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## Absence of vaccinia virus detection in a remote region of the Northern Amazon forests, 2005-2015

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► **To cite this version:**

Galileu Barbosa Costa, Anne Lavergne, Edith Darcissac, Vincent Lacoste, Betania Paiva Drumond, et al.. Absence of vaccinia virus detection in a remote region of the Northern Amazon forests, 2005-2015: Absence of vaccinia virus detection in French Guiana. Archives of Virology, 2017, 162 (8), pp.2369 - 2373. 10.1007/s00705-017-3353-x . pasteur-01585618

**HAL Id: pasteur-01585618**

**<https://riip.hal.science/pasteur-01585618>**

Submitted on 18 Sep 2017

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1 **Title: Absence of Vaccinia virus detection in a remote region from Northern Amazon forests,**  
2 **2005-2015**

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16

17 **Abstract**

18 *Vaccinia virus* (VACV) circulates in Brazil and other South America countries, being responsible  
19 for a zoonotic disease that usually affects dairy cattle and humans, causing economic losses and  
20 impacting animal and human health. Furthermore, VACV was already detected in wild areas from  
21 Brazilian Amazon. To better understand the natural history of VACV, we investigated its  
22 circulation in wildlife from French Guiana, a remote region in Northern Amazon forests. ELISA  
23 and plaque reduction neutralization test were performed to detect anti-*Orthopoxvirus* antibodies.  
24 Real-time and standard PCR targeting C11R, A56R and A26L were applied to detect VACV DNA  
25 in serum, saliva and tissue samples. VACV was not evidenced in any samples tested. These

26 findings provide additional information on the VACV epidemiological puzzle. The virus could  
27 nevertheless be circulating at low levels that were not detected in areas with no human or cattle  
28 presence.

29

30

31 *Vaccinia virus* (VACV) is the prototype of the *Orthopoxvirus* (OPV) genus, *Poxviridae*  
32 family, and was used as a live vaccine around the world to eradicate smallpox. VACV circulates in  
33 zoonotic cycles involving bovine herd and dairy workers in rural areas from Brazil, causing a  
34 disease named Bovine Vaccinia (BV) which accounts for several economical losses and increasing  
35 the public health concerns [1].

36 In the last seventeen years, VACV spread throughout all Brazilian territory [1], though its  
37 occurrence varies among different regions. Furthermore, VACV has been detected in countries  
38 bordering Brazil, such as Argentina [2], Uruguay [3], and Colombia [4]. Although since 1999,  
39 several BV outbreaks affecting mainly dairy cattle and humans have been reported [1], natural  
40 VACV circulation had already been described in a rodent of *Oryzomys* genus captured in the 60's in  
41 the Amazon region [5].

42 During these last years in which reports of BV outbreaks have been observed, VACV  
43 circulation has been described in the Amazon region, in mammal species such as bovines, primates,  
44 coatis, and also humans [6–12]. Nevertheless, there is little information on a possible VACV  
45 sylvatic cycle and on its natural reservoirs since the origin of VACV is still unknown and its natural  
46 reservoir is still unidentified [13–16]. Given that VACV has a broad host range and that wild  
47 animals might be implicated in its transmission chain, we decided to search for VACV circulation  
48 in wildlife animals (rodents, marsupials, and bats) captured in pristine forest areas with the absence  
49 or limited anthropogenic impacts of Northern Amazon forests.

50 We analyzed a total of 2,365 wild mammals from French Guiana, captured between 2005  
51 and 2015 in tropical upland rain forest, Amazon region [17, 18]. Captured sites were located in  
52 remote forests, slightly disturbed forests (facing selective logging, hunting pressure, and low  
53 fragmentation level), secondary forests and edge between forest and open habitats (Figure 1). Wild  
54 mammals included rodents (n = 420), marsupials (n = 606) and Chiroptera (n = 1,339) (Table 1).  
55 All samples underwent molecular testing for VACV. To detect VACV DNA we used real-time PCR  
56 targeting the C11R and A56R genes, and semi-nested PCR targeting C11R and A26L genes [19]. A  
57 molecular approach was performed in serum and tissue samples from rodents and marsupials, and  
58 in DNA extracted from saliva and blood clots of bats. The positive controls used on molecular assays  
59 were DNA extracted from serum and liver of experimental infected Balb/c mice with *Vaccinia virus* Western  
60 Reserve (VACV-WR) strain and Brazilian Vaccinia (Br-VACV) group 1 and 2 viruses [20]. For those for  
61 which we were not able to have an experimental infected animal (marsupials and bats), we provided  
62 an experimentally contamination of serum and liver samples as in the protocol previously described  
63 by Dutra and co-authors [21]. Briefly, it was added different concentrations of VACV-WR and Br-  
64 VACV strains (with a range from  $10^4$  pfu/ $\mu$ L to 1 pfu/ $\mu$ L, respectively). The experimentally  
65 contaminated samples were tested by PCR, compared to the Balb/c controls, and then used as  
66 positive controls for marsupials and bats.

67 In addition, serum samples from the rodents and marsupials were screened for anti-OPV IgG  
68 by ELISA and neutralizing antibodies by  $\geq 50\%$  plaque reduction neutralization test (PRNT<sub>50</sub>) [19].  
69 Sera were grouped into pools of 2–5 serum samples from animals belonging to the same species  
70 that were from the same collection area. No serum samples from bats were available for serological  
71 tests. For serological assays, the positive controls were also those provided by experimental infected  
72 mouse with VACV-WR and Br-VACV strains [20]. For the ELISA assay particularly, we used  
73 purified recombinant A/G protein peroxidase conjugated as secondary antibody. We run rodent and

74 marsupial samples together in the same plate (for ELISA and PRNT), comparing the results with  
75 known positive and negative rodents' serum samples.

76 Most of the rodents captured belong to the species *Proechimys guyannensis* (46,4%),  
77 followed by *Proechimys cuvieri* (11,2%) and *Zygodontomys brevicauda* (9,5%). The main  
78 marsupials studied were *Didelphis marsupialis* (34,1%) and *Marmosa murina* (26,2%). For bat  
79 species, most of them were *Desmodus rotundus* (32,3%), *Carollia perspicillata* (17,2%) and  
80 *Pteronotus* spp. (14,0%). All animals tested by both serological and molecular techniques were  
81 negative for VACV.

82 In this study, we assessed numerous forest mammal species and found no serological or  
83 molecular evidence for VACV circulation among them. Increasing efforts have been done regarding  
84 VACV ecology in South America, and some studies have highlighted the importance of wild  
85 animals and peridomestic rodents as VACV carriers [7, 22, 23, Miranda JB, personal  
86 communication/in peer review]. Our negative results contrast with the above-mentioned ones and  
87 some hypotheses can be considered in explaining the lack of evidence for VACV circulation in wild  
88 animals in French Guiana during the 2005-2015 period. One hypothesis is that wildlife was  
89 investigated in a highly preserved Northern Amazon region, where natural geographic barriers of  
90 unfavorable forest habitats exist, preventing VACV dissemination from Brazil to French Guiana.  
91 This could also be emphasized by the “dilution effect” hypothesis, taking into account that in a  
92 highly preserved environment, the maintenance of a highly diverse mammal community may  
93 prevent infection of putative more sensitive species. A great diversity of mammal species was  
94 studied, but with only a small sampling of species from which VACV has been detected in Brazil,  
95 such as *Mus musculus* [20], *Oryzomys sp* [5], and *Caluromys philander* [Miranda JB, personal  
96 communication/in peer review]. Furthermore, the species *Didelphis albiventris* from which VACV  
97 was recently detected in São Paulo State, Southeast region of Brazil [24], is not present in French

98 Guiana. Nevertheless, two closely related species *Didelphis imperfecta* (n = 2) and *D. marsupialis*  
99 (n = 207) were tested.

100 Another explanation for the absence of VACV detection in this study could be the extremely  
101 low number of bovines in French Guiana. One hypothesis for the broad circulation of VACV in  
102 Brazil is that bovines are acting as VACV amplifiers, excreting high loads of viable particles, being  
103 a source of infection for themselves and other animals [25]. VACV can be detected in animals  
104 without clinical disease since it has been detected in blood samples and feces in animals without  
105 lesions but presenting neutralizing antibodies [25]. Due to the potential role of cattle as a viral  
106 amplifier, the presence of bovines could facilitate VACV persistence and dissemination into the  
107 environment.

108 VACV has recently been identified at the borders of Brazil, in countries such as Argentina  
109 [2], Uruguay [3], and Colombia [4]. Even though the circumstances of VACV detection in South  
110 American countries are still uncertain, the cattle trade and translocation has to be considered, as  
111 highlighted in Amazon region (Mato Grosso and Rondônia states) [9]. Furthermore, some authors  
112 have hypothesized that the seroprevalence of OPV-antibodies in human populations from the  
113 Amazon basin (Acre state) could be linked to agriculture and livestock practices, reinforcing the  
114 role of cattle on VACV maintenance [8].

115 Compared to other South American countries where VACV has been detected, a different  
116 situation is observed in French Guiana where a lower deforestation rate is observed and where only  
117 a few cattle or horses are present [26] (<http://www.globalforestwatch.org/>) (Figure 1). Nevertheless,  
118 our findings do not definitively establish that VACV is not circulating, and also do not exclude the  
119 possibility that small mammals can act as its reservoir in nature. However, data presented here  
120 strongly suggest the absence of circulation of VACV, or at low levels that were not detected, in  
121 remote Amazonian areas with no human impact over a long period of time. The monitoring and

122 surveillance of VACV circulation is important to ensure early warning of zoonotic outbreaks for  
123 vulnerable human populations.

124

125

126 **Legend for figure:**

127 An overview of French Guiana with the location of the studied regions, where were implemented  
128 several trapping sites. The background of the map highlights the human footprint index expected to  
129 summarize the anthropic threats on biodiversity. These threats (accessibility, land use, human  
130 density) are summed and allowed producing an index with lower pressures strenght in light and  
131 higher pressures darker. For each studied region, the dominant habitat is given.

132

133 **Acknowledgements**

134 We thank colleagues from the Laboratoire des Interactions Virus-Hôtes, Institut Pasteur de la  
135 Guyane for their excellent technical support. We also thank Pró-Reitoria de Pesquisa from  
136 Universidade Federal de Minas Gerais (PRPq/UFMG), CAPES, FAPEMIG and CNPq. JS Abrahão,  
137 EG Kroon, and GS Trindade are researchers from CNPq.

138

139 **Funding**

140 This study was funded by two European projects, CAROLIA and RESERVOIRS, through ERDF  
141 funds and a European Commission "REGPOT-CT-2011-285837-STRonGer" grant within the FP7.  
142 This work also benefited from an "Investissements d'Avenir" grant managed by Agence Nationale  
143 de la Recherche, France (CEBA, ref. ANR-10-LABX-25-01).

144

145 **Conflict of interest**

146 Authors declare no conflict of interest.

147

148 **Ethical approval**

149 Captures of rodents, bats and marsupials do not require ethic committee in French Guiana.

150

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