Evidence against Zika virus infection of pets and peri-domestic animals in Latin America and Africa

Edmilson F. de Oliveira-Filho1, Ianei O. Carneiro2, Carlo Fischer1, Arne Kühne1, Ignacio Postigo-Hidalgo1, Jorge R. L. Ribas3, Peggy Schumann4, Kathrin Nowak3, Jan F. Gogarten6,7, Xavier de Lamballerie8, Filipe Dantas-Torres9, Eduardo Martins Netto2, Carlos Roberto Franke2, Emmanuel Couacy-Hymann10, Fabian H. Leendertz5,11 and Jan Felix Drexler1,12,13,*

Abstract

Decades after its discovery in East Africa, Zika virus (ZIKV) emerged in Brazil in 2013 and infected millions of people during intense urban transmission. Whether vertebrates other than humans are involved in ZIKV transmission cycles remained unclear. Here, we investigate the role of different animals as ZIKV reservoirs by testing 1723 sera of pets, peri-domestic animals and African non-human primates (NHP) sampled during 2013–2018 in Brazil and 2006–2016 in Côte d’Ivoire. Exhaustive neutralization testing substantiated co-circulation of multiple flaviviruses and failed to confirm ZIKV infection in pets or peri-domestic animals in Côte d’Ivoire (n = 259) and Brazil (n = 1416). In contrast, ZIKV seroprevalence was 22.2% (2/9, 95% CI, 2.8–60.1) in West African chimpanzees (Pan troglodytes verus) and 11.1% (1/9, 95% CI, 0.3–48.3) in king colobus (Colobus polycomos). Our results indicate that while NHP may represent ZIKV reservoirs in Africa, pets or peri-domestic animals likely do not play a role in ZIKV transmission cycles.

The Zika virus (ZIKV) was first isolated from a sentinel rhesus monkey (Macaca mulatta) in Uganda during the 1950s and later reported in native non-human primate (NHP) species and in forest-dwelling Aedes mosquitos in Africa and therefore transmission in parts of Africa is thought to be maintained through interepidemic sylvatic cycles [1, 2]. ZIKV was not considered a major threat to human health [3, 4] until its emergence in Latin America, which was associated with severe congenital malformations including microcephaly [5]. According to evolutionary reconstructions, ZIKV was likely introduced into Brazil during 2013 [5] and infected approximately 8.5 million people during 2015 and 2016 [6]. Community protective immunity in urban centers likely contributed to cease of the epidemic [7].

Sylvatic transmission cycles may permit ZIKV maintenance until the pool of susceptible humans is replenished by birth and migration, potentially contributing to a resurge of intense urban ZIKV transmission cycles. Whether animal reservoirs play a role in ZIKV transmission in the Americas remains unknown. The available data on ZIKV infection of NHP in the Americas are inconclusive. Some South American NHP species, such as marmosets (Callithrix jacchus) and tamarins (Saguinus labiatus) were susceptible to ZIKV in vivo [8]. Low seroprevalence and low levels of neutralizing antibodies have been reported in capuchin

Received 16 August 2021; Accepted 08 November 2021; Published 25 January 2022

Keywords: Zika virus; flavivirus; serology; animal reservoir; zoonoses; antibody.

Abbreviations: AU, antigenic unit; CI, confidence intervals; ECLIA, electrochemiluminescence immunoassay; NHP, non-human primates; PRNT, plaque reduction neutralization test.

A supplementary table is available with the online version of this article.

001709 © 2022 The Authors

This is an open-access article distributed under the terms of the Creative Commons Attribution License.
monkeys in Northeastern and Central Brazil [9, 10]. Finally, ZIKV has been detected in NHP carcasses in the Southeastern region, but since contamination of the remains could not be excluded, the relevance of those findings still needs to be confirmed [11].

Several flaviviruses can infect pets and peri-domestic animals. For instance, experimental infection and serological evidence indicate circulation of West Nile virus (WNV) and Japanese Encephalitis virus (JEV) in cats and dogs [12–15] and of Wesselsbron virus (WSLV) in cattle, goat and sheep [15–21]. Virus detection by molecular methods, virus isolation and serological evidence substantiate that horses can be infected with WNV, Saint Louis Encephalitis virus (SLEV), JEV and Usutu virus (USV) [22, 23]. Livestock species, such as sheep and goats, were susceptible to ZIKV in vivo [24, 25], and although ZIKV-specific neutralizing antibodies were reported in sero-epidemiological studies in cattle and sheep from Brazil and in horses from French Pacific Islands [26, 27], only few flaviviruses beyond ZIKV were used in those studies to rule out potentially unspecific test results elicited by cross-reactive flavivirus antibodies. Exposure of vertebrates to ZIKV depends on the feeding preferences of the mosquito vectors. In Latin America, ZIKV vectors include predominantly *Aedes aegypti* and, to a lesser extent, *Ae. albopictus* [2, 28, 29]. In Africa, the invertebrate host range of ZIKV is not entirely known. However, major vectors likely include *Aedes aegypti formosus*, *Ae. africanus*, *Ae. albopictus*, *Ae. apicoargenteus*, *Ae. furcifer* and *Ae. vitattus* [28]. Whereas *Ae. aegypti* and, to a lesser extent, *Ae. albopictus* are highly anthropophilic, they are also known to opportunistically feed on other vertebrates, including rodents, cattle, sheep and dogs [29–34]. Those animals are reared in high numbers and in close proximity to humans and may thus be important components of (peri-)urban ZIKV transmission cycles. Therefore, it is currently unclear whether pets and peri-domestic animals play a role in the maintenance of ZIKV in Africa or the Americas.

Here we investigated ZIKV spread in pets (i.e. dogs and cats) and peri-domestic animals (i.e. equids, cattle, sheep and goats) sampled in northeastern Brazil, the 2015–2016 outbreak’s epicentre, and in wild NHP, pets and peri-domestic animals sampled in Côte d’Ivoire, where ZIKV has been documented in forest-dwelling mosquitoes [4, 35].

In Brazil, equid, cattle, sheep, goat and cat sera were collected in the northeastern state of Bahia during 2013–2018 within state routine veterinary surveillance activities or from dogs and cats presenting clinical signs, and in 2015 from dogs in the neighbouring state of Pernambuco for a *Leishmania* spp. serosurvey [36]. In Côte d’Ivoire, peri-domestic animals were sampled in 2012 and 2014. Unhabituated wild NHP such as king colobus (*Colobus polycomos*) and western red colobus (*Piliocolobus badius*) were sampled in the Tai National Park during 2006 and 2016 [37, 38]. Samples were also collected from a habituated group of sooty mangabeys (*Cercocetus atys*) that has been under observation since 2012 [37, 38]. Additionally, nine blood samples from deceased West African chimpanzees (*Pan troglodytes verus*) were included; this population of chimpanzees has been followed daily since 1979 and necropsy samples have been systematically performed on all dead individuals recovered, since the inception of the veterinary programme in 2002 [37, 39].

A total of 1416 sera of pets and peri-domestic animals from Brazil and 298 sera of dogs, peri-domestic animals and NHP from Côte d’Ivoire were screened for ZIKV antibodies using the NS1-based Electrochemiluminescence immunoassay (ECLIA) Elecsys Zika IgG (Roche, Penzberg, Germany). This double-antigen test enables multispecies testing because it does not require species-specific
secondary antibodies, but it was only validated for human sera [40]. ECLIA-positive sera were further tested by a plaque reduction neutralization test (PRNT<sub>90</sub>) to identify anti-ZIKV neutralizing antibodies (Asian ZIKV lineage, strain H/PF/2013; used for samples from Brazil because of limited volume of available sera and African ZIKV lineage, strain MR766; used for samples from Côte d'Ivoire because of limited volume of available sera). Because cross-reacting antibodies against co-circulating flaviviruses can yield false-positive ZIKV test results [41, 42], we compared ZIKV PRNT<sub>90</sub> endpoint titres with endpoint titres against co-circulating flaviviruses representing diverse serocomplexes, including Rocio virus (ROCV; strain UVE/ROCV/1975/BR/5P H34 675), Saint Louis Encephalitis virus (SLEV; strain MSI-7), Bussuquara virus (BSQV; strain BeAn 4073), Wesselsbron virus (WSLV; strain UVE/WESSV/UNK/ZA/SAH-177), Spondweni virus (SPOV; strain UVE/SPOV/UNK/ZA/SM-6 V-1s), Dengue virus 2 (DENV-2; strain Thailand/16681/84), Yellow Fever virus (YFV; strain 17D), and West Nile virus (WNV; strain NY-99). We used antigenic cartography to discern neutralizing antibody reactivity patterns [43]. Due to the lack of sufficient volume of serum samples, chimpanzee sera were only tested by PRNT<sub>90</sub> and not by ECLIA.

PRNT<sub>90</sub> was conducted in 12-well plates seeded 1 day before the infection with 1.6×10<sup>5</sup> Vero FM cells for ZIKV, SPOV, YFV, WSLV, WNV and BSQV, 1.6×10<sup>5</sup> Vero B4 cells for DENV-2 and ROCV, and 1.2×10<sup>5</sup> BHK-21 cells for SLEV. Forty plaque-forming units were incubated with respective serum dilutions of 1 : 40, 1:80, 1:160, 1:320, 1:640 and 1:1280 for 1 h, added onto the cell monolayer, and incubated for 1 h before adding an overlayer containing DMEM with 2% FCS and 1.25% carboxymethyl cellulose. Cells were incubated for 3 days for WNV and SLEV, 4 days for ZIKV, 5 days for SPOV, DENV-2 and BSQV, 6 days for ROCV and WSLV and 7 days for YFV. The overlayer medium was removed, and cells were fixedated with 6% paraformaldehyde and stained with crystal violet. PRNT<sub>90</sub> endpoint reciprocal titres were calculated using a logistic regression function in GraphPad Prism 6 (GraphPad Software, www.graphpad.com).

The initial ECLIA-based ZIKV reactivity rate among pets and peri-domestic animals was 4.6% (65/1416, 95% CI, 3.5–5.8) in Brazil and 3.6% (8/220, 95% CI, 1.6–7.0) in Côte d’Ivoire (Fig. 1, Table 1). However, based on PRNT<sub>90</sub>, fourfold titre differences, commonly considered decisive in flavivirus serology [42], ZIKV-specific antibodies could neither be confirmed in pets and peri-domestic animals from Brazil, nor from Côte d’Ivoire (Fig. 2a; Fig. 2b). Instead, among the Brazilian ECLIA-reactive sera, reciprocal PRNT<sub>90</sub> endpoint titres were fourfold or higher for WNV in five (7.7%), YFV in three (4.6%), SPOV in one (1.5%) and WSLV in one serum (1.5%). A total of 31 ECLIA-positive sera (47.7% of all ZIKV ECLIA-reactive sera from Brazil) were negative for all flaviviruses tested by PRNT<sub>90</sub> likely due to differential sensitivity of those tests. Among the eight ECLIA-reactive

**Table 1.** Sampling table and Zika virus antibody test reactivity rate

| Country sampled | Group     | Animal species                  | Total | ECLIA % (95% CI) | PRNT<sub>90</sub> % (95% CI) | Year        |
|----------------|-----------|---------------------------------|-------|------------------|-----------------------------|-------------|
| Brazil         | Peri-domestic | Donkey                         | 55    | 9.1 (3.0–19.9)   | 0                           | 2013–2018  |
| Brazil         | Peri-domestic | Male                           | 95    | 4.2 (1.2–10.4)   | 0                           | 2014–2018  |
| Brazil         | Peri-domestic | Horse                          | 620   | 4.0 (2.6–5.9)    | 0                           | 2015–2018  |
| Brazil         | Peri-domestic | Cow                            | 278   | 6.5 (3.9–10.0)   | 0                           | 2016–2018  |
| Brazil         | Peri-domestic | Sheep                          | 64    | 4.7 (1.0–13.1)   | 0                           | 2017, 2018 |
| Brazil         | Peri-domestic | Goat                           | 231   | 2.6 (1.0–5.6)    | 0                           | 2018       |
| Brazil         | Pet        | Cat                            | 5     | 20.0 (0.5–71.6)  | 0                           | 2015, 2018 |
| Brazil         | Pet        | Dog                            | 68    | 4.4 (0.9–12.4)   | 0                           | 2015, 2018 |
| CIV            | Peri-domestic | Cow                            | 16    | 12.5 (1.6–38.4)  | 6.3 (0.2–30.2)              | 2012–2014  |
| CIV            | Peri-domestic | Goat                           | 97    | 3.1 (0.6–8.8)    | 1.0 (0.1–5.6)               | 2012–2014  |
| CIV            | Peri-domestic | Pig                            | 18    | 0.0              | 0                           | 2012–2014  |
| CIV            | Peri-domestic | Sheep                          | 89    | 2.2 (0.3–7.8)    | 0                           | 2012–2014  |
| CIV            | NHP        | King colobus (Colobus polycomos) | 9     | 66.7 (29.9–92.5) | 11.1 (0.3–48.3)             | 2006–2016  |
| CIV            | NHP        | Western red colobus (Piliocolobus badius) | 13   | 23.1 (5.0–53.8)  | 0                           | 2006–2016  |
| CIV            | NHP        | Sooty mangabey (Cercocebus atys) | 17   | 0.0              | 0                           | 2006–2016  |
| CIV            | NHP        | Chimpanzees (Pan troglodytes verus) | 9    | 100.0 (66.4–100.0) | 22.2(2.8–60.1)            | 2006–2016  |
| CIV            | Pet        | Dog                            | 39    | 2.6 (0.1–13.5)   | 0                           | 2006–2016  |
| Total          |            |                                 | 1723  | 5.3 (4.1–6.1)    |                             |             |

Detailed information is available in supplementary table S1. CI, confidence intervals; ECLIA, electrochemiluminescence immunoassay; NHP, non-human primates; PRNT, plaque reduction neutralization test.
sera from pets and peri-domestic animals from Côte d’Ivoire, we found a monotypic reaction to YFV in one serum and to SPOV in another serum (Table S1, available in the online version of this article).

Antigenic cartography did not provide a robust separation of flavivirus serocomplexes in pets and peri-domestic animals from either Côte d’Ivoire or Brazil (Fig. 2d). The detection of sera with monotypic reaction or titres ≥fourfold for SPOV, and WSLV in Brazilian animals must be carefully interpreted. WSLV has not been reported in the Americas, and although SPOV has been isolated in *Culex quinquefasciatus* in Haiti [44] it is unlikely that either SPOV or WSLV would be widely dispersed among pets or peri-domestic animals from Brazil because there are no robust data on isolation or molecular detection of those viruses in vertebrates from Latin America. A more plausible explanation for those PRNT results are cross-reactive flavivirus antibodies, which complicate the interpretation of flavivirus serological assays in hyperendemic settings, even when considering only ≥fourfold PRNT titres as decisive serological support [42, 45]. This interpretation is supported by the overall low titres against all tested flaviviruses and by previous evidence showing the difficulties to confirm human ZIKV infection in DENV-endemic areas [42, 46].

In NHP, ZIKV ECLIA reactivity was observed in 23.1% (3/13, 95% CI, 11.1–39.3) of red colobus, 66.7% (6/9, 95% CI, 29.9–92.5) of king colobus and in none of the 17 sooty mangabeys, while 88.9% (8/9, 95% CI, 51.8–99.7) of the chimpanzees tested positive for ZIKV using PRNT titres compared to other flaviviruses were found in 11.1% (1/9, 95% CI, 0.3–48.3) of king colobus and in 22.2% (2/9, 95% CI, 2.8–60.1) of chimpanzees (Table 1, Fig. 2c, e) and in none of the previously ECLIA-reactive red colobus. Moreover, one chimpanzee (1/9, 95% CI, 0.3–48.3) presented titres ≥fourfold for WNV compared to all other flaviviruses. The difference in NHP ZIKV seroprevalence might be related to differential host susceptibility or different ecological niches occupied by those NHP since the mosquito species from which ZIKV was isolated in Côte d’Ivoire (i.e. *Aedes vitattus*, *Ae. furcifer* and *Ae. aegypti formosus*) are predominantly found in the tree canopy [28, 35, 47]. Notably, ZIKV was linked to NHP already in the initial viral isolation from a sentinel Rhesus macaque caged in the canopy [48]. Our data suggest that
some NHP species like chimpanzees and king colobus, which are at least partly arboreal, might play a role as ZIKV reservoirs in Côte d’Ivoire. Our interpretation is supported by previous studies indicating ZIKV exposure of native African NHP by viral isolation from Patas (Erythrocebus patas) and Vervet (Chlorocebus pygerythrus) monkeys in Senegal [49] and by the detection of ZIKV-neutralizing antibodies in different NHP species such as in red-tailed monkey (Cercopithecus ascanius), grey-cheeked mangabeys (Lophocebus albigena), mantled guereza (Colobus guereza) in Uganda [50], and tantalus (Chlorocebus tantalus) and Mona (Cercopithecus mona) monkey in Nigeria [51]. On the other hand, our data do not provide conclusive evidence on whether those NHP species may serve as amplification or reservoir hosts, because of our small sample size and because our methods were not targeted towards detecting active infections. Future longitudinal studies including younger NHP, as performed for CHIKV reservoir studies in Senegal [52], could clarify the role of those NHP species for ZIKV transmission in Africa.

Our study was limited by the lack of longitudinal sampling from the different animal species in both locations in Brazil and Côte d’Ivoire, since long-term titre comparison would be more effective for understanding antibody responses after multiple flavivirus infections. We also did not test other synanthropic animals such as marsupials, known to be relevant arbovirus hosts, e.g. for the alphavirus Ross River virus [53] and which may be exposed to ZIKV vectors [54]. However, while most of the previous studies on ZIKV animal reservoirs either used tests with lower specificity only (e.g. ELISA, complement fixation and haemagglutination) or assessed flaviviral cross-reactivity using fewer viruses in PRNT [27, 50, 51], we provided data from a large number of sera of pets and peri-domestic animals from two continents and performed extensive neutralization testing, considered the gold-standard technique.

In brief, our data confirm that co-circulating flaviviruses challenge unambiguous ZIKV antibody test results in hyper-endemic areas [41, 42]. Despite those technical limitations, our data imply that while some NHP species in Côte d’Ivoire may serve as ZIKV reservoir hosts, pets and peri-domestic animals are neither involved in ZIKV transmission cycles in Brazil, nor in Côte d’Ivoire.

Funding information
J.F.G. was supported by the Deutsche Forschungsgemeinschaft (DFG) Research Group ‘Sociality and Health in Primates’ (FOR2136; CA 1108/3-1). This work was supported by the European Union’s Horizon 2020 research and innovation programme through the ZIKAlliance project (grant agreement no. 734548) to J.F.D. and X.d.L. and by Roche Diagnostics through the investigator-initiated study ZikaStock to J.F.D.

Acknowledgements
We thank Sandra Junglen and Anne Kopp for providing the SLEV strain. We thank the authorities in Côte d’Ivoire for their support of this research, in particular the Ministry of the Environment and Forests and the Ministry of Research, the Ivorian Office of National Parks (Office Ivorian des Parcs et Reserves), the directorship of the Taï National Park and the Centre Suisse de Recherches Scientifiques en Côte d’Ivoire in Abidjan. We are grateful to the field assistants of Taï National Park for their commitment and efforts that enabled this study.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
In Brazil, sampling and analyses were approved by the Federal University of Bahia’s animal ethics committee under authorization no. 55/2017, by the Animal Ethics Committee (CEUA) of the Aggeu Magalhães Institute (Fiocruz) for dog sampling under authorization no. 56/2013. In Côte d’Ivoire, sampling and peri-domestic animals from two continents and performed extensive neutralization testing, considered the gold-standard technique.

References

1. Petersen LR, Jamieson DJ, Honein MA. Zika Virus. N Engl J Med 2016;375:294–295.
2. Mayer SV, Tesh RB, Vasilakis N. The emergence of arthropod-borne viral diseases: A global prospecive on dengue, chikungunya and zika fevers. Acta Trop 2017:166:155–163.
3. Fayé O, Freire CCM, Lamarino A, Fayé O, de Oliveira JVC, et al. Molecular evolution of Zika virus during the emergence in the 20(th) century. PLoS Negl Trop Dis 2014;8:e2636.
4. Valentine MJ, Murdock CC, Kelly PJ. Syltivus cycles of arboviruses in non-human primates. Parasit Vectors 2019;12:463.
5. Faria NR, Azevedo R do S da S, Kraemer MUG. Souza R, Cunha MS, et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science 2016;352:345–349.
6. Brady OJ, Osgood-Zimmerman A, Kassebaum NJ, Ray SE, de Araujo VEM, et al. The association between Zika virus infection and microcephaly in Brazil 2015-2017: An observational analysis of over 4 million births. PLoS Med 2019;16:e1002755.
7. Netto EM, Moreira-Soto A, Pedroso C, Höser C, Funk S, et al. High Zika Virus Seroprevalence in Salvador, Northeastern Brazil Limits the Potential for Further Outbreaks. mBio 2017;8:e01390-17.
8. Berry N, Ferguson D, Ham C, Hall J, Jenkins A, et al. High susceptibility, viral dynamics and persistence of South American Zika virus in New World monkey species. Sci Rep 2019;9:14495.
9. de Oliveira-Filho EF, Oliveira RAS, Ferreira DRA, Laroque PO, Pena LJ, et al. Seroprevalence of selected flaviviruses in free-living and captive capuchin monkeys in the state of Pernambuco, Brazil. Transbound Emerg Dis 2018;65:1094–1097.
10. Moreira-Soto A, Carneiro I de O, Fischer C, Feldmann M, Kümmere BM, et al. Limited evidence for infection of urban and peri-urban nonhuman primates with zika and chikungunya viruses in Brazil. mSphere 2018;3.
11. Terzian ACB, Zini N, Sacchetto L, Rocha RF, Parra MCP, et al. Evidence of natural Zika virus infection in neotropical non-human primates in Brazil. Sci Rep 2018;8:16034.
52. Althouse BM, Guerbois M, Cummings DAT, Diop OM, Faye O, et al. Role of monkeys in the sylvatic cycle of chikungunya virus in Senegal. *Nat Commun* 2018;9:1046.

53. Stephenson EB, Peel AJ, Reid SA, Jansen CC, McCallum H. The non-human reservoirs of Ross River virus: a systematic review of the evidence. *Parasit Vectors* 2018;11:188.

54. Bueno MG, Martinez N, Abdalla L, Duarte Dos Santos CN, Chame M. Animals in the Zika Virus Life Cycle: What to Expect from Megadiverse Latin American Countries. *PLoS Negl Trop Dis* 2016;10:12.

---

**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

**Find out more and submit your article at microbiologyresearch.org.**