

Counotte et al. systematically reviewed all available evidence on the risk for sexual transmission of Zika virus (5). Data from case reports, case series, cohort studies, in vitro work, and animal studies indicate that the infectious period for sexual transmission of Zika virus is considerably shorter than the period during which viral RNA can be detected in semen. As a result, the World Health Organization now recommends male travelers with potential Zika virus exposure delay conception for ≥ 3 months rather than ≥ 6 months (6).

In our case, Zika virus RNA might have persisted in semen because of failed immune clearance secondary to the patient's MRH or his immunosuppressive drug treatment. However, when advising returning male travelers in couples planning pregnancy, clinicians should be aware that Zika virus RNA shedding in semen might be intermittent and persist for longer in patients with immunosuppression.

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No Evidence for Role of Cutavirus in Malignant Melanoma

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Cutavirus was previously found in cutaneous melanoma. We detected cutavirus DNA in only 2/185 melanoma biopsies and in 0/52 melanoma metastases from patients in Germany. Viral DNA was localized in the upper epidermal layers. Swab specimens from healthy skin were cutavirus positive for 3.8% (9/237) of immunocompetent and 17.1% (35/205) of HIV-positive men.

Cutavirus, a novel human protoparvovirus with linear single-stranded DNA, has been detected in fecal samples from children with diarrhea and in cutaneous T-cell lymphomas (CTCL) (1,2). Recently, Mollerup et al. reported the identification of cutavirus in 1 of 10 cutaneous malignant melanomas using viral enrichment methods with high-throughput sequencing and real-time PCR (3). This discovery raised questions concerning tropism and pathogenicity of cutavirus in human skin. We performed a retrospective study to determine cutavirus DNA prevalence and viral load in a large collection of formalin-fixed paraffin-embedded tissue biopsy specimens of malignant melanomas and in forehead swabs of healthy skin of immunocompetent and HIV-positive persons in Germany.

We used 185 cutaneous malignant melanoma biopsy specimens from 179 patients and 52 melanoma metastases from 42 patients from Germany for analyses with cutavirus real-time PCR (Appendix, <http://wwwnc.cdc.gov/EID/article/25/8/19-0096-App1.pdf>). We detected cutavirus DNA only in 2 nodular malignant melanomas, located on the abdomen of a 64-year-old man (MM-A) and on the cheek of an 85-year-old woman (MM-B). Viral DNA loads in these biopsies were 0.3 (MM-A) and 2.8 (MM-B) cutavirus DNA copies per β -globin gene copy. None of the 52 analyzed metastases carried cutavirus DNA (Table). The cutavirus PCR results of the 2 melanomas could be confirmed by sequencing and by in situ hybridization. In both melanomas, the cutavirus DNA-specific signals could be

Table. Cutavirus DNA detection and DNA load in cutaneous malignant melanomas, melanoma metastases, and forehead swabs of healthy nonlesional skin from persons in Germany*

Sample type	No. samples analyzed	No. cutavirus DNA-positive samples† (%; 95% CI)	Median cutavirus DNA load (IQR)‡
Malignant melanoma tumor biopsies§¶	185	2 (1.1; 0.3–3.9)	0.30; 2.82#
Malignant melanoma metastases§**	52	0 (0; 0–6.9)	NA
Skin swabs of HIV-positive men§	205	35 (17.1; 12.5–22.8)	0.33 (0.66–3.81)
Skin swabs of healthy male controls§	237	9 (3.8; 2.0–7.1)	2.31 (0.19–11.72)

*Bold type indicates statistical significance. IQR, interquartile range; NA, not applicable.

†All samples were analyzed with CUTA-UPL5 real-time PCR as described in the Appendix (<https://wwwnc.cdc.gov/EID/article/25/8/19-0096-App1.pdf>). The formalin-fixed paraffin-embedded (FFPE) biopsies (melanomas and metastases) were also analyzed with 2 different real-time PCRs targeting the cutavirus nonstructural 1 gene (Appendix). These PCRs did not detect further cutavirus DNA-positive biopsies.

‡Cutavirus DNA load was determined in all cutavirus DNA-positive samples and was defined as cutavirus DNA copies per β -globin gene copy.

§Details of the biopsies and skin swab specimens are provided in the Appendix.

¶From 21 cutavirus DNA-negative malignant melanomas, fresh frozen tissue could be analyzed in addition to the FFPE tissue samples (CUTA-UPL5-PCR). Cutavirus DNA was not detected in any of the 21 fresh frozen tissue samples. The cellular input of the fresh frozen tissue samples ranged from 1,230 to 40,600 β -globin gene copies per 2 μ L extracted DNA (median 8,330, mean 10,892), indicating a high cellular input.

#Shown here are the viral DNA loads found in the 2 cutavirus DNA-positive nodular malignant melanomas, MM-A and MM-B.

**For 6 of the melanoma metastases, the primary tumor was also analyzed and was cutavirus DNA negative. The 2 patients with cutavirus DNA-positive melanoma biopsies (MM-A and MM-B) did not have metastatic disease.

detected only in the superficial layers and on the surface of the skin but not in the tumor cells (Appendix Figure).

To analyze the prevalence of cutavirus on healthy nonlesional skin, we used 442 forehead swab specimens from 237 immunocompetent men and 205 HIV-positive men that were available from a previous study (4) (Appendix). We found cutavirus DNA significantly more frequently on the skin of HIV-positive men than on the skin of healthy controls (17.1% vs. 3.8%; $p < 0.001$ by 2-sided χ^2 test; Table). Among HIV-positive men, we found a trend for a higher cutavirus prevalence in patients with AIDS compared with those without AIDS (14/59 [23.7%; 95% CI 14.7–36.0] vs. 19/140 [13.6%; 95% CI 8.9–20.2]; $p = 0.078$ by 2-sided χ^2 test). The range of viral DNA loads found in the 44 cutavirus-positive skin swabs was 0.004–268.75 (median 0.41; interquartile range [IQR] 0.0–3.57); there was no significant difference between HIV-negative and HIV-positive men ($p = 0.389$ by Mann-Whitney-U test; Table).

Mollerup et al. found cutavirus DNA in 1 of 10 melanomas from Denmark and suggested investigating the role of cutavirus in cutaneous cancer (3). We detected cutavirus DNA in only 2 of 185 melanoma biopsy specimens and in none of 52 metastases. In situ hybridization localized the viral DNA on the surface of the 2 cutavirus-positive melanomas and not within the malignant cells. Our data therefore argue against an oncogenic role of cutavirus in malignant melanoma.

Väisänen et al. found cutavirus DNA in 2.9% of 136 skin biopsy specimens from 123 organ transplant recipients and in none of 159 skin biopsy specimens of 98 healthy adults (5). In accordance with Väisänen et al., we also found cutavirus more frequently in immunosuppressed patients than in healthy (immunocompetent) adults. Their finding related to healthy adults is in contrast to our results; however, we analyzed not skin biopsy specimens but widespread skin swab specimens covering ≈ 10 cm² of forehead skin (4). Our cutavirus DNA prevalence data on normal skin of immunocompetent adults (3.8%) are in line with cutavirus IgG seroprevalence

rates reported for adults in Finland, Iran, and Kenya (4.2%–5.6%). Lower cutavirus IgG seroprevalence rates have been found in the United States (0%) and Iraq (1%) (6).

A pathogenic role of cutavirus has been investigated in further malignancies. Concerning CTCL, conflicting results have been reported. Phan et al. have found cutavirus DNA in 23.5% (4/17) (1) and Väisänen et al. in 16% (4/25) of CTCL of the mycosis fungoides type (5). Our group recently analyzed 189 biopsies of various cutaneous B- and T-cell lymphoma types and detected cutavirus DNA only in 5.8% of 104 mycosis fungoides biopsy specimens (7). In contrast, Bergallo et al. could not detect cutavirus in 55 CTCL samples (8). The in situ hybridization results of a cutavirus-positive mycosis fungoides sample analyzed by Phan et al. pointed to a localization of the viral DNA in the superficial parts of the lesion (1), similar to the results we show. Therefore, it remains unclear whether cutavirus plays a role in the development of CTCL. Recently, Dickinson et al. could not detect cutavirus in oropharyngeal and oral cavity squamous cell carcinomas (9).

In summary, our data on cutavirus DNA prevalence and localization argue against an oncogenic role of cutavirus in malignant melanoma. However, oncolytic properties of this virus or viral hit-and-run oncogenesis cannot be excluded (10). Cutavirus seems to be more frequent on healthy skin of immunosuppressed patients than on the skin of immunocompetent persons and could be part of the human skin virome. It is possible that cutavirus is an apathogenic virus shed from human skin.

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Intrafamily Transmission of Monkeypox Virus, Central African Republic, 2018

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Monkeypox is a rare viral zoonotic disease; primary infections are reported from remote forest areas of Central and West Africa. We report an investigation of a monkeypox outbreak in Lobaye, southwest Central African Republic, in October 2018.

Monkeypox, a zoonotic disease caused by an *Orthopoxvirus*, has clinical signs and symptoms in humans similar to smallpox and a case-fatality rate of 10% (1). The specific reservoir species for monkeypox virus remains, to a large extent, unidentified (2). Spillover events of monkeypox have been reported in remote forest areas of Central and West Africa. After zoonotic infection, the virus can be transmitted from person to person (1).

To date, human monkeypox outbreaks in the Central African Republic (CAR) have been small: ≈10 cases, restricted to a family or village. Primary infection in these outbreaks occurred from contact with wild fauna, with secondary transmission among close contacts in the community (3,4) and limited nosocomial transmission (5). Since 2000, the Virology Laboratory of the Institut Pasteur de Bangui (IP Bangui), a regional reference center for monkeypox, has reported 20 monkeypox outbreaks across several regions of CAR, totaling ≈100 cases, particularly in the region of Lobaye (3,4). In 2018 alone, IP Bangui investigated 6 different outbreaks in CAR, indicating a possible increase in frequency of outbreaks (6,7).

On September 27, 2018, a healthcare worker from Zomea Kaka healthcare center in Lobaye reported to IP Bangui about 3 cases of suspected monkeypox in an Aka Pygmy family. A 25-year-old female sought care at the health center, 10 km from her village, for maculopapular rash and lesions. She was afebrile. Her signs and symptoms indicated

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Appendix

Materials and Ethics Statement

Formalin-fixed paraffin-embedded (FFPE) biopsies of 185 cutaneous malignant melanomas and 52 melanoma metastases were available for analyses by cutavirus real-time PCR. The 185 melanoma biopsies (69 superficial spreading, 45 nodular, 16 lentigo-maligna, 5 acral, and 50 malignant melanomas without further subtyping) were collected from 179 patients from Germany (70 females, 109 males) during 2002–2017. The patients' mean age was 64.8 years (range 21–93). Because using fixed tissue can reduce the sensitivity of the PCR, matched fresh frozen tissue biopsies from 21 melanomas were analyzed in addition to the FFPE tissue samples. The 52 biopsies of melanoma metastases (28 subcutaneous, 16 lymph nodes, and 8 organ/tissue metastases) were collected from 42 patients (15 females, 27 males) during 2007–2017. The patients' mean age was 68.2 years (range 39–96).

From a previous study, 442 forehead swabs from 237 healthy men and 205 HIV-positive men were available. Characteristics of the HIV-positive patients and of the control subjects have been described in detail (1). All men were Caucasians from Germany and were free of skin cancer, skin infections, and acute or chronic inflammatory dermatoses.

The study was approved by the ethics review boards of the University of Witten/Herdecke (no. 166/2017) and of the University of Cologne (no. 14–132).

Methods

Real-Time PCR

Cutavirus real-time PCRs were designed using the Roche Universal ProbeLibrary—Assay Design Center tool (https://lifescience.roche.com/en_de/brands/universal-probe-library.html-assay-design-center).

All samples (biopsies and skin swabs) were screened for cutavirus VP1 gene sequences using primers CUTA-UPL5 fw: 5'-AACCAAACACACCGAACCAG-3' and CUTA-UPL5 rev: 5'-TGAAAAGGCTTACCTCTTTTGG-3', together with locked nucleic acid (LNA) probe UPL#5: 5'-CAGCCACA-3' (cat. no. 04685024001, Roche Diagnostics, <https://www.roche.com>).

In addition to CUTA-UPL5 PCR, all biopsies were analyzed with 2 further real-time PCRs targeting cutavirus NS1 gene sequences, CUTA-UPL48 PCR and a PCR using primers and probe as described by Mollerup et al. (2). CUTA-UPL48 PCR was performed with primers CUTA-UPL48 fw: 5'-CAAATTGAACCAAATGAAGTTGA-3' and CUTA-UPL48 rev: 5'-CATGGATGTGTAGGCCTGTG-3' with LNA probe UPL#48: 5'-ACTGGGAA-3' (Roche, cat. no. 04688082001). β -globin gene PCR was performed from all samples to rule out DNA degradation caused by tissue fixation and to determine the cellular input. Primers β -403f: 5'-TGGGTTTCTGATAGGCACTGACT-3' and β -532r: 5'-AACAGCATCAGGAGTGGACAGAT-3' with probe β -471pr: 5'-6FAM-TCTACCCTTGGACCCAGAGGTTCTTTGAGT-BBQ-3' were used, as previously described (3), with one modification to the probe: the TAMRA quencher was replaced by a BlackBerry quencher (BBQ).

All PCR reactions (20 μ L) were set up in Light Cycler 480 Probes Master (Roche) with 2 μ L of extracted template DNA (QIAamp DNA Mini Kit, QIAGEN, <https://www.qiagen.com>). The UPL assays contained 0.2 μ M of the respective forward and reverse primers and 0.1 μ M of the respective Universal LNA Probe (UPL; Roche). For β -globin gene PCR, 0.3 μ M of β -403f and β -532r and 0.2 μ M β -471pr were used. Cycling conditions on a Light Cycler 480 II (Roche) were 95°C, 60 sec followed by 45 cycles 95°C, 10 sec; 60°C, 30 sec; 72°C, 5 sec. The real-time PCR according to Mollerup et al. (2) was set up in LightCycler 480 Probes Master reagent (Roche), including 500 nM target specific primers and 200 nM fluorescently labeled probes, 1–2.5 μ L template DNA and H₂O ad 25 μ L (2).

Negative control samples (human placental DNA) were included in each run and never yielded fluorescence signals above background. Ten-fold dilution series from 10⁵ to 10 copies of cutavirus double-stranded (ds) DNA fragments (gBlock, IDT, <https://www.idtdna.com>) were used as standards and for calculation of the viral DNA copy number in cutavirus-positive samples. The slopes of the generated standard curves of the 3 cutavirus real-time PCR assays

demonstrated amplification efficiencies from 1.912 to 2.012. Cutavirus DNA load was defined as the number of cutavirus DNA copies per β -globin gene copy.

The analytical sensitivity of all real-time PCRs (CUTA-UPL5, CUTA-UPL48, Mollerup-PCR) was 1 DNA copy per reaction. Concerning the specificity of the CUTA-UPL5 assay, the forward primer does bind to several known cutavirus strains, as well as to the closely related bufavirus strains 1 and 3. The reverse primer contains 2 mismatches regarding bufavirus sequences. The CUTA-UPL48 forward primer and the LNA UPL48 contain mismatches concerning bufavirus 1 and 3 sequences, and the primers used in the real-time PCR described by Mollerup et al. (2) do not align with bufavirus sequences. We cannot exclude cross-reactivity of the CUTA-UPL5 and CUTA-UPL48 PCRs with bufavirus, but the specificity of the positive cutavirus PCR results of the 2 melanomas in our study was confirmed by sequencing and by in situ hybridization (Appendix Figure). Cutavirus-specific DNA sequences were found in skin swabs, as discussed later. Furthermore, bufavirus has so far been found only in fecal samples and in a nasal swab (5,6).

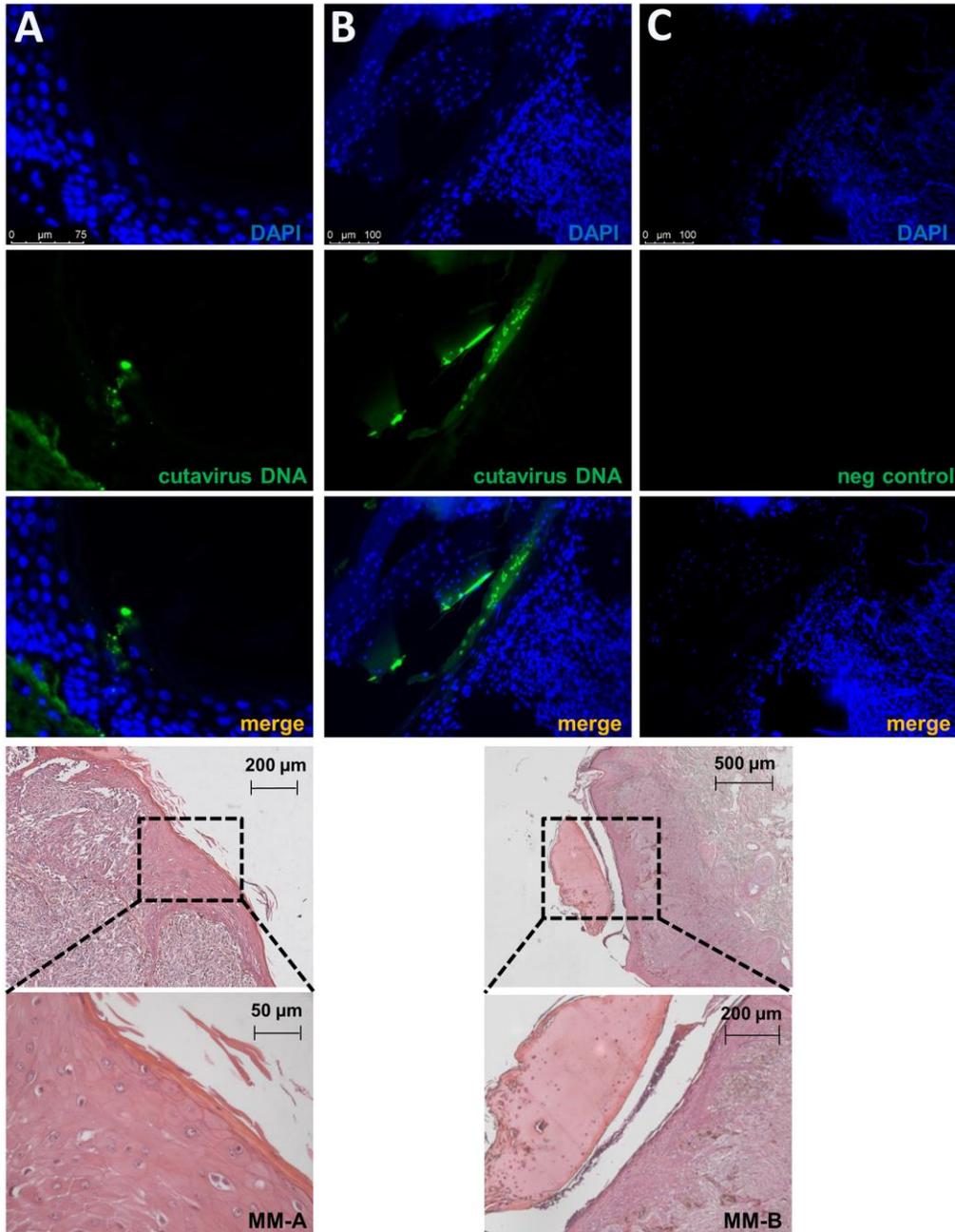
Confirmation of Positive Cutavirus PCR Results by Sequencing

The positive cutavirus PCR results of the 2 melanomas could be confirmed by sequencing (GenBank accession no. MK393127 for MM-B; 849 bp VP1/VP2 genes; 97% [825/849 bp] identity with cutavirus strain FR-D). Additionally, using VP1 primers described by Mollerup et al. (2), we obtained partial cutavirus VP1 gene sequences (306–522 nt) of different cutavirus strains (once each closely related to strains BR-337, BO-46, and FR-D, twice to strain CGG5–268) found in 5 forehead swabs of normal skin to confirm the specificity of the PCR results (GenBank accession nos. MK880125–MK880129).

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Appendix Figure. Detection of cutavirus DNA by in situ hybridization on the surface of two cutavirus PCR-positive malignant melanomas. A) Tumor MM-A; B, C) tumor MM-B. From top to bottom, a nuclear DAPI staining (blue), cutavirus DNA in situ hybridization (green, panels A and B; negative control in panel C), a merge of the green and blue fluorescent signals, and 2 different magnifications of routine histopathology (hematoxylin and eosin staining) highlighting the magnified tissue areas analyzed are shown. For the generation of the cutavirus probe, a dsDNA fragment (gBlock, IDT) spanning nucleotides 2505–2960 of cutavirus strain FR-D was cloned into the vector pJET1.2/blunt (Thermo Scientific,

<https://www.thermofisher.com>). The resulting cutavirus-specific vector and the empty vector (used as a negative control) were used as templates in DIG-Nick labeling reactions using the DIG-Nick translation mix (Roche Diagnostics). In situ hybridization of 4- μ m sections was performed, as described previously (4). Detection of digoxigenin-labeled probes was achieved with anti-digoxigenin-POD Fab fragments (Roche) and the TSA Plus Fluorescein System (Perkin-Elmer, <https://www.perkinelmer.com>). In addition, sections were stained with 4',6-diamidino-2-phenylindole (DAPI) (Merck, <https://www.merck.com>) and visualized by immunofluorescence microscopy.