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Viral loads in clinical samples of men with monkeypox virus infection: a French case series

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5 **Viral loads in clinical samples of men with Monkeypox virus infection: a French case**
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32

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39 transmission

40 **Abstract**

41 **Background.** Monkeypox virus (MPXV) is currently spreading among men who have sex
42 with men outside Sub-Saharan Africa, and close contact during sex seems to be the dominant
43 way of viral transmission in the current outbreak. Our aim was to describe the distribution of
44 MPXV in the human body, as it may play a role in its dissemination through sexual contact.

45 **Methods.** The study population consisted of patients with confirmed MPXV infection
46 attending the Pitié-Salpêtrière Hospital, in Paris, France, between May 20 and June 13, 2022,
47 who had been sampled from multiple anatomical sites, including skin, anus, throat, blood,
48 urine, and semen, at diagnosis and two weeks later. We compared the proportion of positive
49 samples and MPXV viral loads (given as PCR cycle thresholds, Ct) between anatomical sites,
50 and between day 0 (D0) and D14.

51 **Findings.** Overall, 356 samples were collected from 50 men, with a median age of 34 years
52 (IQR 29-40), 22 (44%) being HIV-negative on PrEP, and 22 (44%) living with HIV. MPXV
53 detection was more frequent from skin (44/50, 88%), anus (30/42, 71%), and throat (36/47,
54 77%) than from blood (13/45, 29%), urine (9/41, 22%) or semen (13/24, 54%). Viral loads
55 were significantly higher from skin lesions (Ct 19.8) and anus (Ct 20.9) than from throat (Ct
56 27.2), blood (Ct 32.8), urine (31.1) or semen (Ct 27.8). In the 24 patients with repeated
57 sampling, the proportion of positive samples strongly decreased between D0 and D14 at all
58 sites: skin (4/18, 22%), anus (2/22, 9%), throat (0/21, 0%), blood (1/21, 5%), urine (0/14,
59 0%), and semen (2/11, 9%).

60 **Interpretation.** These data contribute to a better understanding of how the virus may spread
61 between sexual partners over a relatively short period of time.

62 **Funding.** None.

63 **Research in context**

64 *Evidence before this study*

65 Scientific literature on monkeypox began in the 1970s, but articles on virological diagnosis
66 are more recent, and data on the detection of Monkeypox virus (MPXV) from certain
67 anatomical sites are limited. We searched PubMed for the terms “monkeypox AND (shedding
68 OR throat OR oropharynx OR nasopharynx OR saliva OR anus OR urine OR semen OR
69 blood OR plasma)” from inception up to July 31, 2022. We reviewed also epidemiological
70 reports from the World Health Organization (WHO) and the Centres for Disease Control and
71 Prevention (CDC). Almost all cases reported prior to the current monkeypox epidemic in
72 Europe and the USA did not include findings on the distribution of MPXV in the human
73 body, except for a series of seven cases recently reported by English colleagues, providing
74 longitudinal data on MPXV detection from skin, blood, and urine. There are no published
75 prospective cohorts with repeated sampling over time. Since the beginning of the current
76 outbreak (April 2022), the most significant data have included two series of monkeypox
77 cases. MPXV presence in semen has been investigated at least from one sample in 9 and 32
78 patients, with positivity rates ranging 78-91%. Reports on MPXV detection from upper
79 respiratory tract (including throat and nasopharynx) are scarce, as well as for MPXV detection
80 from other anatomical sites such as anus, blood, and urine. The most informative recent
81 publication is by Spanish colleagues, showing almost constant detection of MPXV from anal
82 and oral swabs, and inconsistent detection from urine samples (N=12 patients). Data on blood
83 are very few. We did not find any series of monkeypox cases with systematic multiple
84 sampling over time.

85

86 *Added value of this study*

87 Our case series is representative of the current outbreak of MPXV infection, spreading among
88 men who have sex with men (MSM) in non-endemic countries. The high prevalence of anal
89 and genital lesions suggests that sexual contact is the transmission route. We provide data
90 about MPXV viral loads from 50 French male individuals with confirmed monkeypox, who
91 were sampled from skin, throat, anus, blood, urine, and semen at diagnosis, and 14 days later
92 for 24 of them. We found a high proportion of MPXV-positive oral and anal swabs (71-77%),
93 a lower proportion of positive blood and urine samples (22-31%), and just over half of
94 positive semen samples at diagnosis. MPXV viral loads were high from skin and anus,
95 intermediate in throat and semen, and low in blood and urine. Viral clearance appeared to be
96 relatively rapid, with the vast majority of samples being MPXV-negative within 14 days.

97

98 *Implications of all the available evidence*

99 The detection of MPXV at significant levels in the anal region, in the mouth and in semen is
100 consistent with the sexual practices potentially involved in the spread of the virus among
101 MSM, in addition to skin contact related to sexual or non-sexual proximity. These data should
102 be popularised and disseminated within the gay community, with the aim of promoting
103 appropriate social and sexual behaviour to curb the epidemic.

104 **Introduction**

105 Monkeypox is a zoonotic disease, discovered in monkeys in 1958. The first human case was
106 confirmed in 1970, when the Monkeypox virus (MPXV) was isolated from a child in the
107 Democratic Republic of Congo (DRC).¹ MPXV incidentally causes disease in humans,
108 similar to smallpox, but with a much lower mortality, ranging from 0 to 10% for cases
109 reported after 2000.^{2,3} Symptoms are based on the triad of fever, lymphadenopathy, rash, and
110 very rare complications, such as encephalitis or profuse skin and mucous lesions, are
111 described.⁴

112 Monkeypox is endemic in West and Central Africa,⁵ with sporadic cases or limited outbreaks
113 in high-income countries, linked to the exotic pet trade and/or international travel.⁶ Two
114 distinct clades of MPXV have been characterized: Congo Basin (CB) and West African
115 (WA). The CB clade is associated with higher mortality and seems to transmit more
116 frequently between humans.⁷ The last epidemic with indigenous transmission in a high-
117 income country occurred in the USA in 2003, with 35 confirmed and 36 suspected cases,
118 following the importation of infected animals from Ghana.⁸

119 Since early May 2022, more than 42,000 cases of monkeypox have been reported in countries
120 with no historically reported monkeypox cases, especially in Europe and USA, among
121 individuals who have not travelled to endemic areas.⁹ This current epidemic appears to be
122 concentrated among men who have sex with men (MSM), a significant number of whom have
123 been involved at first in mass gathering events (e.g. the Maspalomas Gay Pride).

124 MPXV is known to be spread through close contact with lesions, body fluids, and respiratory
125 droplets of infected people or animals.¹⁰ However, sexual activity may drive MPXV
126 transmission. First reports show an unexpected number of monkeypox infected patients with
127 anal and genital lesions, which may also be associated with the sexual transmission route.¹¹⁻¹³

128 It is crucial to understand precisely how MPXV circulates from one individual to another, in

129 the context of sex with multiple partners, in order to adapt communication about the disease
130 and a key control of its spread.

131 In this context, we found it essential to investigate the distribution of MPXV, as well as its
132 viral loads according to anatomical locations, in the first patients with a confirmed infection.
133 We report here a series of French individuals with locally acquired cases of monkeypox, and
134 the results of the MPXV detection from several body samples, including semen, at time of
135 diagnosis and 2 weeks later.

136

137 **Methods**

138 This case series includes patients referred the Pitié-Salpêtrière hospital in Paris, France, from
139 20 May to 13 June 2022, with confirmed MPXV infection. Our main objective was to
140 describe the distribution of MPXV in the human body, as well as the viral loads associated
141 with different anatomical sites.

142 The infection was considered as confirmed in patients with compatible clinical lesions
143 associated with a positive MPXV PCR from any anatomical site. All patients who attended
144 our centre during this period were offered to be tested for MPXV from the following
145 anatomical sites: skin, throat, anus, urine, blood, and semen, in order to increase the
146 sensitivity of the diagnosis, and to provide them information on the risk of further
147 transmission. To be included in this series, they had to be tested for MPXV from at least 3
148 different anatomical sites at time of diagnosis (day 0, D0). Anal swabs were inserted 1 to 2
149 centimeters into the anal canal, regardless of the presence of anal lesions, and oral swabs were
150 rubbed on the tonsils, regardless of the presence of oral lesions. All patients were offered to
151 be tested for MPXV two weeks after the diagnosis (D14). The results presented here are from
152 patients who came back for their routine follow-up. Skin samples collected at D14 were from
153 the same lesion tested at D0, when it was still present.

154 Data on medical background, including HIV pre-exposure prophylaxis (PrEP) use, HIV
155 history for people living with HIV (PLWHIV), sexual behaviours, and symptoms evolution,
156 were collected as part of routine care and not as part of any research protocol. All these
157 variables derived from the case definition used in France at time of patients' management.

158 All patients signed a specific written consent form permitting the publication of their clinical
159 and biological data. Data from medical records were aggregated after de-identification.
160 According to the French law (n°78-17 of 6 January 1978 on Computers, Files and Liberties),
161 this study has been conducted in compliance with the CNIL (French National Agency
162 regulating Data Protection, n°2085894), and with the reference methodology 004.

163 All specimens were transported using triple security packaging, and handled in our biosafety
164 level 3 (BSL-3) Virology laboratory, according to French guidelines. DNA extraction was
165 performed on clinical samples using NucliSENS EasyMAG instrument (BioMérieux, Lyon,
166 France). The real-time MPXV PCR assay was performed on the LightCycler®480 platform
167 (Roche Diagnostics, Meylan, France). PCR reactions were carried out with the LightCycler®
168 DNA Hybridization Probes Master mix (Roche Diagnostics). PCR protocol was developed
169 after slight adaptations from the initial protocol previously reported.¹⁴ Results are given as
170 cycle threshold (Ct) values allowing the estimation of the viral load: detection of MPXV was
171 considered as negative in case of $Ct \geq 40$, weakly positive in case of $35 \leq Ct < 40$, and positive in
172 case of $Ct < 35$. MPXV has also been sequenced from several clinical samples by next
173 generation sequencing (MinION, Oxford Nanopore Technologies, Oxford, UK), in order to
174 determine the MPXV clade.

175 Analyses were performed using SPSS, version 25. Aggregate data is presented to maintain
176 case confidentiality. Chi-square tests were used to compare the proportions of
177 positive/negative samples according to anatomical sites. Kruskal-Wallis tests were used to
178 compare the Ct values according to anatomical sites. Wilcoxon tests were used to compare the

179 Ct values from paired samples, between D0 and D14. A p-value <0.05 was considered
180 significant.

181 There was no funding source for this study. The corresponding author had full access to all
182 the data in the study and had final responsibility for the decision to submit for publication.

183

184 **Results**

185 Fifty patients with confirmed monkeypox provided multiple clinical samples. All were males,
186 with a median age of 34 years (interquartile range [IQR] 29-40), and all except one were
187 MSM (Table 1). Twenty-two patients (44%) were HIV-negative on PrEP, and 22 (44%) were
188 PLWHIV. All people living with HIV had a recent (<6 months) undetectable HIV plasma
189 viral load on antiretroviral treatment, with the exception of one individual, in whom HIV
190 infection was discovered concurrently with MPXV infection. This patient is the only one who
191 was managed as an inpatient, to complete the initial investigation of HIV infection, and
192 because of severe anal pain. None received specific antiviral treatment for MPXV infection.
193 MPXV genomes have been sequenced from 24 distinct samples: all viruses belonged to the
194 WA hMPXV-1 clade, lineage B.1.

195 All patients presented with skin lesions, with genital and/or anal involvement in half the cases
196 (Table 2). The majority of eruptions were pauci-lesional, with less than 10 lesions. Eight
197 patients (16%) presented with tonsillitis often associated with intense odynophagia.

198 Overall, 249 samples were collected at time of diagnosis, with a median duration from the
199 onset of symptoms of 5 days (IQR 3-6). MPXV detection was more frequent from skin, anus,
200 and throat than from blood, urine or semen (Figure 1). The five patients with undetectable
201 MPXV from skin samples were positive from samples collected from the throat and/or the
202 anus. Regarding body fluids, MPXV was undetected or weakly detected in more than two
203 third of blood and urine samples, while it was detected in 13/24 (54%) of semen samples.

204 Where MPXV was detectable, viral loads, inversely reflected by Ct, were significantly higher
205 from skin lesions (Ct 19.8) and anus (Ct 20.9) than from throat (Ct 27.2), blood (Ct 32.8) or
206 semen (Ct 27.8) (Figure 2). MPXV viral load was higher from anus of patients with anal
207 ulcers (Ct 19.6 *versus* 25.3, $p=0.007$), higher from throat of patients with tonsillitis (Ct 23.5
208 *versus* 29.0, $p=0.007$), and higher from semen of patients with genital lesions (Ct 26.3 *versus*
209 30.2, $p=0.022$). There were no significant differences in MPXV PCR Ct values according to
210 HIV status.

211 Sampling was repeated in 24 patients 2 weeks after monkeypox diagnosis, with 107 samples
212 collected 19 days in median (IQR 18-21) from the onset of symptoms. The proportion of
213 positive samples, as well as MPXV viral loads, strongly decreased during this interval (Figure
214 1, Figure 3). Anal, oral, blood, urine, and semen samples were found negative or weakly
215 positive for MPXV in 20/22 patients (91%), 21/21 (100%), 14/14 (100%), 20/21 (95%) and
216 9/11 (82%) of cases, respectively. Four out 18 (22%) paired skin swabs remained positive;
217 skin lesions had fully disappeared, and swabbing was not possible in 6/24 patients at D14. Of
218 note, one anal sample was highly positive at D14 (Ct 21.3) while weakly positive at D0, in a
219 patient who presented with anal ulcers at time of diagnosis. We assume that the sample taken
220 at D0 was of poor quality. Overall, only 6 patients had samples with a $Ct < 35$ at D14 (of
221 which 4 were skin samples, 2 were anal samples, and 2 were semen samples). Two patients
222 were HIV co-infected, one with a CD4 cell count of $768/\text{mm}^3$ on antiretroviral therapy, and
223 the other with a CD4 cell count of $55/\text{mm}^3$ without antiretroviral therapy, as he had just been
224 tested positive for HIV. None of them had other concomitant diseases.

225

226 **Discussion**

227 Like a majority of patients infected with MPXV during the current outbreak in Europe and
228 United States, all patients of our series, except one, were MSM. In the 21 days before the

229 onset of symptoms, which corresponds to the maximal incubation period of monkeypox,⁴ they
230 had many sex partners. All previous reports of the current outbreak have identified close
231 sexual contact as the main risk factor of MPXV dissemination. The route of transmission of
232 the virus is therefore a crucial point. Transmission of MPXV has been shown to occur through
233 direct contact with skin lesions, body fluids or respiratory droplets from infected animals or
234 humans, or indirectly through contaminated fomites.¹⁰ By nature, sexual activity involves
235 intimate contact, which one would expect to increase the likelihood of transmission, whatever
236 a person's sexual orientation and irrespective of the mode of transmission. However, there has
237 been little research into whether MPXV can be transmitted sexually until now, i.e. through
238 sexual secretions containing the virus, and through oral, genital or anal mucosa. Herein, we
239 provide data on 356 clinical samples, collected at D0 and D14 from 50 confirmed cases of
240 monkeypox in Paris.

241 We detected MPXV in semen from a majority of men (83% at diagnosis) with confirmed
242 monkeypox, in line with other recent reports,^{13,15,16} suggesting a possible risk of human-to-
243 human transmission through male sexual secretions. This is concordant with previous
244 zoonotic virus like Zika virus^{17,18} and Ebola virus,^{19,20} even if it is probably not the dominant
245 transmission pathway. We also detected MPXV from oropharynx and anus of almost all the
246 patients reported here (87% and 88% at diagnosis, respectively), that can support the
247 transmission through oral and anal sex. A recent Spanish study showed a high rate of MPXV
248 detection in saliva (100%), nasopharynx (83%), and anus (92%), arguing for the same
249 conclusions, and also supporting the transmission of MPXV through breathing and kissing.¹⁶

250 The detection of MPXV was rarer in blood and urine. Our English and Spanish colleagues
251 demonstrated that the detection of MPXV in urine is not constant (never detected in 3/7 and
252 3/12 patients, respectively).^{16,21} The presence of viremia associated with monkeypox
253 symptoms has been studied by a few teams. In the 2003 US epidemic, only 3/12 patients

254 investigated were viremic,²² and of the 2 cases in Nigeria in 2018, both were viremic.²³ In the
255 English series, MPXV was detected in 6/7 patients at least once, rather at the beginning of the
256 disease, and the viremia seemed to be undetectable within 1-4 weeks.²¹ In our series, MPXV
257 was undetectable in urine from more than half of patients at diagnosis, and in blood from
258 about 20% of them.

259 We also found differences in MPXV viral loads depending on the anatomical sites and body
260 fluids collected: they were significantly higher from skin and anus than from throat, blood,
261 urine, and semen. MPXV detection from clinical samples performed 14 days after the
262 diagnosis seemed to support a rapid viral clearance in body fluids and anatomical
263 compartments.

264 We speculate that there may be a short viremia, accompanied by viral shedding in body fluids
265 such as urine and semen, at the beginning of the symptomatic phase of monkeypox, and that
266 systemic viral circulation ends before healing of the skin lesions. However, studies about
267 Ebola and Zika viruses have demonstrated that viral shedding in semen can continue for
268 several months after the viremic phase and after the end of symptoms, with a risk of late
269 secondary sexual transmission.¹⁷⁻²⁰

270 Our findings should be taken with caution. In one hand, we only had one sample per
271 anatomical site, and differences in sampling procedures could alter the outcome (especially
272 for skin scrubbing). We also did not have samples at D14 for half of the patients, which could
273 lead to a bias in the interpretation of the results, and not having samples available after D14
274 limits the ability to gather more information about viral kinetics. On the other hand, what we
275 detect with a molecular biology test is DNA and we have no evidence of the presence of
276 infectious viral particles, which is a key point in concluding on the potentiality of sexual
277 transmission of this disease. However, very recent data argue in favour of the infectivity of
278 MPXV in this context. Indeed, Italian colleagues have just demonstrated the viral viability of

279 MPXV isolated from semen (Ct=29.3) in a case of monkeypox, showing a clear cytopathic
280 effect 48 hours after inoculation of semen in Vero E6 cells.²⁴ These results should precede
281 longitudinal cohort studies exploring viral secretion in different body fluids over time,
282 associated with assessment of viral infectivity using cell culture.

283 We hope that these findings, in addition to other recent reports, will contribute to the debate
284 on sexual transmission of MPXV, will contribute to a better understanding of the risk of
285 MPXV dissemination to sexual partners, and will help define the best communication and
286 prevention strategies for the most affected communities. All sexually active MSM should be
287 informed about the presence of MPXV on the skin, in the throat, in the anus, and in body
288 fluids in case of infection, in order to avoid risky transmission contacts, whether sexual or
289 related to physical promiscuity during festive events.

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292 of the Infectious Diseases and Virology departments of the Pitié-Salpêtrière hospital who
293 provided care for the patient, and performed virological explorations.

294

295 **Transparency declaration**

296 The authors declare no conflicts of interest.

297

298 **Funding**

299 There was no funding source for this study. The corresponding author had full access to all
300 the data in the study and had final responsibility for the decision to submit for publication.

301

302 **Ethical statement**

303 All patients signed a specific written consent form permitting the publication of their clinical
304 and biological data. Data from medical records were aggregated after de-identification.
305 According to the French law (n°78-17 of 6 January 1978 on Computers, Files and Liberties),
306 this study has been conducted in compliance with the CNIL (French National Agency
307 regulating Data Protection, n°2085894), and with the reference methodology 004.

308

309 **Contributors**

310 RP, SB, GM, AN, AGM and VP contributed to conceptualisation of the article. RP, SB, GM,
311 AN, AB, SS, VB, CB, AG, YW, NG, AF, YT, TG, MO, ET, VL, SM, VC, AGM and VP
312 contributed to investigations for the article. RP, SB, AGM and VP supervised the
313 investigations. RP, SB, AGM and VP contributed to writing the original draft. GM, AN, AB,
314 SS, VB, CB, AG, YW, NG, AF, YT, TG, MO, ET, VL, SM and VC contributed to reviewing

315 and editing the manuscript. All authors had access to the data. RP, SB, AGM and VP accessed
316 the original data and vouch for its authenticity.

317

318 **Data sharing**

319 Individual participant data that underlie the results reported in this article may be shared, after
320 de-identification, for individual participant data meta-analysis. Proposals may be submitted up
321 to 36 months following article publication, and should be directed to the corresponding author
322 (RP).

323 **Table 1. Patients' characteristics at monkeypox diagnosis.**

Male, n (%)	50 (100)
Age, median (IQR)	34 (29-40)
History of anti-Smallpox virus vaccination, n (%)	6 (12)
Suspected transmission route, n (%)	
- Sex with men	49 (98)
- Sex with women	1 (2)
HIV coinfection, n (%)	22 (44)
PrEP use, n (%)	22 (44)
Number of sex partners in the previous 3 weeks, median (IQR)	5 (2-10)
Chemsex use in the previous 3 weeks, n (%)	15 (30)
Attendance at a sex on site event in the previous 3 weeks, n (%)	23 (46)
Medical setting at first admission, n (%)	
- Infectious Diseases Department	15 (30)
- Emergency Department	9 (18)
- General Practitioner	14 (28)
- Sexual Health Clinic	4 (8)
- Other Hospital	8 (16)

324 *NOTES.* IQR: interquartile range.

325

326 **Table 2. Patients' clinical presentation.**

Reported clinical features, n (%)	
- Skin lesions	50 (100)
- Fever	26 (52)
- Exhaustion	27 (54)

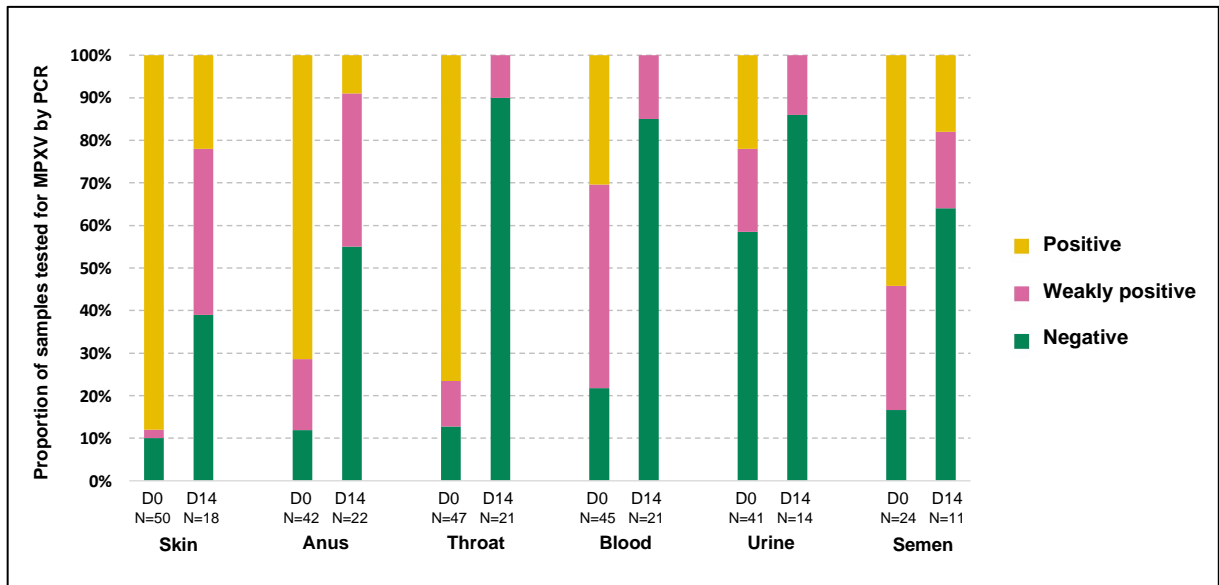
- Lymphadenopathy	27 (54)
- Headache	23 (46)
- Myalgia	19 (36)
- Rectitis ^a	16 (32)
- Tonsillitis	8 (16)
- Cough	3 (6)
Prodromes (general signs before skin lesions), n (%)	30 (60)
Number of skin lesions at time of monkeypox diagnosis, n (%)	
- 1	5 (10)
- 2-5	16 (32)
- 6-10	15 (30)
- 11-50	13 (26)
- >50	1 (2)
Localization of skin lesions at time of monkeypox diagnosis, n (%)	
- Body	36 (72)
- Face	22 (44)
- Palms and/or soles	12 (24)
- Genitals	25 (50)
- Anal margin	23 (46)

327 *NOTES.* IQR: interquartile range. a. Including anal pain, bleeding, and diarrhea.

328

329

330 **Figure 1. Proportion of clinical samples found positive, weakly positive and negative at**
 331 **diagnosis, using MPXV PCR.**

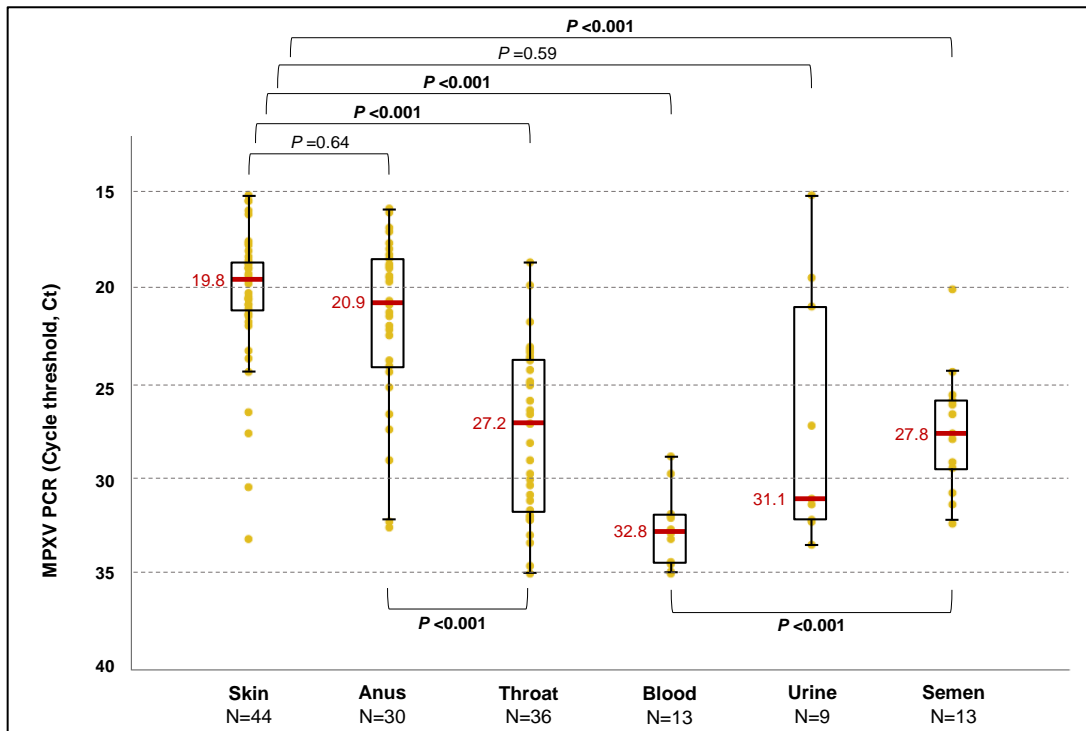


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334 **Figure 2. MPXV viral loads (given as cycle thresholds, Ct) according to sampled sites.**

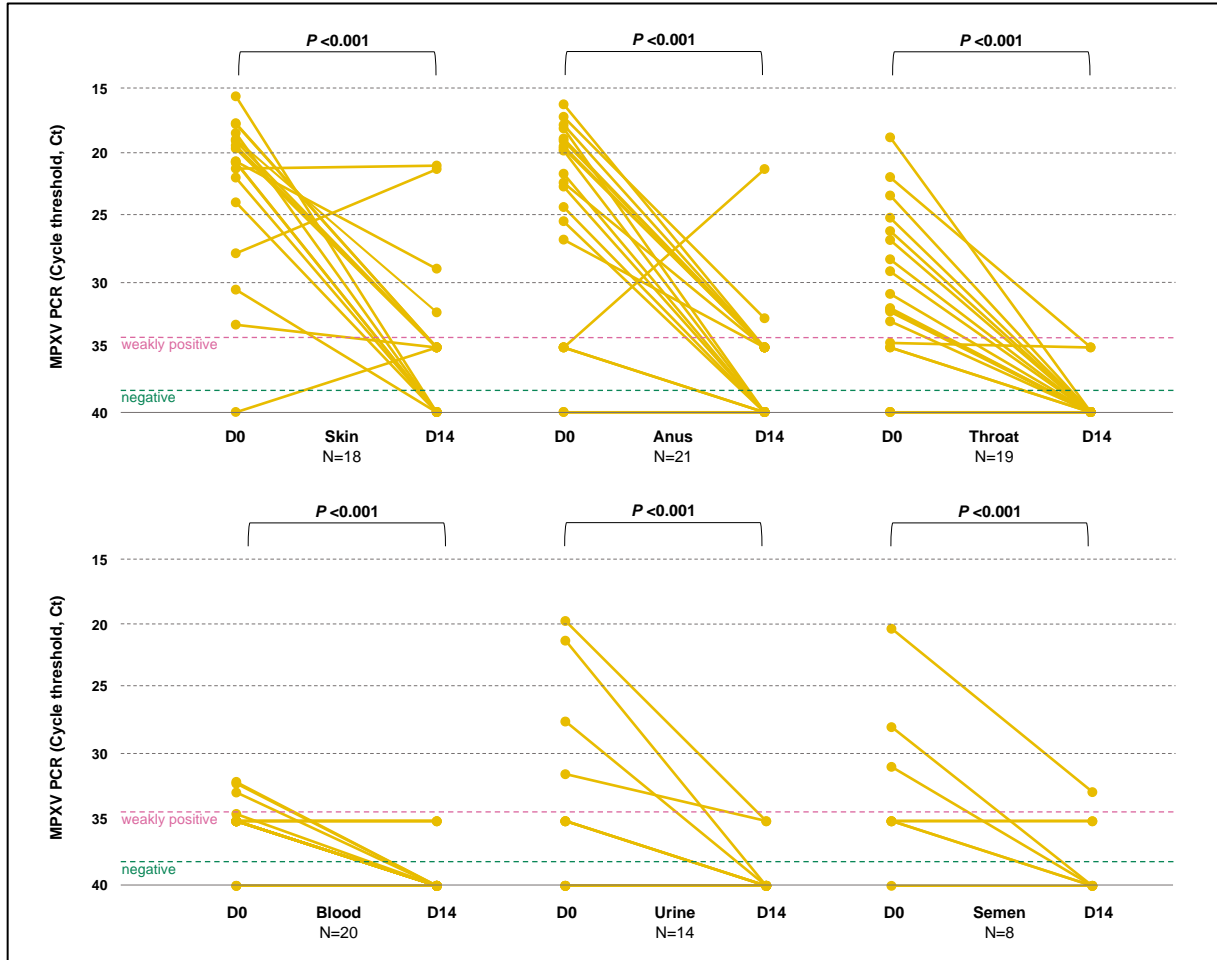
335 Clinical samples found weakly positive ($Ct \leq 35$) or negative using PCR were excluded for this
 336 analysis. Results are given as box plots, where red lines represent median Ct.



337

338

339 **Figure 3. Change in MPXV viral load (given as cycle threshold, Ct) between D0 and D14**
340 **according to sampled sites.** Only patients with paired samples (D0 and D14) were retained
341 for this analysis.



342
343

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