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SHORT REPORT
Herpes simplex virus encephalitis in Peru: a multicentre prospective study

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SUMMARY

Herpes simplex virus (HSV) is one of the most commonly identified infectious aetiologies of encephalitis in North America and Europe. The epidemiology of encephalitis beyond these regions, however, is poorly defined. During 2009–2012 we enrolled 313 patients in a multicentre prospective study of encephalitis in Peru, 45 (14·4%) of whom had confirmed HSV infection. Of 38 patients with known HSV type, 84% had HSV-1 and 16% had HSV-2. Patients with HSV infection were significantly more likely to present in the summer months (44·4% *vs.* 20·0%, $P = 0{\cdot}003$) and have nausea (60·0% *vs.* 39·8%, $P = 0{\cdot}01$) and rash (15·6% *vs.* 5·3%, $P = 0{\cdot}01$) compared to patients without HSV infection. These findings highlight differences in the epidemiology and clinical presentation of HSV encephalitis outside of the Northern Hemisphere that warrant further investigation. Furthermore, there is an urgent need for improved HSV diagnostic capacity and availability of intravenous acyclovir in Peru.

Key words: Central nervous system infections, encephalitis, epidemiology, herpes simplex virus, virology.

Encephalitis is a potentially devastating condition in which the aetiology is often not identified [1, 2]. Studies of the aetiologies of encephalitis have been historically difficult because cases are sporadic and dispersed over large geographical areas, symptoms may be heterogenous, and diagnostic capacity in existing laboratories is often limited. The annual incidence of encephalitis in Western industrialized countries is about 5·2 cases/100 000 persons overall, or 2·2 cases/100 000 adults and 10·5–13·8 cases/100 000 children [3, 4]. In tropical settings, annual incidence in adults and children combined is an estimated 6·3 cases/100 000 [4]. Although several prospective studies of encephalitis have been conducted in developing countries [5–8], comprehensive information on the epidemiology and aetiologies of encephalitis outside of Western industrialized countries – and particularly in Latin America – is extremely limited.

Viral central nervous system (CNS) pathogens are most often identified using polymerase chain reaction

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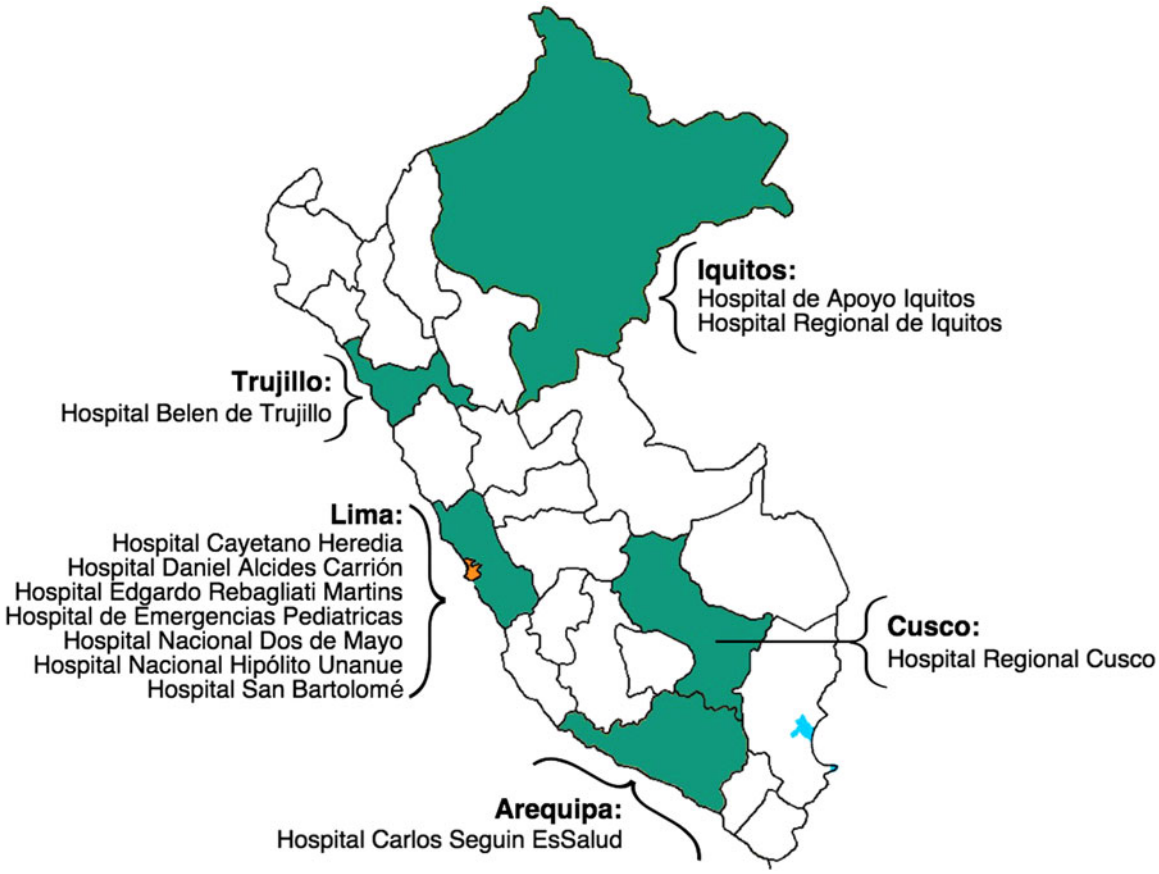

Fig. 1. Map of the study area showing hospitals in five Peruvian cities.

(PCR) to detect viral RNA or DNA [9]. As PCR technology becomes more widely available, herpes simplex virus (HSV) is increasingly recognized as one of the most commonly identified aetiologies of encephalitis across the world [10–13]. Two types of HSV affect humans: HSV-1 infection is acquired through oral transmission and is associated with cold sores, while HSV-2 infection is primarily acquired vertically or via sexual transmission. Both HSV-1 and HSV-2 encephalitis can occur during initial infection or as a result of reactivation, when a herpes virus that is dormant in ganglia of the nervous system reactivates to produce CNS infection [14]. Although CNS infection with HSV-2 after the neonatal period is uncommon, one study reported that 36% of women and 13% of men developed symptoms of meningitis during primary genital HSV-2 infection [15].

HSV encephalitis is a treatable form of encephalitis. Prior to the advent of intravenous acyclovir, mortality from HSV encephalitis was >70%; through treatment with intravenous acyclovir, mortality 18 months post-infection has declined to 28% [16]. Outcome is greatly improved when acyclovir is initiated within 2 days of symptom onset [14].

Current knowledge of aetiological agents of encephalitis in Latin America is primarily based on acute febrile illness surveillance [17] or small studies of limited geographical scope and duration [7, 18]. The objectives of this study were to determine the relative frequency of herpes as a cause of encephalitis in Peru and describe characteristics of patients with and without herpes encephalitis enrolled in a prospective study of CNS infections.

We conducted a prospective, multicentre, hospital-based study from 2009 to 2012 at 12 hospitals in five Peruvian cities: the capital city of Lima (seven hospitals), Iquitos in the Amazonian jungle (two hospitals), Trujillo in the coastal desert (one hospital), and Arequipa and Cusco in the Andes mountains (one hospital each) (Fig. 1).

A case was defined as any patient aged $\geqslant$28 days with acute onset (<2 weeks) of neurological symptoms, such as change in level of consciousness, seizure, altered coordination, or dysphasia. In addition, one or more of the following signs or symptoms was required: fever (temperature $\geqslant$38 °C), headache, cerebrospinal fluid (CSF) white blood cell count >5

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leukocytes/ml, or neuroimaging or electroencephalography abnormalities suggestive of encephalitis. This case definition is similar to that proposed by the International Encephalitis Consortium but incorporates a slightly broader major criterion (neurological symptoms rather than mental status change) and requires one minor criteria rather than two [19]. Subjects were excluded if they weighed <4 kg or had clinical, CSF, or neuroimaging findings consistent with pathology other than encephalitis (e.g. stroke or bacterial meningitis). All patients with presumptive HSV encephalitis received empirical intravenous acyclovir, with treatment discontinued if HSV PCR testing was negative.

All patients were interviewed using a standardized questionnaire to collect information on demographics, medical history, and clinical symptoms. The study physician performed a complete physical and neurological examination. Computerized tomography (CT) and/or magnetic resonance imaging (MRI) were performed when a mass lesion or other pathology was suspected. Patients were asked to return for a convalescent blood draw and interview 14 days after initial presentation.

Serum and CSF samples were collected from patients at initial presentation. CSF glucose, protein, and cell count with differential were determined at local laboratories and aliquots were shipped to the U.S. Naval Medical Research Unit-6 in Lima for further testing. PCR assay for HSV-1/2 was performed on all acute CSF and serum samples and convalescent serum samples when available. Nucleic acid was extracted using the Qiagen QIAamp DNA Mini kit (Qiagen Sciences, USA) and PCR performed using specific primers KS30 and KS31 [20]. Positive results were confirmed by sequencing of the amplicon, which is specific to HSV-1/2, using the BigDye Terminator v. 3.1 Cycle Sequencing kit and 3100 Genetic Analyzer (Applied Biosystems, USA). Acute and convalescent serum samples were tested for HSV-1/2 IgM and IgG antibody using HerpeSelect ELISA test (Focus Diagnostics, USA).

We used a modified version of the case definition proposed by Granerod *et al.* to classify subjects with confirmed, probable or possible HSV encephalitis: confirmed if HSV was detected in CSF by PCR assay; probable if HSV IgG seroconversion was present; and possible if HSV was detected by PCR assay in serum on initial presentation or IgM serology was positive [21].

For univariate analyses, we included patients with confirmed, probable or possible HSV encephalitis. For the comparison of categorical variables among subjects, we used Pearson's χ^2 test for independence and Fisher's exact test. The Mann–Whitney U test was used for comparison of continuous variables when variables were not normally distributed. Statistical significance was defined as $P \leqslant 0{\cdot}05$; all statistical tests were calculated two-sided. Data were analysed using Stata v. 12 (StataCorp., USA).

All study procedures were approved by study hospitals and by the Institutional Review Boards of the University of Washington and the U.S. Naval Medical Research Unit No. 6. Written consent was obtained from each subject or from a parent or legal guardian for subjects aged <18 years. Written assent was obtained from subjects aged 8–17 years.

Three hundred thirteen patients with encephalitis were enrolled in the study (Table 1). Most enrolled patients were from Lima (54·6%), followed by Trujillo (20·1%) and Iquitos (13·4%). The majority (62·3%) of patients were male; median age was 24·0 years (S.D. = 22·1, range 1 month-85 years). Subjects aged <20 years comprised 44·5% of the study population. The most common presenting symptoms and signs were: altered level of consciousness (96·1%), headache (80·9%) and fever (68·1%). The classic triad of fever, headache, and altered consciousness was present in 164 (52·4%) patients. Seizure was reported in 145 (46·3%) patients, cranial nerve deficit in 97 (30·1%), and hemiparesis in 69 (22·0%).

Of the 313 patients with encephalitis, 45 (14·4%) had confirmed ($n = 32$), probable ($n = 1$), or possible ($n = 12$) HSV encephalitis. The highest proportion of patients with HSV infection were aged 30–39 years (26·2%), followed by patients aged 20–29 years (18·0%). Of these, 62·2% had fever and 50·0% had seizures on initial presentation. Thirty-two (71·1%) patients had HSV-1, six (13·3%) had HSV-2, and the remaining seven (15·6%) were inconclusive by HSV typing. HSV-2 encephalitis was detected only in patients aged <49 years.

Patients with ($n = 45$) and without ($n = 268$) HSV encephalitis were similar with respect to age, gender, and most presenting signs and symptoms. Compared to the 268 patients without HSV infection, those with HSV infection were significantly more likely to present in the summer months (44·4% *vs.* 20·0%, $P = 0{\cdot}003$) and have nausea (60·0% *vs.* 39·8%, $P = 0{\cdot}01$) and rash (15·6% *vs.* 5·3%, $P = 0{\cdot}01$). In addition, patients with HSV encephalitis had significantly higher CSF white blood cell count (34 *vs.* 10 white blood cells/μl, $P = 0{\cdot}005$) and percent lymphocytes

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Table 1. *Demographic and clinical characteristics of patients with and without HSV encephalitis in Peru, 2009–2012*

Characteristic	HSV infection		*P* value
	Yes (*n* = 45)	No (*n* = 268)	
Demographic characteristics			
Age, years, median (range)	24·5 (0–67)	24·0 (0–85)	0·9
Male, *n* (%)	31 (68·9)	164 (61·2)	0·4
Location, *n* (%)			
Lima	25 (55·6)	146 (54·5)	0·09
Trujillo	14 (31·1)	49 (18·3)	
Iquitos	2 (4·4)	40 (14·9)	
Cusco	2 (4·4)	26 (9·7)	
Arequipa	2 (4·4)	7 (2·6)	
Season, *n* (%)*			
Summer (warmer and wetter months)	20 (44·4)	59 (22·0)	**0·003**
Winter	25 (55·6)	209 (78·0)	
Presenting symptoms and signs, *n* (%)			
Neurological			
Altered consciousness	43 (95·6)	256 (95·5)	0·7
Nuchal rigidity	25 (55·6)	120 (44·8)	0·3
Obtunded	25 (55·6)	171 (63·8)	0·2
Language disturbance	24 (53·3)	162 (60·4)	0·3
Seizure	22 (48·9)	123 (45·9)	0·7
Abnormal coordination	22 (48·9)	169 (63·1)	0·06
Cranial nerve defect	17 (37·8)	80 (29·9)	0·3
General			
Malaise	39 (88·6)	215 (82·3)	0·2
Headache	39 (86·7)	207 (79·9)	0·4
Anorexia	30 (66·7)	182 (67·9)	0·9
Fever	28 (62·2)	186 (69·9)	0·3
Nausea	27 (60·0)	105 (39·8)	**0·01**
Pharyngeal injection	13 (28·9)	78 (29·1)	1·0
Cough	15 (33·3)	95 (36·1)	0·9
Rhinorrhoea	13 (29·6)	71 (27·0)	0·4
Rash	7 (15·6)	14 (5·3)	**0·01**
Adenopathy	6 (13·3)	15 (5·8)	0·1
Triad of headache, fever, and altered consciousness	23 (51·1)	141 (52·6)	0·9
CSF findings, median (IQR)†			
WBC count, cells/μl‡	34 (7–154)	10 (2–53)	**0·005**
% Lymphocytes‡	100 (90–100)	92 (50–92)	**0·04**
% PMNs‡	2 (0–15)	1 (0–30)	0·5
Protein, mg/dl‡	42 (25·5–94)	40 (18·2–99)	0·4
Glucose, mg/100 ml (S.D.)	61·4 (51·2–71·7)	52·5 (50·1–56·8)	0·09

CSF, Cerebrospinal fluid; IQR, interquartile range; PMNs, polymorphonucleocytes; RBC, red blood cell; WBC, white blood cell.

* There are two seasons in Peru – summer (December–March) and winter. In Lima and Trujillo the summer months are warmer than usual; in Iquitos, Arequipa, and Cusco the summer months are warmer and wetter than usual.

† Results available for CSF findings: white cell count (*n* = 281), % lymphocytes (*n* = 236), % PMNs (*n* = 222), protein (*n* = 268), glucose (*n* = 254).

‡ Non-parametric Wilcoxon rank-sum tests for continuous variables and Fisher's exact test for categorical variables.

(100% *vs.* 92%, $P = 0{\cdot}04$). CSF protein and glucose levels, however, did not differ significantly between the groups.

Forty-four (14·1%) of the 313 enrolled patients returned for follow-up. Although information on patients' outcome was limited, we are not aware of any deaths of patients with HSV encephalitis; two patients with non-HSV encephalitis died.

This is the first report of community-acquired encephalitis from a prospective, multicentre study in

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Latin America. The proportion of subjects presenting with fever (68·1%) and seizure (46·3%) was similar to that reported by studies conducted in high-resource countries. However, a lower proportion presented with rash (6·7% compared to 11–13%) and a higher proportion with respiratory symptoms of rhinorrhoea, cough, or dyspnoea (48·9% compared to 20–33%) [1, 2].

The relative proportion of patients with encephalitis caused by HSV infection (14·4%) is higher than in some reports [1, 2] but consistent with others [11, 12, 22]. Of patients with HSV infection, the proportion with HSV-2 (13·3%) was similar to other reports [1, 14]. Similarly, the higher prevalence of HSV-2 in younger patients is consistent with our current understanding of disease [1, 16]. The seasonal predilection of HSV encephalitis found in this study was surprising but held true when the analysis was limited to patients with confirmed HSV encephalitis; this warrants further investigation since it has not been reported in other series [1, 22].

Our study had several limitations. First, Peru is very diverse in both its geography and populations, so subjects in our study may not be representative of the country as a whole. Furthermore, only patients who presented at hospitals were enrolled, so our sample was likely biased towards patients with more severe infections and access to transportation. Last, CT/MRI results were only rarely recorded and follow-up data was limited so we do not have complete information on patient imaging and outcomes.

Our findings highlight the importance of HSV infection as a commonly identified infectious cause of encephalitis in Peru. Diagnosis of HSV infection through PCR technology and treatment with intravenous acyclovir – the current standard of care – was not available in most Peruvian hospital prior to this study. Development of increased diagnostic capacity for detecting HSV and provision of intravenous acyclovir treatment required collaboration and support from regional and national hospitals and governments.

The high prevalence of HSV infection detected in patients across Peru emphasizes the need for expansion of HSV testing and increasing availability of intravenous acyclovir for treatment of HSV encephalitis in Peru and likely other countries. Furthermore, additional information about regional and seasonal differences in the aetiologies of encephalitis could guide physicians in clinical management and selection of empirical therapy in areas where access to diagnostic tools is limited.

APPENDIX. Meningoencephalitis Working Group

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Dr Montano and Dr Tilley are employees of the U.S. Government. This work was prepared as part of their official duties.

DECLARATION OF INTEREST

None.

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