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1 Article – **R2b**

2 **Chikungunya virus-associated encephalitis: a cohort study on La Réunion**  
3 **island, 2005-2009**

4

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9 Dr Borgherini reports no disclosure.

10 Dr Staikowksy reports no disclosure.

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## 1 **Abstract**

2 **Objectives:** To estimate the cumulative incidence rate (CIR) of Chikungunya virus (CHIKV)-  
3 associated Central Nervous System (CNS) disease during the La Réunion outbreak, and  
4 assess the disease burden and patient outcome after three years.

5 **Methods:** CHIKV-associated CNS disease was characterized retrospectively in a cohort of  
6 patients with positive CHIKV RT-PCR or anti-CHIKV IgM antibodies in the cerebrospinal  
7 fluid and fulfilling International Encephalitis Consortium criteria for encephalitis or  
8 encephalopathy. Neurological *sequelae* were assessed after three years.

9 **Results:** Between September 2005 and June 2006, 57 patients were diagnosed with CHIKV-  
10 associated CNS disease, including 24 with CHIKV-associated encephalitis, the latter  
11 corresponding to a CIR of 8.6 per 100,000 persons. Patients with encephalitis were observed  
12 at both extremes of age categories. CIR per 100,000 persons were 187 and 37 in patients  
13 below 1 year and over 65 years, respectively, both far superior to those of cumulated causes  
14 of encephalitis in the USA in these age categories. The case fatality rate of CHIKV-associated  
15 encephalitis was 16.6% and the proportion of children discharged with persistent disabilities  
16 estimated between 30% and 45%. Beyond the neonatal period, the clinical presentation and  
17 outcomes were less severe in infants than in adults.

18 **Conclusions:** In the context of a large outbreak, CHIKV is a significant cause of CNS  
19 disease. As with other etiologies, CHIKV-associated encephalitis case distribution by age  
20 follows a U-shaped parabolic curve.

21

22 **Keywords:** Chikungunya virus; case fatality rate; cohort studies; disease burden; encephalitis;  
23 encephalopathy; incidence studies; neurological sequelae; prognosis; viral infections.

24

# 1 **Introduction**

2 Chikungunya virus (CHIKV) is a re-emerging alphavirus.<sup>1</sup> Alphaviruses are divided into  
3 “arthritogenic” viruses (Old World) and “encephalitogenic” viruses (New World) including  
4 equine encephalitis viruses.<sup>2</sup>

5 Until its reemergence in the Indian Ocean in 2004 and the worldwide spread that followed,  
6 beyond the burden of arthritis, known for lasting weeks to years,<sup>3</sup> Chikungunya was  
7 considered as a non-fatal disease with spontaneous resolution, not causing lifelong  
8 disabilities, even though rare cases of central nervous system (CNS) disease had been  
9 reported.<sup>4,5</sup>

10 The major outbreaks that have occurred since 2005 in the Indian Ocean islands were  
11 attributable to a new “Indian Ocean lineage” (IOL) that evolved from the East Central South  
12 African (ECSA) lineage and selected the mutation E1-A226V, which favors transmission by  
13 *Aedes Albopictus*.<sup>6,7</sup> Recently, Asian lineage CHIKV emerged in the Caribbean and expanded  
14 to the Americas, and recent clinical and experimental data indicate differences in the  
15 pathogenicity between both Asian and lineages.<sup>8-10</sup>

16 The 2005-2006 epidemic on La Réunion Island, affected 300,000 persons and enabled the  
17 observation of severe forms of the disease.<sup>11</sup> These included rare severe or fatal cases with  
18 CNS involvement, both in adults and neonates.<sup>12-16</sup>

19 Here, we report the results of an ambispective cohort study aimed at characterizing  
20 clinical and biological features of CHIKV-associated encephalitis, disease burden and three-  
21 year neurologic outcome of patients with this condition.

22

## 1 **Methods**

2 We conducted this study in the Groupe Hospitalier Sud Réunion, the largest hospital of the  
3 four general hospitals on the island, which covers a population of 277,602 inhabitants.<sup>17</sup>

### 4 ***Retrospective cohort study***

5 We considered all patients hospitalized between the 1<sup>st</sup> of September 2005 and the 30<sup>th</sup> of  
6 June 2006 with CHIKV infection and neurological symptoms that led to perform a lumbar  
7 puncture (LP) eligible to the study. Patients with positive cerebrospinal fluid (CSF) for  
8 CHIKV RNA or anti-CHIKV IgM antibodies were further studied.

9 Anti-CHIKV IgM assay in the CSF was performed by ELISA using the ETIMAX 3000 ®  
10 (Diasorin, Italy). A one-step TaqMan real-time quantitative RT-PCR was performed from  
11 CSF samples using the Light Cycler 2.0 system® (Roche Diagnostics).

### 12 ***Ethical requirement***

13 Each patient provided oral consent for the use of clinical, biological and imaging data, in  
14 accordance with the recommendations of the local Committee for Clinical Research.<sup>13</sup>

### 15 ***Case definition***

16 We used positive CSF findings (CHIKV RNA or IgM) to provide the more specific case  
17 definition. Additionally, we used IEC (International Encephalitis Consortium) criteria to  
18 classify our patients according to an up-to-date definition of encephalitis.<sup>18</sup> These combine the  
19 major criterion “altered mental status” (defined as decreased or altered level of consciousness,  
20 lethargy or personality change lasting  $\geq 24$  hours with no alternative cause identified), with a  
21 set of minor criteria: (i) fever ( $\geq 38$  °C) within the 72 hours before or after presentation, (ii)  
22 general or partial seizures not fully attributable to an epilepsy, (iii) new onset of focal  
23 neurologic signs, (iv) CSF white blood cells (WBC) count  $\geq 5/\text{mm}^3$ , (v) brain parenchyma on  
24 neuroimaging suggestive of encephalitis either new from prior studies or appears acute in

1 onset, (vi) electroencephalography consistent with encephalitis and not attributable to another  
2 cause.

3 Exclusion criteria were the main causes of encephalopathy and of non-infectious  
4 encephalitis, listed as follows: positive HIV status, pyogenic meningitis, thrombophlebitis,  
5 brain abscess, empyema, cerebral malaria, acute disseminated encephalomyelitis, voltage-gate  
6 potassium channels, N-methyl-D-aspartate receptor antibodies, systemic vasculitis, multiple  
7 sclerosis, or paraneoplastic-related encephalitis, prion disease, encephalopathy of primary  
8 tumor, hematological, toxic or metabolic origin.<sup>19</sup>

9 Thus, we defined probable CHIKV-associated encephalitis in presence of the major  
10 criterion and at least three minor criteria, possible CHIKV-associated encephalitis in presence  
11 of the major criterion and two minor criteria,<sup>18</sup> and “non-encephalitic CHIKV-associated CNS  
12 disease” (NECACD) in the presence major criterion alone or with one minor criterion, or in  
13 the presence of two minor criteria other than fever.

#### 14 ***Prospective follow-up study***

15 We followed-up each patient with CHIKV CNS disease to search for neurological *sequelae*  
16 over a three-year period using the framework of the extended Glasgow Outcome Scale (adult  
17 and pediatric versions).<sup>20</sup> For children, trained psychometrists assured neuropsychological  
18 evaluation using the revised neurodevelopmental scale of Brunet-Lézine, a standardized  
19 psychometric test routinely used in francophone countries. For adults, neurologists performed  
20 clinical and electro-encephalogram (EEG) examinations. CT or MRI scans were performed on  
21 clinical indication.

#### 22 ***Statistical analysis***

23 We compared characteristics of CHIKV-associated encephalitis and non-encephalitic CHIKV  
24 associated CNS disease globally and between adults and children using chi-2 or Fisher exact  
25 test for proportions, as appropriate. Distributions were compared using Mann-Whitney or



1 Kruskal-Wallis tests. We tested correlations between CSF and serum viral loads in children,  
2 or between CSF and serum IgM levels in adults, were tested using Spearman's correlation  
3 coefficients.

4 We provided cumulative incidence rates (CIR) for CHIKV-associated encephalitis  
5 (probable, possible or both), overall and by age groups, applying weights for sub-population  
6 structure using data from the 2006 census. We then compared these estimates to US  
7 standards.<sup>21</sup>

8 We assessed the range of neurological *sequelae* in applying the same actual rate or null for  
9 the missing observations, this assumption being likely conservative, given the very low  
10 probability of lost-to-follow-up due to death or *sequelae* in the insular population of the study.

11 We used Stata (v10.0®, StataCorp. 2008, Texas, USA) for comparisons. Statistical  
12 significance was set at  $P=0.05$ .

13

# 1 **Results**

## 2 **Retrospective cohort study**

### 3 **Characteristics of acutely infected CHIKV patients with neurological symptoms**

4 Among the 129 CHIKV-infected patients with CNS disease, biological analysis of the CSF  
5 was positive for 55 CHIKV RNA or 30 anti-CHIKV IgM, in 78 patients and negative in 51  
6 patients (Figure 1).

7 We excluded nine patients because they exhibited additional conditions, which invalidated  
8 CHIKV as a unique cause for the neurological symptoms. Briefly, these consisted in  
9 encephalopathy of primary metabolic origin (n=3), alcohol-related encephalopathy (n=3),  
10 posterior reversible encephalopathy syndrome in systemic lupus erythematosus (n=1),  
11 *Streptococcus pneumoniae* meningitis (n=1), and neurocysticercosis (n=1). The 69 remaining  
12 patients showed at least one IEC encephalitis criteria but 12 of them were excluded because  
13 incomplete charts did not allow definite classification. Thus, a total of 57 patients diagnosed  
14 with CHIKV-associated CNS disease were enrolled in the study. Among them, 24 (42.1%)  
15 patients with altered mental status matched IEC encephalitis definition, whereas 33 (57.9%)  
16 others did not and were designated as NECACD in further analysis.

17 We identified six confirmed cases of CHIKV-associated encephalitis (*i.e.*, altered mental  
18 status plus at least three minor criteria), while 18 patients were classified as possible cases  
19 (*i.e.*, two minor criteria). Both groups shared the same clinical and biological profiles, which  
20 confirmed the appropriateness of the IEC classification that allow these groups to be pooled  
21 for case registration (data not shown).

22 As expected, CHIKV encephalitic cases were more likely to exhibit severe CNS disease,  
23 than cases of NECACD, which consisted almost exclusively in mild to moderate behavioral  
24 changes (Table 1). Two indicators of CSF inflammation, WBC count and protein level, were  
25 higher in patients with encephalitis than encephalopathy, but viral loads or IgM titers in serum

1 or in CSF were not significantly different between these groups of patients (Table 2).  
2 Importantly, encephalitic cases required more intensive care support than NECACD cases  
3 (Table 3).

4 The cohort contained 21 adults (mean age, 63.9 years; SD, 15.6 years; range, 33-88 years)  
5 and 36 infants (mean age, 1.6 month; SD, 1.15 month; range, 4 days - 5.4 months).

6 Five infants were in the early neonatal period (< 7 days) and four in the late neonatal  
7 period (7 to 28 days), corresponding to cases of mother-to-child and post-natal mosquito-  
8 borne transmission, respectively.

9 Infants were more likely to experience a recent onset of fever prior to hospitalization,  
10 behavioral changes, skin rash, or survival and adults were more likely to experience decreased  
11 consciousness, coma, focal neurologic signs, seizures, or a fatal issue (Table e-1). Protein,  
12 glucose and chloride CSF levels were higher in adults than infants (Table e-2). CHIKV loads  
13 in the CSF or serum were higher in infants than adults, whereas it was the opposite for IgM.  
14 These results are in line with the fact that adults were observed later than infants in the course  
15 of the CHIKV-associated CNS disease. CHIKV loads in the CSF and serum for infants and  
16 between IgM levels in the CSF and serum from adults positively correlated (data not shown).  
17 In infants, CHIKV loads in serum negatively correlated with age, while in adults IgM levels  
18 in serum positively correlated with age (data not shown).

19 Except for one neonate exhibiting cerebral edema MRI features, no early (< 7 days)  
20 diffusion-weighted (DWI) MRI scan was available for CHIKV-associated encephalitic cases,  
21 although DWI is increasingly recognized as the most sensitive technique for timely diagnosis  
22 of acute brain parenchyma inflammation.<sup>22</sup> Subsequently, no radiological image evocative of  
23 acute stage of CHIKV-associated encephalitis was observed among the 22 other patients  
24 submitted to brain CT scans, late MRI scans, or both.

## 25 **Cumulative incidence rates of CHIKV-associated encephalitis**

1 The overall CIR estimate of CHIKV-associated encephalitis was 8.6 per 100,000 persons  
2 (95% CI: 6.9–10.4). Importantly, the age distribution pattern of CHIKV-associated CNS  
3 disease (Figure 2a) or CHIKV-associated encephalitis incidence (Figure 2b) exhibited an U-  
4 shaped parabolic pattern with a very clear trend to the highest incidence towards the youngest  
5 age than the oldest.

### 6 **Prospective cohort study**

7 Six adult patients died (mean, 67.5 years; SD, 15.7 years; range, 41-83 years) during  
8 hospitalization (case fatality rate [CFR]: 10.5%). Detailed cause-specific mortality was as  
9 follows: cardiac failure (n=2), septic shock (n=2), respiratory failure (n=1), sudden death  
10 (n=1). Death certificates mentioned Chikungunya as the primary cause for degradation in  
11 each case. As a consequence, 51 patients were eligible to the follow-up study.

12 Eight adults were discharged with neurological *sequelae* and are presented in Table e3.  
13 One deceased three months after discharge (case n°6). He was a 72-year old male individual  
14 free from past medical history presenting altered mental status, classified as NECACD. He  
15 deteriorated gradually towards dementia and deceased in a clinical picture of metabolic  
16 encephalopathy due to dehydration and hypernatremia. EEG revealed a global slowdown  
17 without spike. Sub-acute stage CT scans showed extensive demyelination and cerebral  
18 subcortical atrophy. Four adult survivors were lost in follow-up and the ten others were  
19 assessed clinically at three-year. Of these, we diagnosed three patients with neurological  
20 *sequelae* (epilepsy, post-infectious dementia, cognitive disorder, respectively) and four with  
21 an absence of detectable *sequelae*.

22 Nineteen infants were lost-to-follow up, and 17 evaluated at an average of 38 months of  
23 age. One developed severe cerebral palsy and blindness. He was a full-term normal-for-  
24 gestational age boy free of obstetrical history presenting with hemorrhagic fever on day 4 of  
25 life (case n°2, Table e3). Sub-acute and late stage MRI findings evidenced progressive

1 decrease of cerebral and cerebellar hemorrhages and replacement of brain edema features by  
2 subsequent demyelination of the white matter, whose evolution contrasted with monophasic  
3 or multiphasic patterns of acute disseminated encephalomyelitis (ADEM). Four infants  
4 exhibited poor neurodevelopmental performances (Brunet-Lézine development quotients  
5 [DQ]  $\leq$  85), irrespective of prenatal alcohol exposure, the other eight had age-related  
6 appropriate skills (mean DQ: 98, SD: 9, range: 86-120). Of these five children, two were  
7 infected vertically and three in the post-neonatal period (day 17, day 35 and day 73,  
8 respectively) (Table e3). The medical history of the lost-to-follow-up infants was uneventful,  
9 except for one who developed Langherhans histiocytosis.

10 Given the high attrition in the follow-up and the risk for information bias, the burden of  
11 neurological *sequelae* resulting from CHIKV-associated CNS disease could not be precisely  
12 calculated and was estimated to be in the range of 17.6% (9/51) to 43.1% (22/51).  
13 Nevertheless, lost-to-follow-up children corresponded to the milder forms of CNS disease so  
14 that our estimates are likely conservative owing to the fact that the incidence of *sequelae*  
15 often correlates with the intensity of the acute stage of infection in a previously healthy  
16 population. For CHIKV-associated encephalitis, the CFR was 16.6% (4/24) and the three-year  
17 burden of neurological *sequelae* in the range of 30% (6/20) to 45% (9/20). Importantly, we  
18 observed an age difference in 3-year outcome of CHIKV-associated encephalitis, poor  
19 prognosis (*i.e.*, death or *sequelae*) being predominant in adults (52.6% vs 18.2%,  $P=0.020$ ).

20

## 1 **Discussion**

2 To our knowledge, this study reports unique findings on CHIKV-associated CNS disease  
3 (encephalitis and encephalopathy) using both CSF examination findings and IEC criteria for  
4 encephalitis. Importantly, our data reveal that during the 2005-2006 CHIKV outbreak in La  
5 Réunion island, the incidence of CHIKV-associated encephalitis contributed to a twofold  
6 increase of the regional overall incidence (14.6 *versus* 6.0 cases per 100,000 persons per year  
7 at baseline) of all encephalitis. Remarkably, this burden far exceeds the annual rate of  
8 encephalitis calculated for mainland France in 2000-2002 about encephalitis of infectious or  
9 specified etiology,<sup>23</sup> as well as the rate reported in the USA between 1998 and 2010 for all  
10 encephalitis.<sup>21</sup> Of note, the CIR of CHIKV-associated encephalitis in La Réunion island was  
11 also superior to those observed with West Nile virus (WNV) and other neuroinvasive  
12 arboviral infections in the USA between 1999 and 2007,<sup>24</sup> or to the global incidence observed  
13 with Japanese encephalitis.<sup>25</sup>

14 Though no similar study has been previously reported to our knowledge, our findings are  
15 consistent with earlier report of CNS conditions complicating CHIKV infection, ranging from  
16 mild neurocognitive or behavioral disorders to severe neurological syndromes including acute  
17 stage encephalopathy/encephalitis, post-infective ADEM (*e.g.*, encephalomyeloradiculitis) and  
18 post-infective Guillain-Barré syndrome (*e.g.*, polyradiculoneuritis).<sup>14-17</sup> They are also in  
19 agreement with earlier observational studies, even though the criteria used to define  
20 encephalitis differ from those we used.<sup>5,26-28</sup> CHIKV-associated CNS disease prognosis seems  
21 similar to that of other viral etiologies. It was associated in our setting with more pejorative  
22 figures than previously reported in India,<sup>26,27</sup> or even recently in Thailand.<sup>28</sup> This substantial  
23 toll is compatible with that of other virus-associated encephalitis.<sup>29-31</sup>

24 Interestingly, the age distribution of encephalitis incidence was U-shaped with two peaks  
25 observed in young infants and elder adults, consistent with the distribution of encephalitis in

1 general populations,<sup>21,29,30</sup> or with overall neuroinvasive disease of viral origin reported in the  
2 USA.<sup>21</sup> The CIR of CHIKV-associated encephalitis in these extreme age groups is 25-fold  
3 higher for children under 1 year and 6-fold higher for people over 65 years than those found  
4 for encephalitis of specified etiology in the USA.<sup>21</sup> This also contrasts the bimodal age  
5 distribution of Herpes Simplex virus encephalitis which peaks between 60 and 64 years,<sup>32</sup> that  
6 of WNV, which have a greater effect on the elderly,<sup>24,33</sup> or that of La Crosse virus, which  
7 targets rather the young children.<sup>24,34</sup> Higher susceptibility of children to CHIKV-associated  
8 encephalitis is also supported by the cerebral edema features observed in a neonate infected  
9 by mother-to-child transmission. Accordingly, young mice are more susceptible to CHIKV  
10 than adult mice.<sup>35</sup> CHIKV targets the choroid plexuses, meningeal and ependymal envelopes,  
11 but does not invade brain parenchyma of adult mice deficient for type-1 interferon and adult  
12 monkeys,<sup>35,36</sup> despite the presence of viral RNA or infectious virus in the CSF of animals  
13 during the acute phase of infection, whereas CHIKV infects neurons of neonatal/suckling  
14 mice.<sup>37</sup> A defective host response may contribute to the higher susceptibility of neonates to  
15 CHIKV, as suggested by studies showing that the neonatal immune response is quantitatively  
16 and qualitatively distinct from that of adults.<sup>38</sup> Thus, in contrast to New World alphaviruses  
17 that cause encephalitis in humans and in animal models as a consequence of viral invasion of  
18 the brain parenchyma,<sup>39</sup> CHIKV is not a neurotropic virus in experimentally infected adult  
19 animals, although it disseminates and replicates in the meningeal and ependymal envelopes.<sup>35</sup>

20 In contrast to what observed in adults, CSF and serum of infants contained CHIKV RNA,  
21 which is likely explained by their earlier presentation to hospital in our cohort. Moreover, the  
22 higher the CHIKV load was in the serum, the higher it was in the CSF, and we made the same  
23 observation in the adult with CHIKV-specific IgM (CSF/serum ratios smaller than one).  
24 Therefore a passive diffusion of viral RNA or IgM from the serum to the CSF, either by

1 traumatic LP or as a result of a leakage in the blood-brain barrier, rather than CHIKV  
2 replication or IgM production in the CNS, cannot be excluded.

3 Importantly, although infants appear more susceptible to CHIKV-associated encephalitis,  
4 the clinical presentation and three-year outcome of CHIKV-associated encephalitis were more  
5 severe in adults than in infants, except for one neonate. As also observed in the CHIMERE  
6 cohort study,<sup>40</sup> they consisted exclusively in behavioral changes and neurocognitive  
7 impairment in infants, while they affected cortical functioning and led to disabling *sequelae* in  
8 adults. These data are in line with French national data and WNV encephalitis in the USA  
9 showing milder presentation and better outcomes in children.<sup>20,31,33</sup>

10 Our study has some limitations. First, we have not searched for CHIKV RNA and IgM  
11 systematically in patients presenting neurologic manifestations. Second, LP was not repeated  
12 in the absence of clinical deterioration. We may therefore have missed pathological changes  
13 in CSF protein level or WBC count, notably in neonates who are prone to pro-hemorrhagic  
14 conditions.<sup>14</sup> Third, we have not performed neuroradiologic examination routinely, so that  
15 patients with mild neurologic forms were probably underestimated, while in turn, cases of  
16 major CNS disease could be unstable to undergo timely MRI scans. Thus, as our study was  
17 not population-based, we may have underestimated the real burden and slightly overestimated  
18 the CFR and incidence of neurological *sequelae*. Fourth, the data collection was partially  
19 retrospective and we may have missed some minor symptoms, such as tremors or other  
20 movement disorders indicative of thalamic or basal ganglia involvement.<sup>22</sup> Thereby, we  
21 focused on the symptoms whose presence was constantly notified, so that our description of  
22 CHIKV-associated CNS disease is likely conservative and limit information bias. Fifth, our  
23 study was restricted to a fairly localized area so that we cannot rule out the extent of CHIKV-  
24 associated CNS disease in recent years may reflect a stronger neurovirulence of the ECSA  
25 sub-lineage. Encephalitis has not yet been described everywhere the ECSA genotype has



1 circulated. The occurrence of encephalitis may depend of the magnitude of the outbreak, by  
2 targeting susceptible hosts to CHIKV-associated CNS disease. The study of host and CHIKV  
3 genetic factors underlying CHIKV-associated CNS disease may help better understand the  
4 pathogenesis of CHIKV-associated CNS disease. In this regard, we have much to learn from  
5 current outbreaks throughout the world due to African and Asian lineages viruses.

6 We report here that CHIKV-associated CNS disease, including encephalitis as defined by  
7 the IEC, may complicate CHIKV infection. Altogether these data contribute to improve the  
8 knowledge of CHIKV-associated neuropathology and illustrates the clinical neurotropism of  
9 CHIKV and its deleterious consequences, especially in neonates.

10

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#### 14 **Footnote section**

15 *List of abbreviations.* ADEM: acute disseminated encephalomyelitis; CHIKV: Chikungunya virus;  
16 CFR: case fatality rate; CIR: cumulative incidence rate; CNS: central nervous system; CSF:  
17 cerebrospinal fluid; CT: computed tomography; DQ: development quotient; DWI: Diffusion-weighted  
18 imaging; EEG: electroencephalogram; ECSA: East Central South African; HIV: human  
19 immunodeficiency virus; IEC: international encephalitis consortium; IgM: immunoglobulin M; LP:  
20 lumbar puncture; MRI: magnetic resonance imaging; NECACD: non-encephalitic CHIKV-associated  
21 CNS disease; RNA: ribonucleic acid; RT-PCR: reverse transcriptase polymerase chain reaction; SD:  
22 standard deviation; WBC: white blood cell; WNV: West Nile virus.

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27 Conflicts of Interest.

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1 **Figure legends**

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3 **Figure 1. Study population of CHIKV-associated CNS disease, Réunion island, 2005-**  
4 **2006.**

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6 **Figure 2. CHIKV-associated CNS disease and encephalitis by age categories, Réunion**  
7 **island, 2005-2006.**

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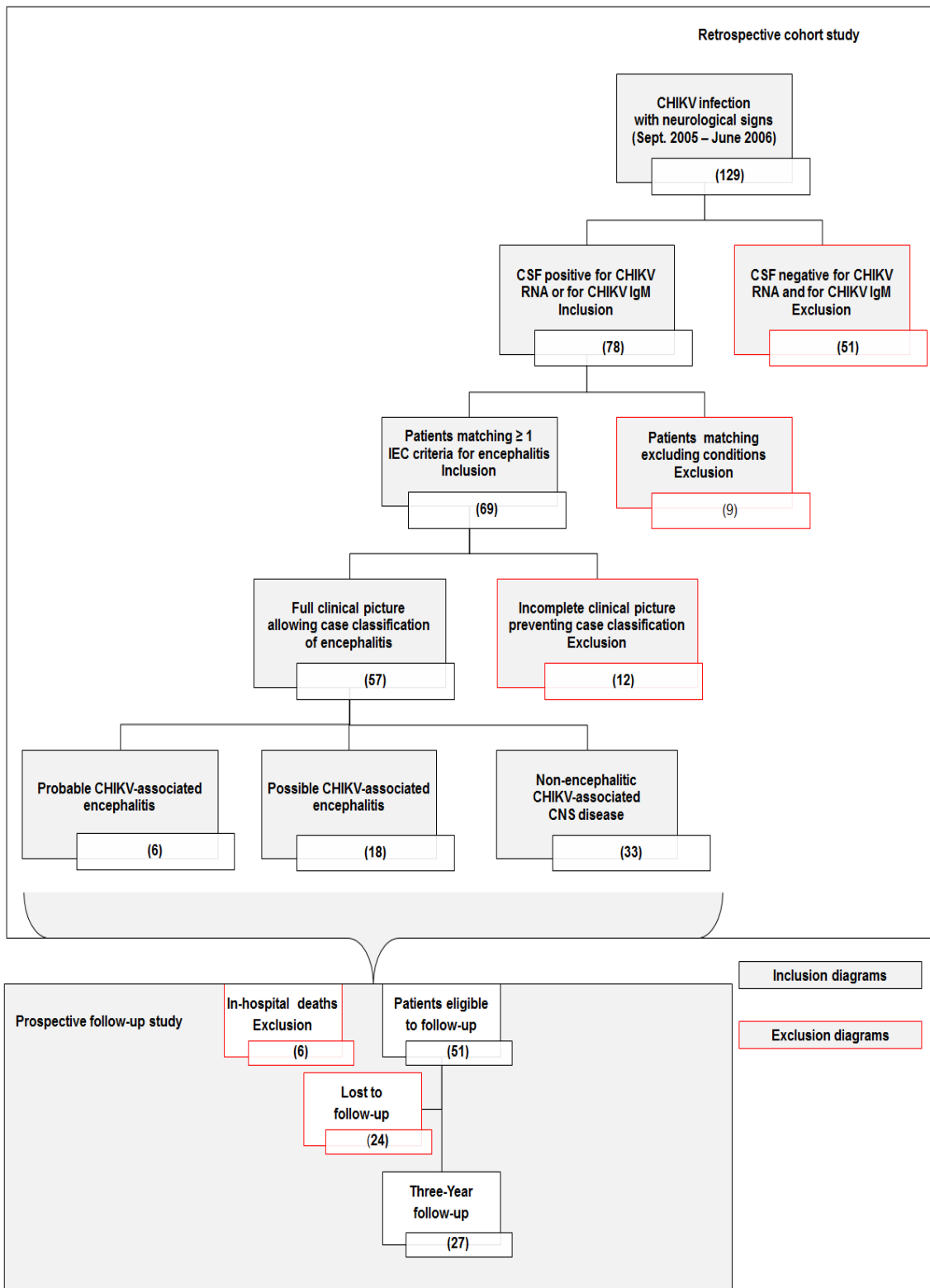
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1 **Figure 1**

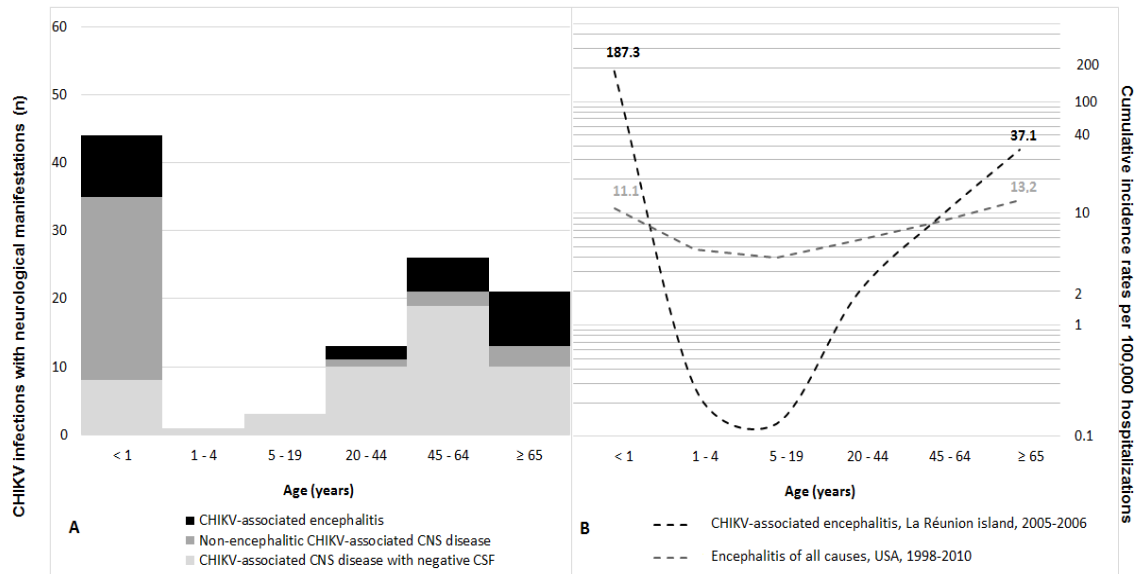


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3 The flow chart classifies the cases reports of CHIKV-associated CNS disease at inclusion and

4 at follow-up.

1 **Figure 2**



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3 Data are the total case reports of CHIKV-associated encephalitis, non-encephalitic CHIKV  
 4 CNS disease and CHIKV-associated CNS disease with negative CSF by age categories

5 (Figure 2a) or the age-stratified cumulative incidence rates of CHIKV-associated encephalitis  
 6 in La Réunion or the age-stratified cumulative incidence rates of all encephalitis in the USA

7 (Figure 2b)

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**Table 1. Clinical features of CHIKV-associated CNS disease in La Réunion island, 2005-2006**

Variables	Encephalitis <sup>#</sup>		NECACD <sup>##</sup>		P value
	N = 24		N = 33		
	n	(%)	n	(%)	
<b>Age group (years)</b>					0.003 <sup>¶</sup>
< 1	9	37.5	27	81.8	
1 – 19	0	0	0	0	
20 – 44	2	8.3	1	3.0	
45 – 64	4	16.7	1	3.0	
≥ 65	9	37.5	4	12.1	
<b>Female sex</b>	11	45.8	16	48.5	0.843
<b>History of fever (≥ 38°C)</b>	24	100	33	100	1
<b>Onset of fever ≤ 7 days</b>	12	60.0	18	85.7	0.063
<b>Altered mental status *</b>	24	100	33	100	1
<b>Decreased consciousness †</b>	8	33.3	2	6.1	0.012
<b>Coma ‡</b>	4	16.7	1	3.0	0.151
<b>General or partial seizures</b>	3	12.5	1	3.0	0.300
<b>Focal neurologic signs</b>	5	20.8	0	0	0.010
<b>Other behavioral changes **</b>	15	62.5	32	97.0	0.001
<b>Skin rash</b>	8	33.3	17	51.5	0.190
<b>Intensive care support</b>	10	41.7	3	9.1	0.009
<b>Length of stay &gt; 4 days</b>	14	58.3	13	39.4	0.187
<b>Deaths</b>	4	16.7	3	9.1	0.439

<sup>#</sup>Probable or possible. <sup>##</sup> Non-encephalitic CHIKV-associated CNS disease. Unless stated, the data were available for 24 encephalitic cases and 33 cases of NECACD. The onset of fever was available for 20 encephalitic cases and 21 cases of NECACD. <sup>†</sup> Glasgow coma score (GCS) < 15. <sup>‡</sup> GCS ≤ 9. \* decreased or altered level of consciousness, lethargy, or personality change (disorientation, agitation). \*\* attention disorders, memory troubles, excessive pain feeling (irritability). <sup>¶</sup> Fisher's exact overall P value testing the five age categories.



**Table 2. Biological parameters of CHIKV-associated CNS disease in La Réunion island, 2005-2006.**

Variables	Encephalitis <sup>#</sup>		NECACD <sup>##</sup>		P value
	N= 24		N = 33		
	n	%	n	%	
<b>CSF white blood cells <math>\geq 5 / \text{mm}^3</math></b>	17	70.8	4	12.1	< 0.001
<b>CSF proteins <math>\geq 40 \text{ mg / dl}</math></b>	21	91.3	16	50.0	0.001
	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>P value</b>
<b>CSF white blood cells (<math>\text{mm}^3</math>)</b>	12.8	21.9	1.7	2.7	< 0.001
<b>CSF proteins (mg/dl)</b>	75.3	39.4	47.2	23.7	< 0.001
<b>CSF glucose (mmol/l)</b>	4.2	1.3	3.7	1.0	0.086
<b>CSF chloride (mmol/l)</b>	123.3	4.3	121.1	5.8	0.039
<b>CSF CHIKV load (cp/ml)</b>	578,815	1,787,072	221,914	796,673	0.126
<b>Serum CHIKV load (cp/ml)</b>	$1.2 \times 10^8$	$2.2 \times 10^8$	$9.0 \times 10^7$	$1.7 \times 10^8$	0.330
<b>CSF anti-CHIKV IgM (ui/l)</b>	101.0	83.0	143.7	168.7	0.885
<b>Serum anti-CHIKV IgM (ui/l)</b>	139.3	115.6	57.3	92.9	0.219
<b>CSF/serum CHIKV loads ratio <sup>†</sup></b>	0.63	0.14	0.55	0.20	0.055
<b>CSF/serum IgM levels ratio <sup>‡</sup></b>	0.41	0.33	0.96	0.57	0.069

<sup>#</sup>Probable or possible. <sup>##</sup> Non-encephalitic CHIKV-associated CNS disease. CSF: cerebrospinal fluid. CSF proteins were available for 23 encephalitic cases and 32 cases of NECACD. CHIKV loads were measured in the CSF available for 52 patients (36 infants and 16 adults). CSF of all infants, of whom 9 are encephalitic, and CSF of 4 encephalitis adults are positive. Among the 12 adults with CSF negative, 9 are encephalitic. CHIKV loads were measured in the serum available for 37 patients (32 infants and 5 adults). Among infants, 31 are positive and 9 of them are encephalitic, while the 5 adults are negative. CHIKV IgM are found in the CSF of 21 patients on 52 (2 weakly positive infants with NECACD and 19 highly positive adults, of whom 10 encephalitis) and in serum of 32 patients on 37 (13 weakly positive infants of whom 6 encephalitis; 19 highly positive adults, of whom 10 encephalitis). <sup>†</sup> Data are complete for 9 infants with encephalitis, 22 infants with NECACD. <sup>‡</sup> Data are complete for 12 adults with encephalitis, 4 adults with NECACD.

**Table 3. Outcomes of CHIKV-associated CNS disease in La Réunion island, 2005-2009**

Variables	Encephalitis <sup>#</sup>		NECACD <sup>##</sup>		P value
	N = 24		N = 33		
	N	(%)	n	(%)	
<b>Intensive care support</b>	10	41.7	3	9.1	0.009
<b>Length of stay &gt; 4 days</b>	14	58.3	13	39.4	0.187
<b>Extended Glasgow Outcome Scale</b>					0.946 <sup>†</sup>
1. Dead	3	18.7	4	21.0	
2. Vegetative State	0	0	0	0	
3. Lower Severe Disability (Lower SD)	1	6.3	2	10.5	
4. Upper Severe Disability (Upper SD)	0	0	2	10.5	
5. Lower Moderate Disability (Lower MD)	1	6.3	1	5.3	
6. Upper Moderate Disability (Upper MD)	3	18.7	2	10.5	
7. Lower Good Recovery (Lower GR)	1	6.3	1	5.3	
8. Upper Good Recovery (Upper GR)	7	43.8	7	36.8	
Not assessed.	8		14		

<sup>#</sup>Probable or possible. <sup>##</sup>Non-encephalitic CHIKV-associated CNS disease. Unless stated, the data were available for 24 encephalitic cases and 33 NECACD cases. The Extended Glasgow Outcome Scale (GOSE) was assessed at discharge for non-survivors and at three years post infection for the survivors. We used both adult and pediatric versions of the GOSE. Percentages are calculated on a total of 35 patients. <sup>†</sup>Fisher's exact overall P value testing seven of the eight outcome categories.

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