



Case Report

Detection of Anti-Orthopoxvirus Neutralizing Antibodies in Three Dogs and One Cat from a Household with a Confirmed Human Mpox Case in Brazil

Mariella Sousa Coêlho Maciel ^{1,2,3,†}, Adriana de Souza Andrade ^{2,3,†}, Ana Luiza Neri de Oliveira ²,
Silvia Hees de Carvalho ^{2,4}, Giliane de Souza Trindade ^{1,3}, Ana Gabriella Stoffella-Dutra ^{1,3}, Erna Geessien Kroon ^{1,3},
Mauricio Teixeira Lima ^{3,5} and Marco Antônio Campos ^{2,3,*}

¹ Departamento de Microbiologia, Universidade Federal de Minas Gerais, Belo Horizonte 31270901, MG, Brazil; mariellamaciel@hotmail.com (M.S.C.M.); gitrindade@yahoo.com.br (G.d.S.T.); anagstoffella@gmail.com (A.G.S.-D.); ernagkroon@gmail.com (E.G.K.)

² Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizonte 30190002, MG, Brazil; andradeadriana.ds@gmail.com (A.d.S.A.); nerianaluiza@gmail.com (A.L.N.d.O.); silviahees@outlook.com (S.H.d.C.)

³ Instituto Nacional de Ciência e Tecnologia em Poxvírus, Belo Horizonte 31270901, MG, Brazil; maurili15@gmail.com

⁴ Serviço de Doenças Infecciosas e Parasitárias, Hospital Eduardo de Menezes, Fundação Hospitalar do Estado de Minas Gerais, Belo Horizonte 30620470, MG, Brazil

⁵ Instituto Octávio Magalhães, Fundação Ezequiel Dias, Belo Horizonte 30510010, MG, Brazil; maurili15@gmail.com

* Correspondence: marcoantonioscampos@gmail.com or marco.campos@fiocruz.br

† These authors contributed equally as first authors.

Simple Summary

Mpox, caused by the monkeypox virus, an orthopoxvirus, is a zoonotic infectious disease of public health concern. While human-to-human transmission of the virus is well established, the role of domestic animals in infection remains poorly understood. Investigating how the virus transmits among humans, dogs, and cats can enhance our understanding of the disease and assist with developing strategies to limit its dissemination during future outbreaks. In this study, blood samples were collected from three dogs and one cat in a household where a confirmed human case of mpox had occurred. A test was performed to detect protective antibodies against orthopoxviruses. Our data demonstrated that these antibodies were present in all blood samples from the domestic animals. These results indicate that domestic animals were exposed to an orthopoxvirus. One possible explanation is exposure following contact with a person with confirmed mpox, raising the hypothesis of human-to-animal transmission within the household. Overall, these findings are consistent with orthopoxviruses exposure in domestic animals; however, they do not provide evidence of active infection, onward transmission, or a role in virus maintenance.

Abstract

Mpox is a zoonotic infectious disease caused by the species *Orthopoxvirus monkeypox* (MPV), which is becoming an increasing public health concern. While human-to-human transmission of the virus is well established, the role of domestic animals in MPV infection remains poorly understood. Investigating how the virus is transmitted among humans, dogs, and cats can improve our understanding of the disease's pathogenesis and help develop strategies to limit its dissemination during future outbreaks. In this study

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samples were collected from four domestic animals (three dogs and one cat) in May 2023. These animals lived in a household where a human mpox case had been confirmed by PCR in March 2023, approximately 2 months before animal sampling. A plaque reduction neutralization test (PRNT) using vaccinia virus was performed to detect anti-orthopoxvirus (OPV) neutralizing antibodies (nAbs). Our data demonstrated that anti-OPV nAbs were present in samples obtained from three dogs and one cat with neutralization titers ranging from 1:5 to 1:20. These results indicate that the domestic animals were exposed to OPVs after contact with an individual with confirmed mpox, suggesting possible human-to-animal transmission within a household. Nevertheless, due to the cross-reactive immune response among OPVs, and the endemic circulation of vaccinia virus in Brazil, it is not possible to determine the specific viral species based solely on PRNT results. Therefore, these serological findings should be interpreted as evidence of OPV's exposure rather than confirmed MPV infection.

Keywords: case report; mpox; domestic animals; orthopoxvirus; neutralizing antibodies; zoonosis

1. Introduction

Viruses from the genus *Orthopoxvirus* (OPVs) are among the main causes of public health concern. Among them, the species *Orthopoxvirus monkeypox* (MPV) is notable for causing mpox, a zoonotic disease linked to epidemiological outbreaks of varying severity, which can lead to serious or even fatal outcomes [1,2]. The clinical presentation of the disease is influenced by factors related to both the virus and the host, such as the viral clade and the host's immune status, respectively, with skin lesions being a hallmark of the disease [3,4]. Infections caused by Clade II MPVs are generally milder, while those caused by Clade I MPVs tend to be more severe, with mortality rates reaching up to 10.6% [5,6].

Since the outbreaks began in 2022, the disease has spread to several countries, including areas that were previously non-endemic [7]. Brazil is among the countries with the highest number of reported cases, documenting over 14,000 mpox cases between 2022 and 2026 [8]. MPV can infect humans, non-human primates, and other animals [9]. However, although human-to-human transmission has been well established, the involvement of domestic animals in MPV infection remains largely unexplored [10,11]. Given the potential public health implications of OPV infections, contact tracing of those with confirmed mpox, including assessment of domestic animals sharing the same environment, may contribute to a better understanding of viral circulation and transmission dynamics. In this context, the present study aimed to investigate evidence of OPV exposure in domestic animals residing in a household where a human mpox case had been confirmed by PCR. We hypothesized that close household contact with an infected individual could represent one possible source of OPV exposure for companion animals.

Based on the above, we evaluated the presence of anti-OPV neutralizing antibodies (nAbs) in samples collected from four domestic animals. Although the detection of nAbs by plaque reduction neutralization test (PRNT) does not allow specific identification of MPV due to the known cross-reactivity among OPVs [12,13], the identification of anti-OPV nAbs in samples from three dogs and one cat that had contact with mpox patients may suggest environmental exposure to MPV in this epidemiological setting, highlighting the need for enhanced surveillance strategies. This study contributes to the understanding of OPV circulation, suggesting that domestic animals may be exposed to OPVs, with possible implications for MPV transmission.

2. Methodology

2.1. Ethics

This study is part of a broader research project to identify cases of mpox, which was approved by the Fundação Oswaldo Cruz (Fiocruz-Minas) Ethics Committee (61832222.1.0000.5091) [14]. The samples from domestic animals analyzed in this research were collected after their owners signed the informed consent form and in accordance with the approval of the Universidade Federal de Minas Gerais (UFMG) Ethics Committee on the Use of Animals, under protocol number: 2/2022.

2.2. Samples and Study Population

Blood samples were collected by a veterinarian from four domestic animals, one male feline (cat) and three female canines (dogs), living in a household with a confirmed mpox case. The owners did not report the animals' ages or breeds. Samples were collected about two months after qPCR confirmation of the human case. Collection was done at home, and the samples were then transported to the Laboratório de Imunologia de Doenças Virais at Fundação Oswaldo Cruz (Fiocruz-Minas), where they were processed and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

The household consisted of 14 residents, including the index case. The index case presented with crusted mucocutaneous lesions affecting the face and oral cavity, as well as penile necrosis associated with dysuria and constipation. Mpox infection was confirmed by qPCR. All individuals within the family cluster reported some type of contact with the index case, ranging from sharing personal hygiene items to prolonged physical contact. Among the 13 identified contacts, four developed cutaneous lesions. Notably, a 3-year-old niece of the index case tested positive for MPV by PCR and viral isolation [14].

During the infectious period, the animals remained in the household and had frequent close contact with the index case, including physical interaction (e.g., petting and handling) and sharing household spaces and furniture. Although the duration and frequency of contact were not formally quantified, household members reported that such interactions occurred daily.

Information regarding whether the animals shared bedding with the patient was not systematically collected. Likewise, the duration and frequency of direct contact between the animals and the index case during the infectious period were not formally documented.

2.3. Cells and Virus

Vero cells (ATCC CCL-81) and BSC-40 cells (ATCC CRL-2761) obtained from American Type Culture Collection (ATCC) were used for viral replication and PRNT, respectively. The experiments were conducted with BSC-40 cells at passage 18. Cells were cultured in high-glucose Dulbecco's modified Eagle medium (DMEM) (Millipore Sigma, Burlington MA, USA) supplemented with 5% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), and 1% Penicillin-Streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). All cells were maintained at $37\text{ }^{\circ}\text{C}$, 5% CO_2 , and 95% humidity. The Western Reserve vaccinia virus (VACV-WR) used in this study was kindly provided by the Laboratório de Vírus at the Universidade Federal de Minas Gerais. The virus was replicated in Vero cells at the Laboratório de Imunologia das Doenças Virais at Fundação Oswaldo Cruz (Fiocruz-Minas), Belo Horizonte, Minas Gerais, Brazil, and titrated in BSC-40 cells, yielding a titer of 10^6 PFU/mL.

2.4. Plaque Reduction Neutralization Test

Neutralizing antibody titers were measured by PRNT using VACV-WR. Serum samples were heat-inactivated at 56 °C for 30 min, serially diluted, and then incubated with 200 plaque-forming units (PFU) of VACV-WR for 18 h. After that, confluent BSC-40 cells monolayers, seeded in 6-well plates, were infected with 300 µL of the serum-virus mixture and incubated at 37 °C for 1 h. Thereafter, 2 mL of DMEM with 2% FBS were added to each well of the plate, which was subsequently incubated at 37 °C, with 5% CO₂ atmosphere and 95% humidity. After 48 h, the plaques were treated with 3.7% formalin for 2 h and stained with crystal violet for 20 min.

Plaques were counted, and the percentage reduction was calculated relative to the virus control [15]. Samples were classified as positive or negative using the PRNT₅₀ cutoff, defined as the highest dilution that neutralized at least 50% of the virus. All analyses were performed in duplicate.

3. Case Report

General Characteristics of Study and Clinical Findings in Domestic Animals

In May 2023, blood samples were collected from four domestic animals (three dogs and one cat) contacts of a patient with a confirmed diagnosis of mpox (index case), in the city of Belo Horizonte, Minas Gerais, Southeast of Brazil. The animals had constant contact with their owners, they lived in the same household as the individual who was diagnosed with mpox and their close family contacts, who had volunteered to participate in a previous study.

Notably, according to medical records, the index case from a previous report experienced an onset of lesions in October 2022, but did not seek emergency care until March 2023, when the lesions had worsened. The individual was admitted to Hospital Eduardo de Menezes (HEM), in Belo Horizonte, Minas Gerais, Brazil, where PCR testing confirmed mpox [14].

Regarding animals, during the veterinary examination conducted on the day of sample collection, no lesions or scarring were observed in the domestic animals. The level of consciousness of the animals, as well as their posture and locomotion, were deemed normal. Veterinary professionals classified animals as normocardiac, normotensive, and normothermic. However, after the analyses, it was observed that all animal samples exhibited anti-OPV nAbs with neutralization titers ranging from 1:5 to 1:20, as determined by PRNT. A titer of 1:20 was observed in the feline, 1:5 in canine 1, 1:20 in canine 2, and 1:10 in canine 3 (Figure 1A,B).

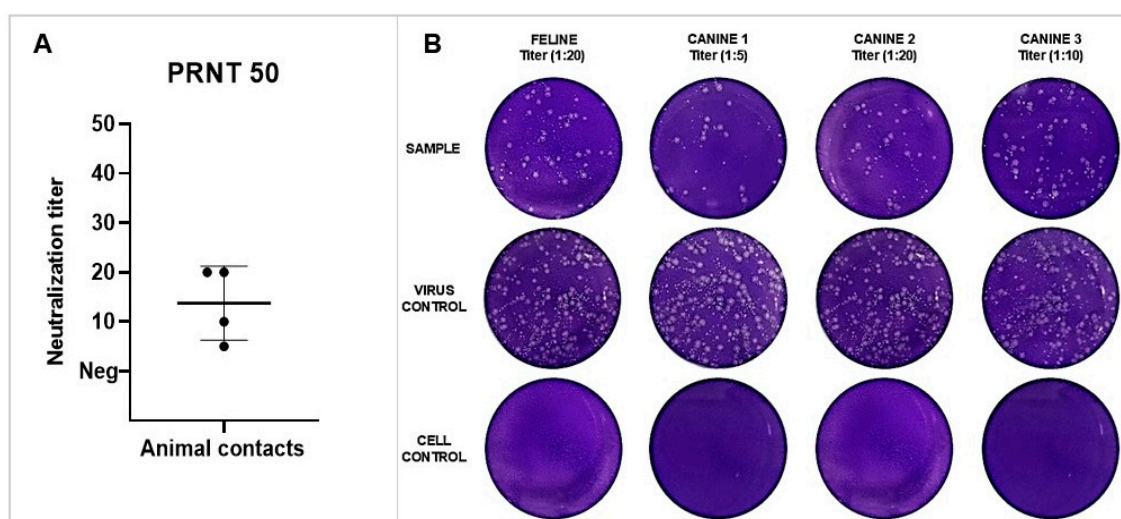


Figure 1. Viral neutralization in serum samples from domestic animals. **(A)** Neutralizing antibody titers measured by the plaque reduction neutralization test (PRNT). Each black dot represents the neutralizing antibody titer of an individual animal. The central black line represents the mean neutralization titer determined by PRNT₅₀, while the upper and lower black bars represent the standard deviation (SD). Neg indicates a negative result **(B)** Representative PRNT plate showing the wells used for titer determination. Virus-only and cell-only controls were included.

4. Discussion

Zoonotic diseases represent an important cause of morbidity and mortality worldwide and remain a constant public health concern. In this context, OPVs are highly relevant to both human and veterinary health, as they have been known to infect a variety of host species [16,17]. As with other viruses, OPV outbreaks may involve changes in transmission dynamics across species; however, the ecological and evolutionary mechanisms underlying these processes are not yet fully understood [17–19].

The detection of MPV or OPV-related material in domestic animals has been reported in different contexts, although the strength of evidence varies depending on the diagnostic approach used. For example, in France, a dog presenting with skin lesions was confirmed positive for MPV by molecular sequencing [20]. In the United States, MPV DNA has also been detected in samples from domestic animals, although detection of viral DNA alone does not necessarily indicate active infection or clinical disease [21]. Taken together, these studies illustrate that human-to-animal exposure to MPV or related OPVs may occur, although transmission pathways, infection dynamics, and clinical relevance in companion animals remain unclear [22,23]. In this study, we analyzed serum samples from one cat and three dogs living in the same household as the mpox index case. While no abnormalities were observed during the veterinary clinical examination, all serum samples tested positive for anti-OPV nAbs, with neutralization titers ranging from 1:5 to 1:20. The feline lived in the same household as the human mpox case, as reported during the home visit; however, detailed information on the frequency and intensity of human–animal contact, as well as potential environmental exposures outside the household, was not available. Additionally, it should be highlighted that antibody levels may have been influenced by the time elapsed between the PCR-confirmed human mpox case [14] and the collection of animal samples, which in this case was approximately two months.

In this context, given the chronology of events and the use of qPCR for diagnosing acute infection, the PRNT stands out as the gold standard for detecting neutralizing antibodies. This may indicate prior environmental exposure to OPVs. In Brazil, anti-OPV nAbs in samples from domestic animals were documented before 2022, including dogs and cats [24–26]. Most studies have focused on VACV, which is endemic in rural areas, although urban circulation has been reported [24,25,27]. This study has limitations, including the limited sample size and absence of controls to assess background OPV seroprevalence, and the lack of longitudinal sampling. Consequently, it was not possible to determine when exposure occurred, whether antibodies were present before the human mpox case, or the source of exposure. Moreover, the serological cross-reactivity among OPVs prevents definitive attribution of the antibody response to a specific OPV species by PRNT. However, the detection of antibodies in all animals from a household that has multiple confirmed MPV cases is noteworthy. Our findings highlight the need for further studies investigating the potential involvement of domestic animals, such as dogs and cats, in MPV transmission dynamics.

5. Conclusions

This case report provides serological evidence of OPV exposure in three dogs and one cat that lived in a household where a human mpox case was diagnosed. Although the absence of molecular confirmation precludes definitive attribution of the antibody responses to MPV, these findings indicate prior exposure to an OPV. The timing, source, and route of exposure cannot be determined from the available data, and both household and environmental sources remain possible. These results underscore the need for further studies to clarify the epidemiology of OPV exposure in domestic animals and to better understand their role in human–animal interface investigations.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Universidade Federal de Minas Gerais, protocol code 02.22, approved on February 21, 2022 for studies involving animals.

Informed Consent Statement: Informed consent was obtained from all owners of the animals involved in the study. The samples from domestic animals analyzed in this research were collected after their owners signed the informed consent form and in accordance with the approval of the Universidade Federal de Minas Gerais (UFMG) Ethics Committee on the Use of Animals, under protocol number: 2/2022.

Data Availability Statement: All manuscript data are enclosed in the manuscript itself.

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