

Dr. Resende is a postdoctoral researcher at the Laboratory of Respiratory Viruses at Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil, National Influenza Center for the World Health Organization. Her research interests are reconstructing the phylogenetic profile of influenza viruses and on identifying genomic viral polymorphisms associated with an increase in virulence and antiviral drug resistance.

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Address for correspondence: Paola Cristina Resende. Laboratório de Vírus Respiratórios e do Sarampo, WHO/NIC, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, RJ, Pav HPP, B104, 21040-360, Brazil; email: paola@ioc.fiocruz.br

Avian Pox in Native Captive Psittacines, Brazil, 2015

Felipe C.B. Esteves, Sandra Y. Marín, Maurício Resende, Aila S.G. Silva, Hannah L.G. Coelho, Mayara B. Barbosa, Natália S. D’Aparecida, José S. de Resende, Ana C.D. Torres, Nelson R.S. Martins

Author affiliation: Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

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To investigate an outbreak of avian pox in psittacines in a conservation facility, we examined 94 birds of 10 psittacine species, including sick and healthy birds. We found psittacine pox virus in 23 of 27 sick birds and 4 of 67 healthy birds. Further characterization is needed for these isolates.

Avian pox is caused by avipoxvirus. Infections occur worldwide in domestic and wild avian species (1), are suggested to be host family- or order-specific, and are modulated by habitat and ecologic niche (2). Avipoxviruses have been described in Brazilian *Amazona* spp. and *Pionus* spp. parrots with severe diphtheritic upper digestive lesions, experimentally causing the formation of cutaneous lesions in chickens; chicken and parrot strains will not provide cross protection (3). The presumptive diagnosis, based on typical pocklike skin lesions of papular or nodular hyperplastic and hypertrophic skin foci or upper digestive diphtheritic form in severe cases (1), may be confirmed by detection of avipoxvirus DNA by PCR (4).

In June 2015, an outbreak of avian pox occurred among 10 species of native Brazilian psittacines (n = 94) maintained in a conservation facility. In addition to the typical pocklike nodular skin lesions, the psittacines had weight loss and reduced activity; 3 died. The outbreak lasted 3 months; remission of lesions occurred within ≈3 weeks in each bird.

Skin scrapings were collected from the cutaneous lesions of affected birds (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/23/1/16-1133-Techapp1.pdf>), and conjunctiva and cloaca swabs were collected from all 94 psittacines showing cutaneous lesions (27 birds) or not (67 birds). Skin samples treated with an antibacterial–antimycotic drug mixture (Gibco; Thermo Fisher Scientific, Waltham, MA, USA) were inoculated onto the chorioallantoic membrane (CAM) of 10-day-old specific pathogen-free chicken embryos and typical pocklike CAM lesions were obtained (online Technical Appendix Figure 2). Cutaneous samples and the CAM of inoculated

embryos were subjected to PCR with specific primers forward 5'-CAGCAGGTGCTAAACAACAA-3' and reverse 5'-CGGTAGCTTAACGCCGAATA-3' (4) to amplify a partial sequence (576 bp) of the gene encoding the core protein P4b (*fpv167* locus) of avipoxvirus (Table). The avipoxvirus P4b gene partial sequence was obtained from skin lesions, conjunctiva and cloacal swabs, and CAM; sequences then underwent phylogenetic characterization (online Technical Appendix Figure 3).

The P4b gene partial sequences obtained from the avipoxvirus isolate Betim-1 of the red-and-green macaw (*Ara chloropterus*) (GenBank accession no. KT187552), dusky parrot (*Pionus fuscus*) (Esteves et al., unpub. data), and golden conure (*Guaruba guarouba*) (Esteves et al., unpub. data) were 100% identical. The obtained sequences were aligned with local avipoxvirus of different species with clinical disease (Esteves et al., unpub. data) and with previously published avipoxvirus sequences in GenBank (online Technical Appendix Figure 3). Evolutionary analyses were performed in MEGA6 (5), evolutionary history was inferred by neighbor joining (6), replicate trees were clustered by using the bootstrap test (7) with 1,000 oversampling, and evolutionary distances were computed by the Tamura 3-parameter method (8). The phylogenetic relationships revealed grouping with psittacine poxviruses, including isolates from the United Kingdom (macaw, GenBank accession no. AM050382.1, and parrot, GenBank accession no. AM050383.1); United States (yellow-crowned amazon [*Amazona ochrocephala*], GenBank accession no. KC018069.1); and Germany (lovebird [*Agapornis* sp.], GenBank accession no. AY530311.1). To enable comparisons, a local strain (GenBank accession no. KX863707) of the Atlantic canary (*Serinus canaria*) (Esteves et al., unpub. data) and an isolate of the Magellanic penguin (*Spheniscus magellanicus*) (GenBank accession no. KF516679.1) (9) were grouped in the canarypox clade. A local chicken isolate (GenBank accession no. KX863706) was grouped within the fowlpox clade, including chicken (GenBank

accession nos. KF722860.1 KF722863.1) and turkey (GenBank accession nos. KF017961.1 and DQ873808.1) strains.

Birds with pocklike lesions represented 28.7% (27/94) of psittacines in the sanctuary. Laboratory diagnosis was implemented at the early stages of the outbreak; birds were clinically observed up to the final cases (3 months). Of the 27 psittacines with lesions, 23 (85.2%) were PCR positive for avipoxvirus. No blue-fronted parrots (*Amazona aestiva*) showed lesions, but all were PCR positive (4/94 [4.2%]), suggesting that they were immune, although they were not tested for an immune response. Among the 25 golden conures, the most abundant species in the premises, 9 showed typical pocklike lesions and were PCR positive, but 1 with lesions was PCR negative and 15 had no lesions and were PCR negative, indicating nonuniform immunity. None of the 14 blue and yellow macaws (*Ara ararauna*) had lesions; PCR results were negative for all, suggesting previous immunity or a lower susceptibility to infection. Four birds with lesions were not PCR positive, possibly indicating an advanced phase of virus-free scars.

The proximity of aviaries suggests that all birds might have had the opportunity for infection during the outbreak. The death rate (3.2%) for the outbreak was low; the death of young golden conures with diphtheritic lesions and 2 adult dusky parrots that demonstrated conjunctivitis and cachexia were accompanied by complications by *Candida albicans* and *Capillaria* sp., suggesting the potential aggravation risk. Most psittacines (63/94; 67.0%) were PCR negative and without lesions, as tested in cloacal and conjunctival swabs.

The affected species are declining in wild populations. Our findings emphasize the risk for avipoxvirus among captive psittacines; their relevance for psittacine rehabilitation and conservation may be considerable regarding pathogenicity. As shown, 2 adult dusky parrots and 1 nestling golden conure died; further characterization is needed for the isolates, including their eventual importance for commercial poultry.

Table. Avipoxvirus detection by PCR according to clinical status and psittacine species, Brazil, 2015*

Psittacine species	Clinical status			
	PCR positive		PCR negative	
	Normal	Cutaneous lesions	Normal	Cutaneous lesions
<i>Amazona aestiva</i> (blue-fronted parrot)	4	–	–	–
<i>Amazona brasiliensis</i> (red-tailed Amazon)	–	1	4	1
<i>Anodorhynchus hyacinthinus</i> (hyacinthine macaw)	–	2	4	–
<i>Ara chloropterus</i> (green-winged macaw)	–	–	2	–
<i>Ara macao</i> (scarlet macaw)	–	2	1	–
<i>Ara ararauna</i> (blue and yellow macaw)	–	–	14	–
<i>Deropterus accipitrinus</i> (red-fan parrot)	–	1	9	2
<i>Guaruba guarouba</i> (golden conure)	–	9	15	1
<i>Pionites leucogaster</i> (white-bellied caique)	–	4	12	–
<i>Pionus fuscus</i> (dusky parrot)	–	3	2	–
Total	4	23	63	4
Ratio, no. birds/no. tested (%)	4/94 (4.2)	23/94 (24.4)	63/94 (67.0)	4/94 (4.2)

*Values are no. birds except as indicated. –, not found.

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Dr. Esteves is a veterinarian dedicated to native wildlife conservation. He is the leader of a nongovernmental agency dedicated to the rescue and rehabilitation of wild animals. On his farm, he maintains and cares for about 170 animals that are not fit to reintroduce into nature.

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Address for correspondence: Nelson R.S. Martins, Universidade Federal de Minas Gerais—Medicina Veterinária Preventiva, Avenida Antonio Carlos 6627, Belo Horizonte 31270-901, Brazil; email: nrsmart@gmail.com

Chikungunya Fever in Traveler from Angola to Japan, 2016

Saho Takaya, Satoshi Kutsuna, Eri Nakayama, Satoshi Taniguchi, Shigeru Tajima, Yuichi Katanami, Kei Yamamoto, Nozomi Takeshita, Kayoko Hayakawa, Yasuyuki Kato, Shuzo Kanagawa, Norio Ohmagari

Author affiliations: National Center for Global Health and Medicine, Tokyo, Japan (S. Takaya, S. Kutsuna, Y. Katanami, K. Yamamoto, N. Takeshita, K. Hayakawa, Y. Kato, S. Kanagawa, N. Ohmagari); National Institute of Infectious Diseases, Tokyo (E. Nakayama, S. Taniguchi, S. Tajima)

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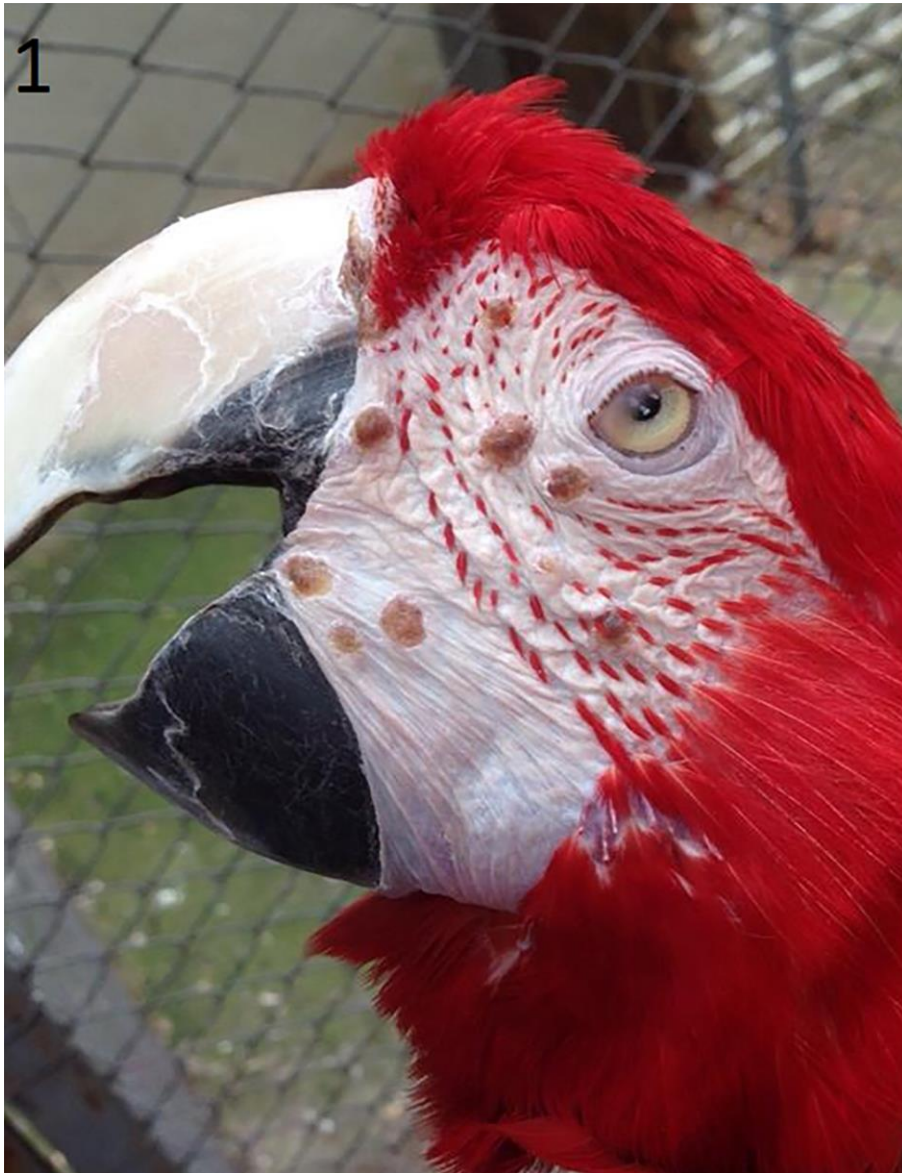
Simultaneous circulation of multiple arboviruses presents diagnostic challenges. In May 2016, chikungunya fever was diagnosed in a traveler from Angola to Japan. Travel history, incubation period, and phylogenetic analysis indicated probable infection acquisition in Angola, where a yellow fever outbreak is ongoing. Thus, local transmission of chikungunya virus probably also occurs in Angola.

Simultaneous circulation of multiple arboviruses has been observed several times in many parts of the world. In 1970, Angola reported an outbreak of a dengue-like syndrome, which turned out to be a concurrent outbreak of yellow fever and chikungunya fever (1). On April 13, 2016, the World Health Organization declared a yellow fever outbreak in Angola. In response to the outbreak, a nationwide yellow fever vaccination campaign was initiated. As of July 29, 2016, a total of 3,818 confirmed and suspected cases were reported (2). In addition, on July 23, 2016, the World Health Organization was notified of a Rift Valley fever case in a man from China working in Luanda, the capital city of Angola, and started an investigation in Angola (3). We describe a case of chikungunya fever in a traveler from Angola to Japan.

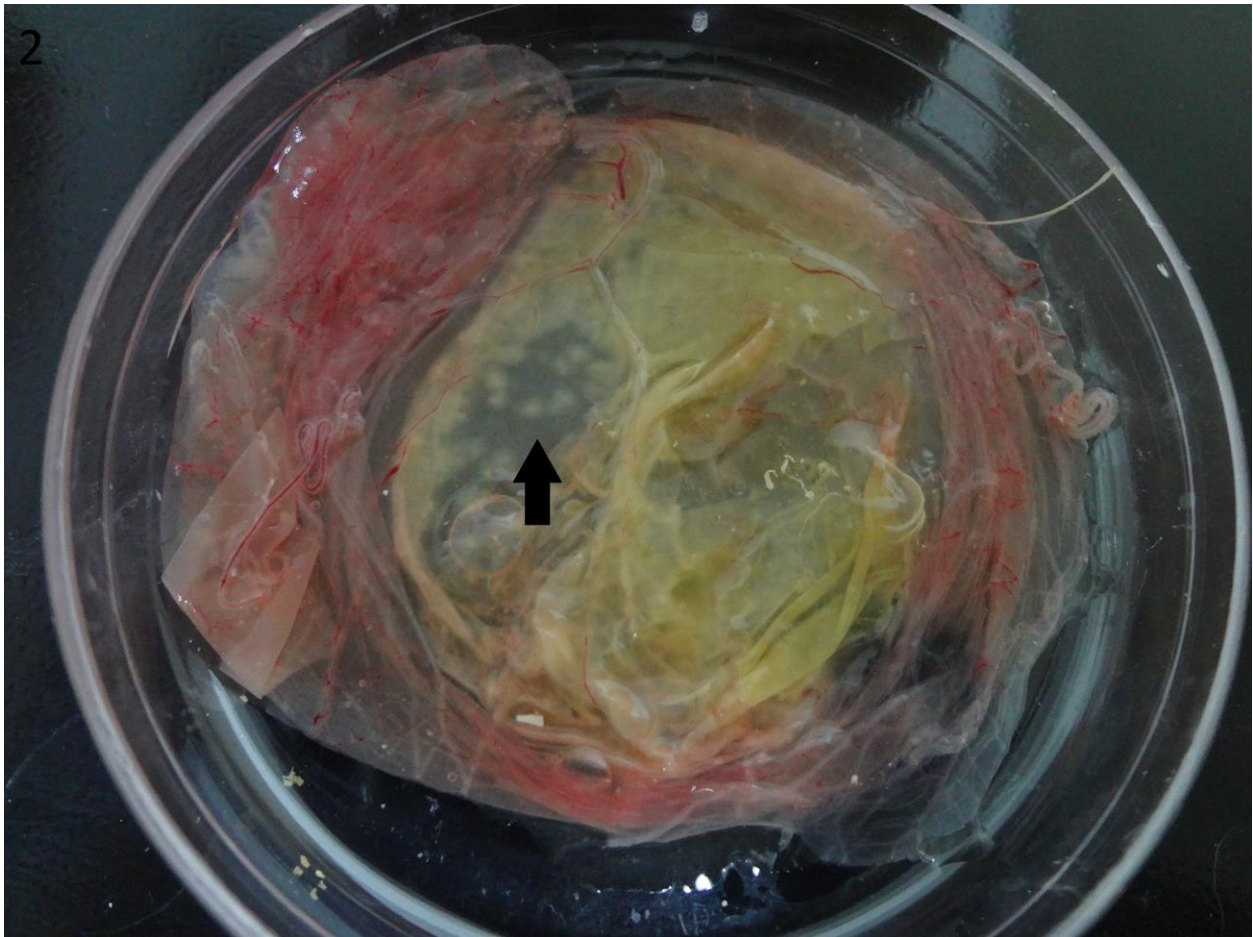
In May 2016, a 21-year-old woman traveled to Tokyo, Japan, from her home in Luanda. She began to exhibit a high fever on the first day of her visit. On the second day, she sought care at the National Center for Global Health and Medicine (Tokyo). She had been previously healthy and had not traveled out of Luanda in the past 6 months. She claimed to have been vaccinated according to the national immunization plan, which included vaccination against yellow fever. At the first visit, she had high-grade fever (40.7°C) without other signs. Her vital signs were otherwise stable, and physical examination revealed no

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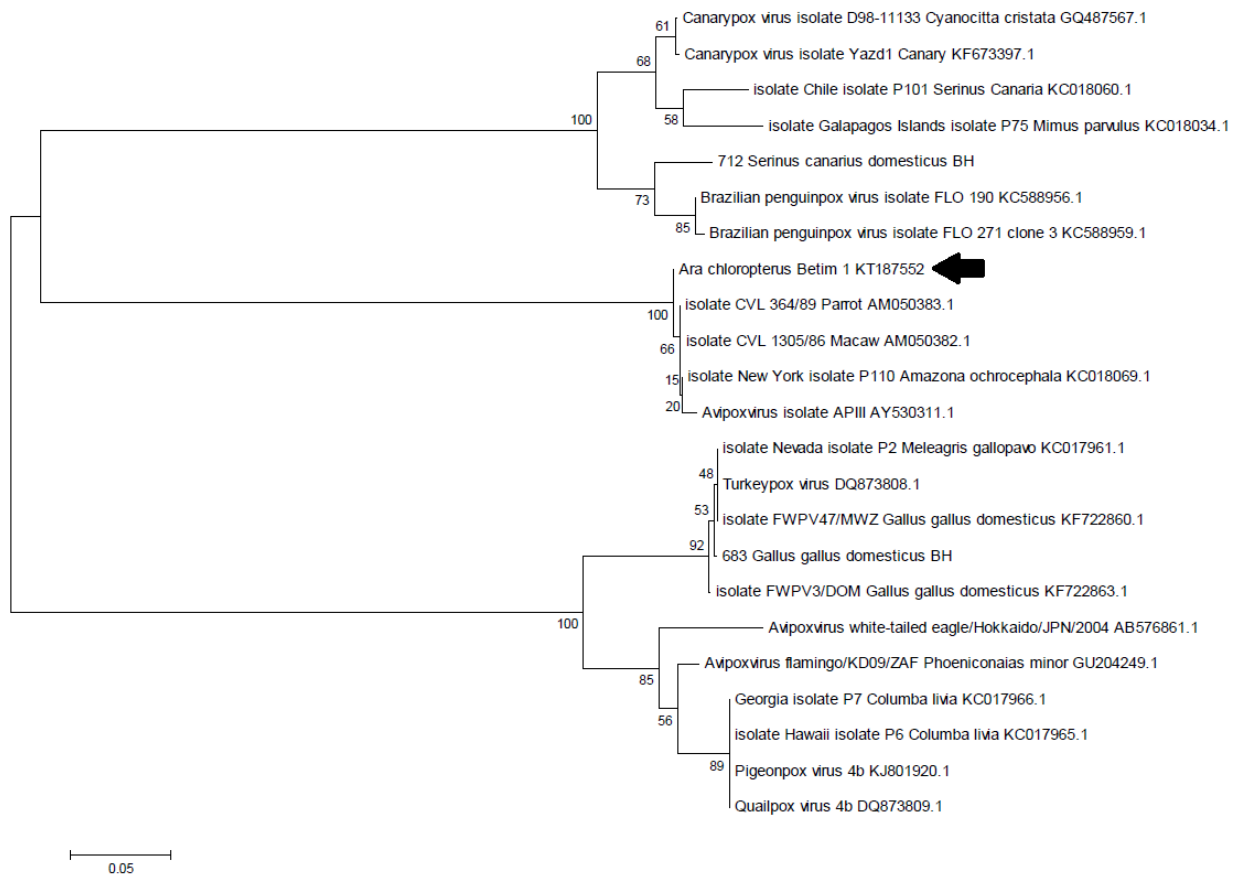
Technical Appendix



Technical Appendix Figure 1. Green-winged macaw (*Ara chloropterus*) with papular lesions on the face. This bird recovered completely.



Technical Appendix Figure 2. Chorioallantoic membrane of 17-day old specific pathogen-free chicken embryo infected at 10 days of incubation with the avipoxvirus isolate of the dusky parrot (*Pionus fuscus*), showing whitish foci (arrow) and large opaque yellowish area with edema.



Technical Appendix Figure 3. Evolutionary relationships of avipoxvirus strains. Three phylogenetic clades were revealed, grouping *Canarypox*, *Psittacinepox*, or *Fowlpox* strains separately. The isolate *Ara chloropterus* Betim 1 (arrow) (GenBank accession no. KT187552) was grouped with *Psittacinepox* viruses, including the New York strain (KC018069.1), obtained from cutaneous lesions of the yellow-crowned amazon (*Amazona ochrocephala*), with the clinical isolates obtained in quarantine (Weybridge, UK), AM050382.1 (unspecified macaw), and AM050383.1 (unspecified parrot), and the isolate AY530311.1 from *Agapornis* (Germany). A local *Canarypox* (KX863707) isolate was grouped with the recent penguin pox strains (KC588956.1 and KC588959.1), and a local *Fowlpox* (KX863706) was grouped with chicken and turkey isolates. The evolutionary history was inferred with the neighbor-joining method (6). The optimal tree with the sum of branch length = 1.31754708 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (7). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed by using the maximum composite likelihood method (8) and are in the units of the number of base substitutions per site. The analysis involved 23 nt sequences. Codon positions included were

1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated; there were 470 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (5).