Short Report: Intrafamilial Transmission of *Vaccinia virus* during a Bovine Vaccinia Outbreak in Brazil: A New Insight in Viral Transmission Chain

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Abstract. Bovine vaccinia (BV) is an emerging zoonosis caused by the *Vaccinia virus* (VACV), genus *Orthopoxvirus* (OPV), *Poxviridae* family. In general, human cases are related to direct contact with sick cattle but there is a lack of information about human-to-human transmission of VACV during BV outbreaks. In this study, we epidemiologically and molecularly show a case of VACV transmission between humans in São Francisco de Itabapoana County, Rio de Janeiro state. Our group collected samples from the patients, a 49-year-old patient and his son. Our results showed that patients had developed anti-OPV IgG or IgM antibodies and presented neutralizing antibodies against OPV. The VACV isolates displayed high identity (99.9%) and were grouped in the same phylogenetic tree branch. Our data indicate that human-to-human VACV transmission occurred during a BV outbreak, raising new questions about the risk factors of the VACV transmission chain.

The *Vaccinia virus* (VACV) belongs to the *Poxviridae* family, genus *Orthopoxvirus* (OPV) and it is related to bovine vaccinia (BV) outbreaks in Brazil. The BV is an emerging zoonosis that circulates between bovines and humans causing economic losses and public health problems.^{1,2}

Since 1999, several BV outbreaks have been reported in Brazil, causing exanthematic lesions in dairy cattle and milkers.^{3,4} Several VACVs have been isolated during BV outbreaks from different Brazilian regions, showing a genetic and biological dichotomy.⁵ The main VACV transmission route is likely direct occupational contact between milkers and sick cattle.⁶ Therefore, in most of the outbreaks, the human lesions have been restricted to the milkers' hands and arms. Other symptoms are also frequent, including fever, myalgia, headache, arthralgia, and lymphadenopathy.¹

Although the lesions usually present high titers of infectious particles,⁷ there is a lack of information about human-to-human transmission of VACV during BV outbreaks. In this study we describe, based on virological, biological, and molecular data, a case of intrafamilial transmission of VACV during a BV outbreak.

During field expeditions conducted in São Francisco de Itabapoana County, in Rio de Janeiro state, in September 2002, our group was notified about the occurrence of a case of exanthematic disease affecting a milker (patient 1). The 49-year-old patient had been working as a milker at three farms belonging to the same farmer. Patient 1 reported that he had not been previously vaccinated against smallpox and did not present a vaccination scar on his left arm. This patient reported the development of lesions on his hands a few days after contact with sick cattle. The lesions evolved from macules to papules, vesicles, pustules and, after some weeks, to scabs. In addition, patient 1 presented a high fever, ranging from 39 to 40°C, myalgia, headache, and axillary lymphadenopathy. Patient 1 did not report the use of ban-

dages for lesion covering. The disease lasted 3 weeks (Figure 1A). Interestingly, ~6 days after the beginning of the healing stage, patient 1 reported that his son (patient 2), a 14-year-old student, presented with similar symptoms, including exanthematic lesions, fever, myalgia, headache, and axillary lymphadenopathy. During part of the acute phase of the disease (vesicle and scab) patient 1 had shared domestic environments with patient 2, keeping direct contact to him (Figure 1A). There is no information about sharing of clothes or devices between patient 1 and 2. Interestingly, patient 2 did not work as a milker and did not have contact with cattle. Patient 2 had been living at a residence located 24 km from the property where patient 1 reported occupational contact with sick cattle.

To investigate this case, our team went to the affected farm and collected scab samples from the hands of patient 1 using sterile devices, as previously described,⁸ and swab samples from the hand lesions of patient 2 using a sterile swab. In addition, sera samples were collected from both patients. The study followed the rules of Ethics Committee of Universidade Federal de Minas Gerais (UFMG). The collection procedures were carried out separately, and the samples were stored and manipulated far from each other to avoid cross-contamination. In our laboratory, the samples (swab and scab) were prepared for virus isolation, as described previously. The samples were inoculated onto a Vero cell monolayer and the chorioallantoic membranes of hen's eggs^{8,9}; after the appearance of cytopathic effects, DNA was extracted by phenol-chloroform and isoamyl alcohol,¹⁰ and then submitted to a polymerase chain reaction (PCR) assay,¹¹ targeting the *a56r* gene. This gene is a molecular marker for Brazilian VACV (VACV-BR), and its sequencing allows both the identification of VACV and indicates in which Brazilian VACV-BR group the new viral isolate can be included. Furthermore, we amplified and sequenced two more viral genes: the A-type-inclusion body gene (*a26l*) and serine protease inhibitor 3 (*k2l*), by using primers designed to amplify 780 and 1100 base pair (bp), respectively. The viral isolates were named VACV-SFI1 (patient 1) and VACV-SFI2 (patient 2). The PCR products

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FIGURE 1. (A) Clinical and epidemiological timeline. (B) Maximum parsimony phylogenetic tree constructed based on the nucleotide sequence of the *Orthopoxvirus* (OPV) *a56r* gene. The SFI1 and SFI2 isolates grouped with other Brazilian *Vaccinia virus* (VACV) in group 1. This clade is the most prevalent in bovine vaccinia (BV) outbreaks. Bootstrap confidence intervals are shown on the branches (1,000 replicates), as are the GenBank accession numbers. (C) Nucleotide alignment of partial *a56r* gene sequences of some VACV and other OPV. The arrow highlights a nucleotide signature (guanine) of the SFI's isolates, whereas all other viruses show a cytosine residue.

were directly sequenced using the MegaBACE platform¹² and compared with nucleotide sequences available in the GenBank database using BLASTN. Based on the *a56r* nucleotide sequences from the VACV-SFI1 and other OPV, we constructed a maximum parsimony tree using the MEGA 4.1 program (Arizona State University, Phoenix, AZ).

Both exanthematous samples showed the amplification of *a56r*, *a26l*, and *k2l* fragments. The alignment of the *a56r* sequences obtained from VACV-SFI1 and VACV-SFI2 displayed high identity (99.9%) (Figure 1C). When compared with nucleotide sequences available, the *a56r* genes from the VACV-SFI isolates were similar to homologous genes from other VACV isolates. The SFI *a56r* inferred amino acid sequence contained a signature deletion also present in the sequences of other VACV-BR, such as Araçatuba virus (ARAV), Cantagalo virus (CTGV), Serro virus (SV2), Mariana virus (MARV), Guarani P2 virus (GP2V), Muria virus (MURV), and others, but absent in GuaraniP1 virus (GP1V), Belo Horizonte virus (VBH), BeAn58058 virus (BAV), and SPAN232 virus (SAV). The SFIs were sub-clustered together among several VACV isolates (Figure 1B). Although the SFIs isolates presented an nt change in *a56r* (A-C), the alignment of the VACV *a56r* regions indicates that the VACV-SFIs share an exclusive nucleotide substitution (C-G) upstream from the 18 nt deletion (Figure 1C). The observed nt change in VACV-SFIs may be a result of viral polymerase error-prone during viral replication in patient 1 or 2. Distinct OPV had been isolated from a same exanthematous lesion,² however, we believe that this is not the case of VACV-SFIs, based on lack of substantial genetic evidence of co-infection, as described in other studies.² Corroborating to this hypothe-

sis, the sequencing of *a26l* and *k2l* genes showed a perfect nt alignment (100% identity) among the two SFI isolates. Therefore, taking together molecular and epidemiological data, we believe that a VACV human-to-human transmission took place in this outbreak.

Additionally, sera samples from the patients were submitted to immunoglobulin G (IgG) and immunoglobulin M (IgM) OPV enzyme-linked immunosorbent assays (ELISAs) and to a 50% plaque-reduction neutralizing test (PRNT₅₀). Our results showed that patients 1 and 2 had developed anti-OPV IgG and IgM antibodies, respectively. Both patients presented neutralizing antibodies against OPV. Furthermore, we also analyzed several bovine sera samples from the farm where patient 1 worked as a milker during the outbreak. The bovine sera also presented anti-OPV IgG antibodies.

The number of poxvirus infections of humans has increased in recent years highlighting the importance of studies in this area. The BV is an occupational disease with no specific treatment or an available vaccine for cattle, the primary known host of VACV in Brazil.^{6,13,14}

During BV outbreaks, both bovines and humans develop exanthematous lesions. The VACV transmission to humans occurs primarily by direct contact with infected animals or occasionally with contaminated fomites. Few studies have reported cases of VACV-BR transmission among humans.¹⁴⁻¹⁶ However, VACV vaccine strains have been associated with several cases of human-to-human transmission in the United States¹⁷ with most related to contact transmission from smallpox vaccines among military personnel and their relatives. To our knowledge, only two cases of natural transmission have been described between humans during BV outbreaks,

although in these cases, no molecular, virological or phylogenetic analyses were used to confirm the intrafamilial transmission.^{15,16} Additionally, there is only one additional case reporting an intrafamilial transmission.¹⁴ In this study, we molecularly showed a case of VACV transmission between humans. We analyzed sera samples taken from a father and son by serological methods, which confirmed the infection by an OPV. Additionally, the molecular and phylogenetic analyses reinforced the diagnosis and suggested that the father and son were infected with the same virus.

Considering our molecular and epidemiological data, we believe that this was a case of VACV intrafamilial transmission during the BV outbreak that occurred in São Francisco de Itabapoana. Some previous studies have shown long-lasting stability of VACV and other OPVs particles under environmental conditions.^{8,18,19} Therefore, two hypotheses could explain such intrafamilial transmission: 1) direct contact between the father's lesion and the son's skin, and 2) VACV transmission occurring through contaminated fomites.

Our study reinforces the risk of non-occupational VACV transmission during BV outbreaks. Further studies are needed to elucidate the importance of human-to-human transmission during BV outbreaks and to indicate how this type of report is under-notified. A relevant question to be explored is why human-to-human VACV transmission after smallpox vaccination is much more frequently described than those observed (or notified) during BV outbreaks in Brazil. Nevertheless, we believe that this report provides one more clinically relevant piece in the VACV transmission chain, particularly considering the possibility of household transmission among infected milkers and immunosuppressed patients.

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