

Recombinant vaccine-derived poliovirus in Madagascar.

Dominique Rousset, Mala Rakoto-Andrianarivelo, Richter Razafindratsimandresy, Bakolalao Randriamanalina, Sophie Guillot, Jean Balanant, Philippe Maucière, Francis Delpeyroux

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TsCysti was exclusively endemic (2). The contaminated areas in Irian Jaya have increased from the central area (Paniai), to the east (Jayawijaya) (3), and then to the west (Manokwari), where 54 TsCysti cases have been reported (Papua Province Health Office Services, 1997, unpub. data). We wanted to know if taeniasis/cysticercosis had been introduced into the eastern half of New Guinea Island, called Papua New Guinea (PNG) (9). We had already serologically confirmed that 16 (3.0%) of 541 local residents and Irianese refugees in Alice River villages along the border in PNG had asymptomatic TsCysti (Ito et al., unpub. data). Follow-up surveys will be crucial in several other districts including Merauke District in Irian Jaya, PNG, and other islands such as Timor Island, where most of the population is Christian and many suspected cases have recently been reported by the District Health Office Services (10). Schoolchildren should also be checked so that cases can be detected and treated early. Sustainable education of the local community in Irian Jaya, Indonesia, and Papua New Guinea is also necessary.

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**Toni Wandra,* Akira Ito,†
Hiroshi Yamasaki,†
Thomas Suroso,*
and Sri S. Margono‡**

*Ministry of Health, Jakarta, Indonesia;
†Asahikawa Medical College, Asahikawa, Japan; and ‡University of Indonesia, Jakarta, Indonesia

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Address for correspondence: Akira Ito, Department of Parasitology, Asahikawa Medical College, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan; fax: +81-(0)166-68-2429; email: akiraito@asahikawa-med.ac.jp

Recombinant Vaccine-Derived Poliovirus in Madagascar

To the Editor: Between October 2001 and April 2002, five cases of acute flaccid paralysis associated with vaccine-derived poliovirus (VDPV) type 2 isolates were reported in the southern province of the Republic of Madagascar. The first patient, an 11-year-old child from the urban district of Toliara, first experienced paralysis on October 29, 2001. Three other chil-

dren, 6, 9, and 14 months of age from Ebakika village, in a rural district of Taolagnaro (250 miles east of Toliara), showed signs of poliomyelitis between March 21 and March 26, 2002. The last case-patient, a 20-month-old child from Ambanihazo village (6 miles north of Ebakika), came into contact with one of the three case-patients in Ebakika in March 2002, and symptoms developed on April 12, 2002 (1). None of the patients had been fully vaccinated against poliomyelitis.

Nine type 2 poliovirus (PV) strains were isolated. A restriction fragment

length polymorphism (RFLP) assay, with three different genomic regions amplified by reverse transcription-polymerase chain reaction (RT-PCR) and four different restriction enzymes (*HinfI*, *DpnII*, *RsaI*, and *DdeI*) were used to characterize the PV isolates at the molecular level (2). The RFLP profiles of all of the isolates in the two capsid protein regions were identical to that of the type 2 strain of the oral polio vaccine (OPV) in the VP1-2A region (nucleotides 2,872 to 3,647) but slightly different in the VP3-VP1 region (nucleotides 1,915 to 2,883). The observed differences allowed us

to distinguish two groups (isolates from Toliara and isolates from Taolagnaro) and two subgroups (isolates from March and isolates from April). The RFLP profiles of isolates in the noncapsid region, at the 3'-terminal end of the genome (polymerase 3D and 3' noncoding regions: nucleotides 6,535 to 7,439) also confirmed the presence of two separate groups. These last profiles were completely different from those of the three reference vaccine strains, suggesting recombination with other enteroviruses.

Partial genomic sequencing confirmed these observations. The entire VP1 region (903 nucleotides) of the type 2 PV strains from Toliara and Taolagnaro differed from the type 2 OPV strain by 1% and 2.5% nucleotides, respectively. This difference may indicate that the two strains had been multiplying or circulating for approximately 1 and 2.5 years, respectively. Taolagnaro strains are closely related to each other (<1% nucleotide difference) but appear to be very different from Toliara strains (2.9% nucleotide difference), indicating the existence of two genetic lineages. The sequencing of the noncapsid

region (440 nucleotides corresponding to nucleotide positions 6,705 to 7,144 of the Sabin 2 genome) confirms the existence of two lineages derived from different recombination events with two nonidentified enteroviruses of the phylogenetic cluster C. This cluster, based on sequence similarity, includes some coxsackieviruses and all PV strains (3).

We tried to identify the donor strains for sequences in the 3' terminal end of these recombinant strains by aligning the nonidentified sequences with homologous enterovirus sequences available in a nucleotide sequence database (FASTA, version 3.3 applied to GenBank) (4). The highest percentages of nucleotide sequence identity were those with PVs and with most other cluster C enteroviruses available in the database (87% to 91% nucleotide identities). No wild PV strains have been isolated in Madagascar since 1997 despite surveillance and investigation of viral causes of acute flaccid paralysis cases (5). Thus, that the detected VDPVs were the product of recombination between OPV strains and two nonpolio enteroviruses is more likely than that they were the product of

OPV strains and two different undetected wild PV strains. However, we cannot exclude the possibility that wild PVs were imported or circulating silently for a while.

In response to the outbreak, the local health authorities conducted house-to-house vaccination with OPV. Further field investigations were carried out to determine the extent to which VDPV had spread and to search actively for other cases. Data analysis is in progress.

As with the other epidemics in Egypt and Hispaniola, VDPV circulated in a province of Madagascar with low OPV coverage (6,7). Because a high OPV coverage rate helps prevent the circulation of both VDPVs and wild PVs, obtaining and maintaining high rates of immunization coverage are essential (8). Moreover, two recombinant VDPV lineages in Madagascar indicate that recombination is frequent between OPV and cluster C enteroviruses. Similar recombinant VDPVs have been implicated in the epidemics in Hispaniola and in the Philippines (6,9). Determining whether the neurovirulence and transmissibility of these VDPVs could be the result of

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the recombination with nonpolio enteroviruses is important. These VDPVs have major implications for the cessation of immunization with OPV after certification that wild PV has been eradicated.

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**Dominique Rousset,*
Mala Rakoto-Andrianarivelo,*
Richter Razafindratsimandresy,*
Bakolalao Randriamanalina,†
Sophie Guillot,‡ Jean Balanant,‡
Philippe Maucière,*
and Francis Delpeyroux‡**

*Institut Pasteur de Madagascar, Antananarivo, Republic of Madagascar; †Ministry of Health, Antananarivo, Republic of Madagascar; and ‡Institut Pasteur, Paris, France

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Address for correspondence: Mala Rakoto-Andrianarivelo, Unité de Virologie, Institut Pasteur de Madagascar, BP 1274, Antananarivo 101, Madagascar; fax: (261.20) 22.415.34; email: mala@pasteur.mg

West Nile Virus Infection in Crocodiles

To the Editor: Recently West Nile virus (WNV) infection has been reported in three alligators (*Alligator* sp.) from central Florida (1) and one captive crocodile monitor (*Varanus salvadori*) with neurologic signs from the District of Columbia and Maryland area (2). These first reports of the virus in American reptiles highlight the possible role of this group of vertebrates in the WNV life cycle. To our knowledge, WNV in a reptile was reported only once before in a serosurvey conducted in Israel from 1965 to 1966, in which 22 reptiles and 96 amphibians were tested for hemagglutination-inhibiting antibodies against several viruses, including WNV; one turtle (*Clemmys caspica*) was seropositive (3). Experimental infection of the lake frog (*Rana ridibunda*) with a Russian strain of WNV resulted in high levels of viremia (4). At

present, the role of reptiles and amphibians in the life cycle and epidemiology of WNV is not known.

We report, for the first time, WNV infection in crocodiles (*Crocodylus niloticus*). To assess the potential role of crocodiles in the life cycle of WNV in Israel, serum specimens were collected from 20 healthy crocodiles on a commercial farm in the Negev Desert, in southern Israel (31°14'N, 34°19'E). The crocodiles came from two separate breeding farms (32°03'N, 35°26'E and 30°18'N, 35°07'E) in the Syrian-African Rift Valley, which is on the main route of bird migration from Africa to Europe. Five males and 15 females, 1–2.5 years of age, were examined. Blood was withdrawn from the crocodiles' ventral caudal vein, separated by centrifugation, and kept at –20°C until analyzed. Neutralizing antibody titers were determined against WN-goose-98 (5) and attempts to isolate the virus were performed by using Vero cell culture (6) and by using direct reverse transcription–polymerase chain reaction (RT-

PCR) on the serum specimens. To eliminate the possibilities of nonspecific reaction, all serum samples were concurrently tested for the only other flavivirus known to be present in Israel; Israeli turkey meningoencephalitis virus (ITV) (7). Because ITV does not produce cytopathic effects (CPE) in Vero cells, virus neutralization was conducted on BHK cells for both WNV and ITV by using WN-goose-98 and ITV (vaccine strain). In this case, the virus stocks ($10^{-4.2}$ 50% tissue culture infective dose) were diluted 1:400, and virus neutralization titers were checked 3 days later.

Viral RNA was extracted from serum samples with the QIAamp RNA blood kit (QIAGEN, Valencia, CA), according to the manufacturer's protocol and resuspended in 30 µl of RNase-free water. The primer pair WN240-Kun848 (respective genome positions 5': 848 and 1,645) was used to synthesize an 800-bp product in the E gene region (8,9). The resulting DNA fragment was visualized on