatible with the surveillance case definition
Probable Bacterial Meningitis	A suspected case with a turbid (“cloudy”) CSF or a CSF with an elevated protein (>100 mg/dl) decreased glucose (100 WBC/mm) with 80% neutrophils
Probable Viral Meningitis	A suspected case with CSF findings including pleocytosis (usually mononuclear, occasionally polymorphonuclear in the early stages), increased protein, normal sugar and absence of other causative organisms
Confirmed	A suspected or probable case which is laboratory confirmed

Table 1: Standard CSF findings in different type of infectious meningitis*

Test Marker	Normal	Viral meningitis	Bacterial meningitis	Fungal meningitis
White blood cell count (no. of cells/mm$^{3)}$	0–5 (up to 30 in neonates)	>100	>1000	Variable
Predominant WBC type	Lymphocytes	Lymphocytes (Polymorphonucleocytes (PMNs) may predominate early in the disease)	PMNs (Lymphocytosis present 10% of the time)	Lymphocytes
Protein (mg/dL)	15-40	Normal to mild elevation	Elevated	Normal to mild elevation
Glucose (mg/dL)	50–80 (two third of the serum level)	Normal to mild less	Low (<40)	Low to normal
CSF to serum glucose ratio	0.44-0.90 in adults and children 0.42-1.10 in neonates	Normal	Low	Normal

*References [30,31,32]

CSF culture and/or blood culture methods are used for the detection of causative agents with clinically suspected bacterial CNS infections. However, conventional bacterial culture methods have low sensitivity and high specificity for the aetiological diagnosis.[7] The viability of bacteria is essential for positive culture results, and use of antibiotics prior to sample collection, and delayed sample transportation and/or delayed sample processing are major factors that lead to the loss of bacterial viability. In one study using a conventional culture method, only five of 76 CSF samples yielded a pathogen,[7] and of those positives, four had been collected prior to antibiotic administration. However, in some studies, none of the CSF samples from patients with suspected bacterial meningitis were positive with conventional bacterial test methods, including Gram stain, and culture.[9,10] The authors note that prior antibiotic usage, low CSF sample volume, and delay in CSF sample collection are significant factors for the above findings. Balachandra *et al.* (2021) found that lumbar puncture (LP) was delayed in 68.75% of the patients, antibiotic was given prior to LP in 60% of patients, and the volume in 82.5 % of CSF samples was less than 1 ml and considered as highly insufficient.[10]

The organism's viability is not an influencing factor for molecular assays. Empirical antibiotic treatment therefore has no impact on the results. In addition, these methods have high specificity and sensitivity, and the turnaround time is limited to a few hours. A study with the ME film array had a 54% (27/50) definitive diagnosis, while only 6.5 % of patients (7/108) had a definitive diagnosis with the conventional culture method.[7] However, viral nucleic acid detection in some infections, especially arboviral infections, has high specificity but low sensitivity due to the short viraemic phase and low viral load after the onset of the symptoms.[16]

Although antibiotics used prior to sample collection have no impact on PCR results, low volume of CSF samples and delay in LP significantly affects PCR results.[10] In this study with 82.5 % of insufficient sample volume (<1 ml), only eight samples (10%) were positive for bacterial nucleic acids by a multiplex PCR assay targeting only *H. influenzae, S. pneumoniae and N. meningitidis.*[10]

Antigen detection for *H. influenzae, S. pneumoniae* and *N. meningitidis* in CSF samples of patients with clinically suspected meningitis has been available in Sri Lanka. However, bacterial antigen detection in CSF samples has up to only 7% sensitivity and there is no

added advantage of routine use of latex agglutination test for the diagnosis of bacterial CNS infections and management of the infections.[33] There is an added advantage with molecular assays in CSF samples compared to antigen detection assays for diagnosing the aetiology in CNS infections as the sensitivity is higher with molecular assays. For example, although 10% positivity has been given with the PCR assays, none of the CSF samples were positive for the tested antigens.[10]

Antibody detection against some pathogens is done mainly for research purposes, and not as a routine diagnostic practice. For example, WNV was detected with WNW-specific IgM antibodies in serum or CSF samples and confirmed with WNW-specific PRNA,[16] and *M. pneumoniae* IgM antibody was detected in serum samples.[26] However, false positives are common due to cross-reaction among pathogens in the same family, and antibodies may last for months in the serum and CSF. In addition, PRNT and serological assays to detect antibodies in CSF samples are not commercially available and need special laboratory requirements to perform the test.[16]

Limitations in aetiological diagnosis of CNS infections and recommendations

The diagnosis of viral CNS infections is based mainly on molecular assays targeting herpes viruses, enteroviruses, and dengue virus and is available in only a few laboratories in Sri Lanka. Up to date, no state hospital laboratories have started molecular diagnosis for bacterial infections of the CNS. Studies conducted so far in Sri Lanka have demonstrated the low sensitivity of conventional test methods in diagnosing bacterial CNS infections. However, these studies have been limited to a few organisms in a limited geographical area. Prior use of antibiotics, insufficient volume, improper storage, and delays in collection, transportation and processing of CSF samples are considered the main factors for low sensitivity of conventional methods.[7,10] Implementation of available guidelines and protocols for sample collection and transportation is essential to enable laboratory confirmation of these CNS infections.

A few other factors may contribute to the low level of aetiological diagnosis, such as the spread of new neurotropic microorganisms, and poor quality of the samples. The main predisposing factors for emerging new microorganisms are behavioral changes of humans, climate changes, natural disasters, and gene mutations of the microorganisms. It is therefore crucial to conduct more prospective studies covering the whole country to identify any new aetiological agent causing CNS infections and regularly update the epidemiological data. Expansion of diagnostic capability due to the COVID-19 pandemic has resulted in the introduction of newer molecular assay facilities, including real-time PCR machines to government hospitals which could contribute to improved aetiological diagnosis of CNS infections. Additionally, laboratory staff have gained the knowledge and skills to perform molecular diagnostic assays during the pandemic. It is therefore possible to establish molecular assays for diagnosis of CNS infections with multiplex PCR test panels covering a broad spectrum of neurotropic organisms.

We would also recommend the development of rapid, cheap and effective immunoassays such as immunochromatographic tests and agglutination assays with sensitivity and specificity approaching 100% which can be used in both rural and urban areas of the country. More sophisticated advanced molecular techniques are suitable only for laboratories already using these techniques. As a low-income developing country, the cost of molecular diagnostics may be beyond the means of the state sector health services.

Scientists, laboratory experts, microbiologists and virologists are responsible for developing innovative techniques and identifying emerging neurotropic microorganisms to expand knowledge and awareness of neurotropic organisms in Sri Lanka.

"Editorial declaration: The two authors for this manuscript (RM and VL) are editors of the journal. This manuscript was handled by the other co-editor and the managing editor through the initial evaluation, reviewer assignment and decision-making process. RM and VL were not involved in the editorial process and decision making."

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