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Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002

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Summary An atypical outbreak of West Nile virus (WNV) occurred in Ngorban County, South Kordophan, Sudan, from May to August 2002. We investigated the epidemic and conducted a case–control study in the village of Limon. Blood samples were obtained for cases and controls. Patients with obvious sequelae underwent cerebrospinal fluid (CSF) sampling as well. We used enzyme-linked immunosorbent assay (ELISA) and neutralization tests for laboratory diagnosis and identified 31 cases with encephalitis, four of whom died. Median age was 36 months. Bivariate analysis did not reveal any significant association with the risk factors investigated. Laboratory analysis confirmed presence of IgM antibodies caused by WNV in eight of 13 cases, indicative of recent viral infection. The unique aspects of the WNW outbreak in Sudan, i.e. disease occurrence solely among children and the clinical domination of encephalitis, involving severe neurological sequelae, demonstrate the continuing evolution of WNV virulence. The spread of such a virus to other countries or continents cannot be excluded.

keywords encephalitis, epidemic, Nuba Mountains, Sudan, West Nile virus

Introduction

West Nile virus (WNV), first isolated in 1937 in Uganda, is part of the family of Flaviviridae, genus *Flavivirus* (Campbell *et al.* 2002). The virus belongs to the Japanese encephalitis serocomplex, which includes other viruses causing human encephalitis, such as Japanese encephalitis, Murray Valley encephalitis and St Louis encephalitis. Two lineages of WN strains have been identified: viruses from lineage I caused the main recent epidemics with human encephalitis and are distributed worldwide (Africa, India, Europe, Asia, the Middle East, North America), while viruses from lineage II have been identified only in Sub-Saharan Africa (McLean *et al.* 2002). The virus is maintained in a cycle of wild birds and mosquitoes, especially *Culex* species. Humans are infected through mosquito bites (Berthet *et al.* 1997).

The disease's clinical presentation has changed since the mid-1990s (Campbell *et al.* 2002). Earlier, WNV epidemics were mainly, although not exclusively, characterized by cases of fever, accompanied by rash and lymphadenopathy (Marberg *et al.* 1956; Hayes 1989). In contrast, during recent epidemics in Romania 1996 (Tsai *et al.* 1998), Russia 1999 (Platonov *et al.* 2001), the United States 1999 (Anonymous (MMWR) 1999) and Israel 2000 (Chowers *et al.* 2001; Weinberger *et al.* 2001), encephalitis has dominated the clinical presentation while rash or lymphadenopathy have rarely been observed. Change in the virus' virulence, population age structure or background immunity may contribute to this transformation (Hubálek 2001; Petersen & Marfin 2002). Although it has been purported that all ages are equally susceptible to infection with WNV, elderly persons (over 60 years) are at greater risk of neurological complications and death (Campbell *et al.* 2002; Solomon & Vaughn 2002).

The Nuba Mountains are situated in central-southern Sudan. The area is rural, remote, and has no motorized transport. The population consists primarily of subsistence farmers but people may own various farm animals in their living compound. Some cattle camps exist in the region. The medical non-governmental organization Médecins Sans Frontières (MSF) has been based in Limon, Ngorban County, since February 2002, to ensure permanent medical support and emergency preparedness. Measles is endemic in the region and according to MSF data, an epidemic peaking in May and June 2002 took place at about 1 week walking distance west from Limon (Western Jebels). The national polio immunization days were held in May, June and July 2002 with an estimated coverage of 72%, 99%, and 95% respectively (A Henekamp, MSF country manager, personal communication). A meningitis epidemic commenced in January 2002 and peaked in March and April. Patients were successfully treated with oily chloramphenicol intramuscular

(IM). The first cases of clinical encephalitis were reported at the tail end of the meningitis epidemic.

Methods

Description of the outbreak

A retrospective field investigation of the encephalitis cases in Ngorban County in the Nuba Mountains took place 3 weeks after the last case was identified. Data were collected through extensive in-depth interviews with the medical team in Limon, and comprehensive revisions of available patient medical files. For patients with missing information or lacking written documentation on the medical history, we relied on the recollection of the medical team. We recorded age, village of origin, date of onset of symptoms, date of admission and disease outcome of all patients. The caretakers of 11 previously identified cases were extensively interviewed on the patient's clinical history and related environmental factors. Each of these patients underwent a thorough clinical examination. Based on this compiled information, a 'probable case' was defined as any person present in Ngorban County, who had an episode of fever, prolonged fits and loss of consciousness since 1 May 2002. We also drew a spot map of all known cases as well as an epidemic curve of the outbreak. Attack rates were calculated using population estimates per village, based on population figures of the 2000 polio vaccination campaign (Elnayer 2000).

Case–control study

To better understand the epidemic we conducted a case–control study. Due to long distances between houses and villages, the mountainous terrain, and the limited time available, it was restricted to the village of Limon (2-h walking radius from the centre of the village), where the majority of cases resided. The study enrolled all cases from Limon, as identified from the health centre's records, and using the case definition described above. For each case, two age-group matched (≤12 years) controls were randomly selected from the population of Limon. Information on medical history, nutritional habits, exposure to animals and clinically ill children since the beginning of the rainy season (corresponding to 1 May), was obtained via questionnaires applied to the head of the household, at home or at the health centre.

Laboratory analysis

Duplicate samples of venous blood and cerebrospinal fluid (CSF) were taken from cases with neurological sequelae residing in Limon, and from one convalescent patient. For all other convalescent cases, and for the controls included in the case–control study, only blood samples were taken. Samples were taken after the child's caretaker had given written informed consent. All samples were frozen at −70 °C for viral analysis.

We did enzyme-linked immunosorbent assays (ELISA) to detect IgM and IgG antibodies in serum and CSF samples (IgM antibodies are responsible for the early immune response and indicate an early infection, whereas IgG antibodies correspond to the secondary immune response: Murgue *et al.* 2001). The analysis included flaviviruses West Nile, Dengue (four serotypes) and Japanese Encephalitis, and alphavirus O'Nyong Nyong. To confirm the results, Plaque Reduction Neutralization Tests ($PRNT_{80}$) were performed with serial twofold dilutions of serum samples starting at 1:20, using Vero cells and the France 2000 WN strain (Murgue *et al.* 2001). $PRNT_{80}$ titres of 80 and above strongly suggest WNV infection.

Statistical analysis

Proportions were compared using chi-square test (Fisher exact test for expected frequencies below 5) and medians using Mann–Whitney test. To examine the risk associated with exposures, we calculated bivariate odds ratios (OR) with 95% confidence intervals (CI) (Epi Info 6.04, CDC Atlanta, GA, USA).

Results

Clinical presentation of cases

Based on the 11 patient interviews, it was concluded the disease started with a sudden onset of high fever, followed by convulsions within 24–72 h. Convulsions were several to many times a day, and mostly generalized. They lasted for several days, up to weeks, but seemed to diminish in frequency and severity over time, tending to become more focalized to one part of the body (e.g. eye, hand, leg). Loss of consciousness or lack of responsiveness in between convulsions was reported. No haemorrhagic features were noted. A history of rash was recorded for two patients. Severe neurological sequelae such as agitation, spastic contractions or paralysis, limited or no response to pain, as well as leg and neck stiffness were observed. The combination of aggressive antibiotic therapy (chloramphenicol, ampicillin and/or gentamycin) and steroids led to clinical improvement in only two of the nine patients for whom the information was available. No recovery was observed with antimalarial treatment.

Description of the outbreak

A total of 31 patients were withheld as probable cases. All occurred in Ngorban County between 23 May and 6 August 2002, and involved children between 6 months and 12 years of age (median: 36 months); 80.6% (25 of 31) were 5 years or younger. The sex ratio (M/F) was 1.1 (17 of 14). The attack rate was 1.8 per 1000 population under 15 years, higher among children aged 5 years or younger (3.3 *vs.* 0.7, $P < 0.001$). Cases came from 10 villages up to 8 h walk away from Limon. The highest attack rate was found in the village of Limon (6.0 per 1000 population <15 years), followed by Saraf Jamus (3.4 per 1000 population <15 years) and Tangal (2.4 per 1000 population <15 years). The remaining villages had attack rates between 1.2 and 0.5 per 1000 population <15 years. Four patients (12.9%) died, seven cases (22.6%) recovered, six (19.3%) retained slight, and nine (29.0%), severe neurological sequelae. For five patients (16.1%), the final outcome was unknown. The epidemic curve showed a first peak in the week of 20 May, a second during the week of 17 June, and a third in the week of 29 July (Figure 1). The first and second peak correspond to cases mainly from the western part of the County (Saraf Jamus, and Tangal, Um Cerdiba and Kurchu), whereas the third peak is caused by the cases in Limon.

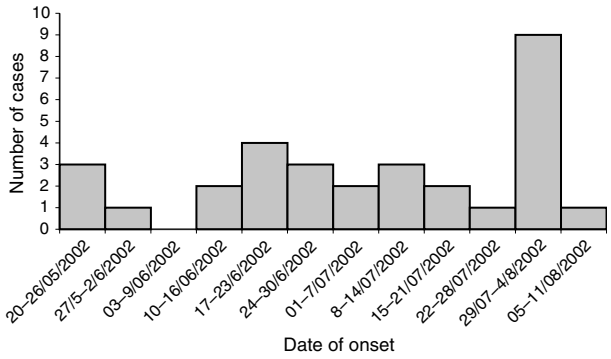

Figure 1 New encephalitis cases per week and per date of onset, Nuba Mountains, Sudan, 2002.

Case–control study

Ten cases and 19 controls were enrolled in the case–control study. The proportion of males was 40.0% for the cases (four of 10) and 63.1% for the controls (12 of 19) ($P = 0.42$). Cases and controls had a median age of 18 (range: 6–84) and 36 months (range: 6–60) respectively ($P = 0.12$). A history of fever and fits was confirmed for all cases, but for four the disordered consciousness could not be validated and they were considered suspected cases. All controls that stated having been sick since the beginning of the rainy season ($n = 17$) confirmed a history of fever, but none reported fits or loss of consciousness.

All cases and all controls had been vaccinated for polio during the last mass vaccination campaign. Cases and controls did not differ either by history of measles vaccination or meningitis in the past year, or contact with sick children. Contact with sick persons was not identified as a potential risk factor for encephalitis. No significant association could be shown for any of the other factors investigated (Table 1).

Table 1 History of personal contact, nutritional intake and contact with animals

	All cases (%) ($n = 10$)	Probable cases (%) ($n = 6$)	Controls (%) ($n = 19$)	All cases (OR)	Probable cases (OR)
Know child who had meningitis	2 (20.0)	1 (16.7)	3* (16.7)	1.2 (0.1–12.7)	1.0 (0.0–17.1)
Know a 'case'	1 (10.0)	1 (16.7)	2 (10.5)	0.9 (0.0–17.1)	1.7 (0.0–35.6)
Know child who had diarrhoea or vomiting or cough	1 (10.0)	0 (0.0)	6 (31.6)	0.2 (0.0–2.8)	0.0 (0.0–3.0)
Breastfeeding	6 (60.0)	4 (66.7)	7 (36.8)	2.6 (0.4–17.1)	3.4 (0.4–38.0)
Intake of goat milk	3 (30.0)	1 (16.7)	5 (26.3)	1.2 (0.2–8.8)	0.6 (0.0–8.0)
Intake of cow milk	1 (10.0)	0 (0.0)	3 (15.8)	0.6 (0.0–8.6)	0.0 (0.0–8.7)
Intake of pork meat	8 (80.0)	4 (66.7)	13 (68.4)	1.8 (0.2–17.6)	0.9 (0.1–10.1)
Intake of chicken meat	6 (60.0)	5 (83.3)	18 (94.7)	0.1 (0.0–1.1)	0.3 (0.0–12.8)
Intake of goat meat	4 (40.0)	3 (50.0)	13 (68.4)	0.3 (0.0–2.0)	0.5 (0.0–4.2)
Intake of cow meat	2 (20.0)	2 (33.3)	10 (52.6)	0.2 (0.0–1.7)	0.4 (0.0–4.2)
Contact with goats	6 (60.0)	4 (66.7)	8 (42.1)	2.1 (0.3–13.3)	2.7 (0.3–29.8)
Contact with sheep	1† (11.1)	0‡ (0.0)	1 (5.3)	2.2 (0.0–98.9)	0.0 (0.0–81.2)
Contact with pigs	4† (44.4)	3‡ (60.0)	4 (21.0)	3.0 (0.4–24.3)	5.6 (0.5–80.5)
Contact with chicken	8 (80.0)	5 (83.3)	17 (89.5)	0.5 (0.0–6.0)	0.6 (0.0–20.8)

* $n = 18$ (1 missing value); † $n = 9$ (1 missing value); ‡ $n = 5$ (1 missing value).

Laboratory results

Blood samples of 30 children were examined: eight were cases with neurological sequelae, five were convalescent and 17 were controls. Samples of CSF were taken for seven cases with neurological sequelae and for one convalescent. ELISA results for Dengue serotypes 1, 2, 3 and 4, and Japanese Encephalitis were negative, or slightly positive. For the cases, positive results were found only for WNV (Table 2). Seven of the eight children (87.5%) with neurological sequelae were positive for blood IgM and IgG of a flavivirus of the group West Nile; the CSF was IgM-positive for six of seven cases with neurological sequelae. As for the convalescent cases, one blood sample showed signs of recent infection, and all others were negative for WNV, while the one CSF sample available showed inconclusive results (borderline IgG and IgM) (Table 2). Neutralization tests were done for six patients with sequelae, for whom WNV $PRNT_{80}$ titres ranged from 80 to >640. For the one recovered case, who had WNV IgM but no IgG antibodies, a $PRNT_{80}$ titre of 40 was found.

Thirteen of 17 controls (76.5%) were negative for WNV infection, three (17.6%) showed signs of an old infection which was confirmed by $PRNT_{80}$ (titres 40, 160 and 320). One patient (5.9%) showed signs of a recent infection with a flavivirus of the group West Nile and had a $PRNT_{80}$ titre of 80. IgG antibodies against the alphavirus O'Nyong Nyong were detected in six controls (35.3%), but none of the cases.

Discussion

A WNV outbreak occurred in Ngorban County, in the Nuba Mountains of Sudan. The outbreak involved 31 children aged up to 12 years and occurred during the national polio immunization days. The epidemiological curve showed three peaks: the magnitude of the first two peaks might be underestimated due to distances from the health centre; the third peak might be overestimated because of sensitization of the medical personnel.

A relationship between the epidemic and the concurrent polio vaccination campaign was unlikely, due to the rare occurrence of post-vaccination incidents (one case/1.4–4.1 million OPV doses administered), and the characteristic clinical presentation of post-vaccination incidents (i.e. acute-onset flaccid paralysis), which was absent in this subgroup of the population (Kohler *et al.* 2002). The case–control study suggested that the outbreak was no consequence or continuation of the meningitis epidemic, which subsided in June. Neither a history of meningitis, nor previous contacts with a meningitis case were found to represent a risk for encephalitis. This finding corresponded to the clear distinction observed in the clinical presentation of cases, as well as in their lack of response to antibiotic treatment. The case–control study permitted to consider as unlikely any person-to-person type of transmission, including measles. No other conclusions could be drawn from the case–control study, as the number of participants was small, and OR had wide CI.

The laboratory analysis, showing WNV IgM antibodies in serum and CSF, and neutralizing antibodies in serum, identified WNV as the most probable cause of this outbreak. However, the close antigenic relation of the Japanese encephalitis serocomplex viruses causes important serological cross-reactions (Hubalék & Halouzka 1999), possibly complicating diagnosis. Although the serological results of the outbreak in the Nuba Mountains were compelling, no irrefutable method such as virus isolation was attempted. Recently in the United States, WNV genome was detected in a CSF sample whereas serology was negative (Huang *et al.* 2002).

The WNV antibodies were documented as early as 1956 in children aged up to 14 years in the same region of the Nuba Mountains: In 23% in Kadugli, 68% in Um Dorein and 29% in Talodi (Taylor *et al.* 1956). Data for older ages were not reported. More recently, WNV antibodies occurred in Northern Province (Watts *et al.* 1994) and Khartoum (McCarthy *et al.* 1996). However, no epidemics have been reported for the area, and information on the mosquito species present in the Nuba Mountains was not available.

Generally, in endemic countries children usually present mild symptoms when infected with WNV, whereas a large proportion of the adult population has neutralizing antibodies (Murgue *et al.* 2002). During the latest epidemics, neurological complications were predominantly observed in the elderly (>60 years). The clinical presentation of WN virus infection observed in the Nuba outbreak corresponds to the evolution of previous epidemics, with a progressive increase of neurological involvement and encephalitis, although neurological sequelae have not been described (Hubálek 2001; Petersen & Marfin 2002). However, the outbreak in the Nuba Mountains, to our knowledge, only concerned children, the majority of whom was under the age of 5. Similar findings have been observed in Algeria in 1994, where serology was undergone for 18 clinically ill patients; 13 were children between 10 months and 9 years and one was an adolescent of 14 years, all positive for WNV (Le Guenno *et al.* 1996). In India, WNV was isolated from the brains of three children who had died of encephalitis, originating from two districts within the same state, 20 months apart (March 1980 and November/December 1981) (George *et al.* 1984). In contrast, in other recent outbreaks, children with encephalitis were absent or

Table 2 Presence of WNV IgG and IgM antibodies in frozen blood and CSF samples of cases

ID	Sex	Age	Onset of symptoms	Sample	WN IgG	WN IgM	WN $PRNT_{80}$	JE IgG	JE IgM	DEN1 IgG	DEN1 IgM	DEN2 IgG	DEN2 IgM	DEN3 IgG	DEN3 IgM	DEN4 IgG	DEN4 IgM	ONN IgG	ONN IgM	Result
Cases with neurological sequelae																				
1	F	2 years	21/05	Blood	0.62	0.09	160	0.31	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.18	0.00	0.00	0.00	Possible RI WN
2	M	2 years	23/05	Blood	0.37	0.52	>640	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ND	RI WN
				CSF	0.10	0.11		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	
3	M	3 years	23/06	Blood	0.09	0.17	80	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	RI WN
4	F	2 years	24/06	Blood	0.72	0.11	320	0.43	0.00	0.00	0.00	0.00	0.00	0.08	0.05	0.29	0.00	0.00	0.00	RI WN
				CSF	0.30	0.11		ND	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	
5	F	2 years	01/07	Blood	0.76	1.37	ND	0.41	0.41	0.00	0.07	0.00	0.16	0.00	0.32	0.22	0.20	0.00	0.00	RI WN
				CSF	0.35	1.08		ND	ND	0.00	0.08	0.00	0.16	0.00	0.28	0.06	0.12	0.00	0.00	
6	M	7 years	14/07	Blood	0.91	0.28	>640	0.36	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00	RI WN
				CSF	0.43	0.42		ND	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	
7	M	3 years	04/08	Blood	0.02	0.33	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	RI WN
				CSF	0.00	1.32		ND	ND	0.00	0.00	0.00	0.07	0.00	0.13	0.00	0.12	ND	ND	
8	F	8 months	04/08	Blood	0.08	0.49	640	0.06	0.07	0.00	0.05	0.00	0.08	0.00	0.12	0.00	0.15	0.00	0.00	RI WN
				CSF	0.01	0.20		ND	ND	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.07	0.00	0.00	
Convalescent cases																				
9	*F*	*6 months*	*30/07*	*Blood*	*0.00*	*0.01*	*ND*	*ND*	*ND*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*ND*	*0.00*	*N*
10	*M*	*9 months*	*31/07*	*Blood*	*0.00*	*0.04*	*ND*	*ND*	*ND*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*N*
11	M	18 months	01/08	Blood	0.00	0.02	ND	ND	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Inconcl.
				CSF	0.10	0.08	ND	ND	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
12	*M*	*9 years*	*02/08*	*Blood*	*0.00*	*0.00*	*ND*	*ND*	*ND*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*N*
13	*F*	*2 years*	*02/08*	*Blood*	*0.00*	*0.55*	*40*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*RI WN*

For the cases in italic, the presence of disordered consciousness was not validated, and were therefore considered as 'suspected' cases. Sample on filter paper:
RI WN, recent infection with West Nile; N, negative; ND, not done; PRNT, plaque reduction neutralization test; JE, Japanese encephalitis; DEN, dengue; ONN, O'Nyong Nyong.

exceptional: during the 1996 outbreak in Bucharest (Romania), no children under 9 years of age were reported among 393 patients with confirmed WNV infection (Tsai *et al.* 1998); during the 1999 outbreak in Volgograd (Russia), <15% of the patients were children under 15 years, whereas more than 50% of all cases were 50 years or older (Platonov *et al.* 2001). In the USA, in 1999, among the 78 hospitalized patients with confirmed WNV infection, there was only one child of 5 years and one of 15 years old (Horga & Fine 2001). Of the 26 patients with neurological involvement reported in a regional hospital during the outbreak in Israel in 2000, five were children, all between 9 and 15 years (Klein *et al.* 2002).

No vaccine exists to prevent WNV infection in humans. Vector control and health education on how to prevent mosquito bites are the only preventive methods available. Whereas WNV epidemics used to be sporadic and with rare severe implications, the number of outbreaks reported has exploded since the 1990s. The recent strains seem more virulent with greater epidemic potential and spread to new regions (Campbell *et al.* 2002; Gubler 2002). As history has shown, new virus strains might be transported from one region to another (Solomon & Cardosa 2000; Petersen & Roehrig 2001). Whether this happens via migratory birds, bird trade or international travel is not clear (McGuigan 2002). An extended and internationally coordinated surveillance system would help to better understand the epidemiology and pathogenicity of West Nile virus, and to contain the risk for these new epidemics.

Conclusion

The West Nile virus outbreak in the Nuba Mountains affected only children and was characterized by serious neurological involvement, both demonstrating the evolution of the virulence of WNV. The rapid change of viruses increasingly challenges our current knowledge about known diseases, as well as our preparedness for new emerging diseases. It is not unlikely that similar outbreaks in developing countries have passed unnoticed, due to lack of investigative and diagnostic capacities. Recent events have illustrated the ease of worldwide spread of such viruses, and should serve as a lesson demanding an organized response, increasing the efforts put on surveillance and investigation, as well as monitoring possible spread to other continents.

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