Encephalitis in Nepal: The Visitation of The Goddess of The Forest

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SUMMARY: Examination of hospital records from BMH Dharan and the large Nepalese hospital at Biratnagar has revealed a worrying trend in the incidence of acute encephalitis in the area of the British Military Cantonment at Dharan. The weight of available evidence strongly favours Japanese Encephalitis Virus (JEV) as the aetiological agent. Serological studies from healthy Nepalese from the Dharan area and animals from the Cantonment farm indicate that intense transmission of JEV occurs within the Cantonment area. Japanese Encephalitis (JE) must now be regarded as a serious threat to British Military personnel and dependants living in or visiting Nepal. Protective measures, particularly active immunisation are discussed.

Introduction

The British Military Cantonment at Dharan lies on the northern fringe of the Terai region of eastern Nepal in the Koshi Zone. The Terai is a flat fertile rice growing plain bordering the northern states of India. The Cantonment supports a population of British and Gurkha soldiers and their families and a large force of local Nepalese who live or work within its area.

Although acute encephalitis, known to the locals as the "visitation of the goddess of the forest," has probably always been a feature of life on the Terai, it appeared to be uncommon in the Dharan area before 1970. Since then each summer, cases have been admitted to the BMH and to the local Nepalese hospitals in increasing numbers. This in conjunction with the appearance of severe epidemics of Japanese Encephalitis (JE) in neighbouring parts of the Indian subcontinent^{1, 2, 3} suggested a threat might exist to British Military personnel living in or visiting Nepal. This paper explores the extent of the encephalitis threat, presents evidence implicating the Japanese Encephalitis Virus (JEV) as the principal casual agent and suggests measures that might be taken to reduce the risk of JEV transmission to man and the consequences of this.

Materials and Methods

The study was carried out in 4 phases.

PHASE I: The clinical records of all patients admitted to BMH Dharan with acute encephalitis were examined. The case finding was probably incomplete due to the absence of a data retrieval system. Clinical and laboratory data were extracted

from the records and where available the results of flavivirus serology were examined. Serum was tested for evidence of flavivirus antibodies either at the Special Pathogens laboratory, Porton or the Virus Reference laboratory, Colindale.

PHASE II: Dharan lies on the northern fringe of the Terai in the Koshi Zone. To determine the extent of the encephalitis problem in the southern Terai the inpatient analysis data from Biratnagar hospital was examined. Biratnagar is the largest town in the Koshi Zone and lies almost due south of Dharan near the Indian border. It is important from the military view-point in that all military personnel and families flying to and from Katmandu do so via Biratnagar airport.

PHASE III: To detect evidence of Japanese Encephalitis Virus (JEV) transmission on the Dharan Military Cantonment blood was taken from known amplifying hosts on the Cantonment resettlement farm. Serum from 16 pigs of varying ages was tested by Haemagglutination Inhibition (HAI) for JEV antibody at the Arbovirus Unit, Winches Farm* London and serum from adult buffaloes, chickens and ducks tested by HAI at the Armed Forces Research Institute of Medical Sciences (AFRIMS) Bangkok. All the animals tested had spent their entire lives on the farm.

PHASE IV -- To detect evidence of inapparent JEV transmission to local Nepalese serum from a sample of 20 Nepalese men working on the Cantonment was tested by HAI at AFRIMS. Serum from 20 UK soldiers and families was similarly tested to gauge their susceptibility to JEV.

*Winches Farm is a field station near St Albans of the London School of Hygiene & Tropical Medicine.

Results

PHASE I — Clinical data

The notes of 43 patients were reviewed, of whom eight were seen personally in 1982.

Age and Sex. The mean age of the group was 15 years (range 5 months to 63 years). 27 (62%) were under 20 years of age. The sex incidence was similar (20 males, 23 females).

Place of Domicile. Dharan — 24, Terai — 14, Hills — 3, Military Cantonment — 2.

Season. Although occasional cases occurred throughout the year the majority occurred in relation to the summer monsoon (Fig. 1). 74% of cases occurred between the months of August and October.

Fig. 1 Monthly incidence of encephalitis

Yearly Incidence. The disease appears to have been uncommon before 1970. Since then the annual incidence has been increasing (Fig. 2).

Fig. 2 Annual incidence of encephalitis

Mode of onset. The majority had a non-specific prodrome lasting a few days before the onset of definite encephalitic features. In addition to malaise, anorexia and fever, 58% had severe headache and 32% vomiting. In 19% the prodromal symptoms were not well recorded. Towards the end of this

stage and often precipitating hospital admission came symptoms of severe cerebral dysfunction. Disturbance of consciousness occurred in 100%, aphonia in 42% and grand mal fits in 40%. In a minority the disease was explosive with high fever and a rapidly fatal acute brain syndrome.

One patient demonstrated wildly disturbed behaviour for 3 days before lapsing into fatal coma.

Physical Findings

(a) *Fever.* 76% were febrile on admission, often to an alarming degree. Their mean oral or rectal temperature was 39°C (range 37.8-40.6). The fever had a swinging character and lasted on average 7 days (range 1-24 days).

(b) *Tachycardia:* Sinus Tachycardia was the rule with a mean heart rate of 113 beats/minute. In only one patient was an inappropriate bradycardia noted (rate 50 B/M).

(c) *Neurological Findings:* See Table I. No other abnormal signs were noted.

Table I Neurological findings on Admission	
SIGN	PERCENTAGE OF TOTAL
MENINGISM	81%
DROWSINESS	60%
SPASTICITY	44%
EXTENSOR PLANTARS	44%†
HEMIPLEGIA	42%
COMA	40%*
FITS	40%
FLACCIDITY	37%
ABNORMAL MOVEMENTS	27%
CRANIAL NERVE PALSIES	2%

†Excludes children of less than 1 year
*Implies unresponsive to pain

Outcome. Eighteen (42%) died in hospital, mostly within a few days of admission. Age and sex did not relate to prognosis but the presence of coma on admission accurately predicted a fatal outcome. Of those in coma 88% died, while in drowsy patients the death rate was only 11%. Of the 25 who survived to leave hospital only 28% made a good recovery. The remainder had varying degrees of neurological dysfunction which was judged as severe in 52%. Long term follow-up was not possible.

Management. Supportive and nursing care formed the cornerstone of management. Some patients received corticosteroids or antimicrobials but no consistent policy was employed.

(a) *Laboratory Investigations:* As the study was largely retrospective the laboratory data in places was incomplete.

(b) *Chest Radiology*: Most patients had an early chest X-ray. None was abnormal.

(c) *Biochemistry*: Liver function tests were normal. The mean serum sodium was 131 mmol/l (range 118-142), mean potassium 3.8 mmol/l (range 1.9-5.6) and mean urea 6.8 mmol/l (range 2.3-17.6). In those with an elevated blood urea the abnormality was corrected by rehydration.

Haematology (Table II)

(a) *Malarial Parasites*: Thick and thin blood films from 15 patients were examined for malarial parasites with negative results.

(b) *Electrocardiogram*: ECG was recorded in 9 cases. Apart from sinus tachycardia no abnormalities were noted.

(c) *Cerebrospinal fluid*: CSF was examined in 35 patients. Ziehl-Neelsen and Gram stains were negative. In 34% the initial CSF was normal but in 45% there was a modest lymphocytosis and in 37% an elevated protein. In the 14 patients where a simultaneous blood and CSF sugar was measured the mean blood — CSF difference was 1.6 mmol/l (range 0.7-2.6). In four cases a traumatic puncture obscured the results.

(d) *Arbovirus Serology*: Serum from a few patients was tested for antibodies to Dengue I, Tembusu, West Nile, Kunjin and Chikungunya viruses with negative results. In 25 cases serum was tested for antibody to Japanese Encephalitis Virus (JEV). In six only acute specimens were tested due

to early death, all were negative. Of the 19 paired sets 11 were reported as negative. Eight cases showed rising antibody titres against JEV, six of which were personal cases from the 1982 outbreak. The results are shown in Table III.

PHASE II — Inpatient analysis data from Biratnagar Hospital

Accurate data was available for patients admitted with acute encephalitis since 1975. A total of 673 cases were recorded. The sex ratio showed a slight male preponderance of 1.5:1. The mortality was 34%. Follow-up data of survivors was not available. The overall trend revealed an increasing annual incidence since 1975 (Fig. 3).

The monthly incidence showed occasional cases occurred throughout the year with a very large increase in admissions in the early phase of the summer monsoon (Fig. 4).

The age specific attack rate showed a marked preponderance of school-aged children (Fig. 5).

Examination of the records of the small Nepali hospital in Dharan showed a pattern of incidence very similar to that of BMH.

Table II Haematological Parameters on Admission			
Parameter	Number Tested	Mean	Range
Haemoglobin gm/dl	36	12.2	4.0-17.1
WBC X 10 ⁹ /l	37	14.6	5.4-45
Percentage neutrophils	30	78	49-95
ESR	19	29	8-56

Table III Japanese Encephalitis Antibody Titre			
1	1978	Negative	1:160*
2	1978	Negative	1:80
3	1982	<1:10	1:20
4	1982	1:40	1:80
5	1982	1:20	1:80
6	1982	1:10	1:40
7	1982	1:10	1:40
8	1982	1:10	1:160

*Specific JEV IgM

Fig. 3 Annual incidence of cases of acute encephalitis admitted to Biratnagar Hospital

Fig. 4 Monthly incidence of acute encephalitis

Fig. 5 Age specific incidence of acute encephalitis

PHASE III. Animal Serology

Serum from a variety of animals from the Dharan Cantonment farm was examined by Haemagglutination Inhibition (HAI) for antibody to JEV and West Nile Virus (WNV). The latter acted as a non-specific marker of flavivirus activity, to exclude the possibility of the JEV antibody merely representing a general flavivirus response rather than a specific JEV response. The results are shown in Table 4.

Serum from five adult buffaloes also showed JEV antibody up to a titre in 1:80. Interestingly none of the pigs or buffaloes tested was negative. Five ducks and six chickens from the large population of farm birds were tested with negative results.

Table IV
Flavivirus Serology in Cantonment pigs

Pig	JEV Titre	WN Titre
A1	640	40
A2	640	<20
A3	1280	80
A4	640	80
A5	640	80
B1	160	20
B2	320	40
B3	320	40
B4	160	40
B5	320	<20
C1	160	80
C2	320	80
C3	160	80
C4	120	<20
C5	160	40
C6	160	<20

JEV—Japanese Encephalitis Virus.

WN—West Nile Virus. A—Pigs four years old.

B—Pigs 11 months old. C—Pigs six months old.

PHASE IV. Healthy Human Serology

Twenty UK military personnel, 10 newcomers and 10 residents of at least 12 months standing, were tested for JEV antibody by HAI. All were negative.

Twenty Nepalese adults working on the Cantonment were similarly tested. 55% showed low titres of JEV antibody (titre of 10-80) indicating previous (remote) inapparent infection with JEV.

Discussion

There can no longer be any doubt that a serious threat from flavivirus encephalitis exists in the Terai region of southern Nepal. The Koshi Zone which includes the area around the British Military Cantonment at Dharan is one of the most severely affected areas⁴. Sporadic encephalitis, known to the local people as the "visitation of Goddess of the forest," has probably always been a feature of life in the Terai but in the last decade the disease appears to have taken on an epidemic form, not only in Nepal^{3, 4} but in nearby areas of India³ and West Bengal^{1, 2}. In addition examination of hospital records from Biratnagar on the southern fringe of the Koshi Zone and from Dharan to the north has revealed an alarming annual increase in reported cases of acute encephalitis. Small numbers of cases are reported throughout the year with a sharp increase occurring in relation to the summer monsoon. Interestingly the epidemics occur earlier in the southern Terai (Biratnagar) where 61% of the cases are reported between May and July compared with the northern Terai (Dharan) where 74% of cases occur between August and October.

The strongly seasonal character of the epidemics favour an arbovirus aetiology. Early clues as to the nature of the casual agent came from West Bengal and India^{1, 2, 3, 4} where Japanese Encephalitis Virus (JEV) was identified as the cause of summer encephalitis. In 1978 JEV was identified on serological grounds as cause of summer encephalitis in Nepal with confirmation in following years^{3, 4}. The ecological substrate for JEV transmission is ideally met in the Terai. There are vast expanses of rice paddy so favoured by the prime vectors *Culex tritaeniorhynchus* and *Culex vishnui*^{2, 5, 6, 7} which are the dominant species⁴. The main amplifying hosts, pigs, buffaloes, cattle, wild and domestic birds which are essential to JEV transmission abound.

The JEV cycles involves mosquito vectors and animals with man being involved incidentally. In most JEV endemic areas pigs seem to be main amplifying host^{8, 9, 10, 11}. Newly born pigs lose their maternal antibody by 6 months¹² and become fully susceptible to JEV. In areas of high transmission all susceptible pigs are infected within 12 months¹³. Once infected the young pig develops an intense

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essential during the months of May to November when the risk is highest. It is however unsafe to assume visits at other times are without risk as *Culex tritaeniorrhynchus* is active at a low level throughout the year³⁵ and cases of encephalitis do occur in the cool months.

3. *The farm.* A potential pool of JEV exists on the cantonment farm where an interface of susceptible people, mosquitoes and amplifying hosts exists. The domestic fowl probably act as amplifying hosts^{15,18} and could be removed at little economic cost. Certainly the keeping of fowl in the residential quarters should be discouraged. The adult pigs and buffaloes are immune to viraemia and thus not only of no risk to man but by acting as an immunological brake of JEV transmission may be protective^{14,15}. Young pigs however greatly amplify the virus pool and represent a threat. It has been shown that pigs immunised against JEV are much less likely to produce a mosquito-infecting viraemia when challenged with JEV¹². Consideration should be given to active immunisation of young pigs against JEV.

4. *Human Immunisation.* Unfortunately anti-mosquito measures are never fully effective. The Dharan Cantonment is surrounded by ideal mosquito breeding terrain where amplifying animal hosts abound. The flight range of the prime vector *Culex tritaeniorrhynchus* of at least 1800m³⁶ implies that the Cantonment mosquito population can be constantly reinforced by potentially infected mosquitoes flying in from outside, so local anti-mosquito measures are unlikely to abolish the risk. This raises the question of active immunisation. Serological testing of 20 British Military personnel living on the Dharan Cantonment showed all to be fully susceptible to JEV. This contrasts with the locally employed Nepalese civilians 55% of whom had JEV antibody in their serum. The risk to British soldiers and their families is therefore great, not only in the context of susceptibility but in the light of a 10-20 fold increase in the encephalitis rate compared with local people^{13,31,32,33}. A killed mouse brain (BIKEN) vaccine which employs the Nakayama-NIH strain of JEV exists. In its present form over 82 million doses have been issued since 1966³⁷. It appears to be extremely safe^{37,38,39} and effective^{38,40} at least against Japanese JEV. Unfortunately there is no certainty that the wild Nepalese JEV is of that strain. Indeed evidence from Thailand³⁷ and India^{38,41} suggests that local strains of JEV exist and that although the vaccine may offer some protection, it appears to be less effective against local strains than the Japanese strain^{37,38}. However until other vaccines become available it would

seem sensible to offer Biken vaccine to British Military personnel and families living in or visiting Nepal.

The introduction of mass immunisation by inducing serum antibodies might cloud the serological diagnosis of JE in vaccinees. Both immunisation and wild infection with JEV (apparent and inapparent) induce serum antibodies, but it has recently been shown in Thailand that only wild infection producing clinical encephalitis induces antibody formation in CSF. The antibody titres are tiny but are quite diagnostic even at an early stage of the disease using an enzyme linked immunosorbent assay technique⁴². This new technique therefore offers a rapid diagnosis of JE even in people previously vaccinated.

The current research needs to be extended to evaluate the herd immunity of British Gurkha soldiers serving in Nepal and to attempt virus isolation either from patients or mosquitoes, not only to confirm identification of JEV but to test the protective effect of neutralising antibody raised by BIKEN vaccine against the wild virus.

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