



Detection of Merkel cell polyomavirus on environmental surfaces

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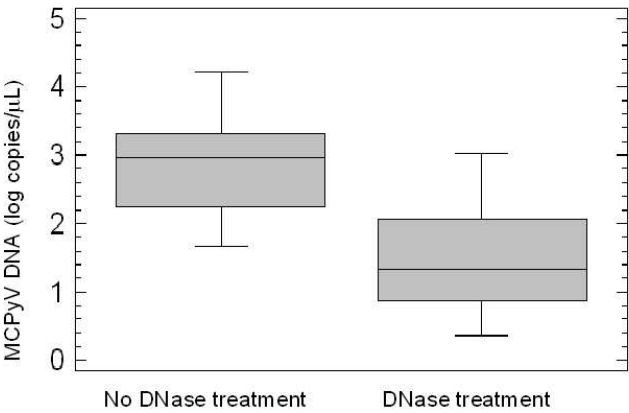


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**Detection of Merkel cell polyomavirus on environmental surfaces**

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Table 1 : Detection of human DNA, MCPyV DNA on environmental surfaces

		Human DNA			MCPyV
Environmental surfaces		Beta-globin target	LT3 target	VP2/3 target	Viral load (copies/ng of human DNA)
Lab.1	Incubator handle 1	+	+	+	2,875
	Incubator handle 2	+	+	+	20,226
	Incubator handle 3	+	+	+	52
	Main door handle	+	+	+	2,250
	Stairway banister	+	+	+	2,588
	Door handle 1	+	+	+	611
	Door handle2	+	+	+	167
	Freezer handle	+	+	+	100
	Lift keyboard	+	+	+	1,600
	Hood working surface	+	-	-	na
	Wall1	-	-	-	na
	Wall2	+	-	-	na
Lab.2	Drink dispenser keyboard 1	+	+	+	2,941

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	Drink dispenser keyboard 2	+	+	+	3,083
	Lift keyboard	+	+	+	1,333
	Computer keyboard and mouse	+	+	+	2,583
	Stairway banister	+	+	+	2,500
	Copy machine keyboard	+	+	+	829
	Wall	-	-	-	na
Lab.3	Main door handle	+	+	+	51,667
	Door handle 1	+	-	+	na
	Door handle 2	+	+	+	17,154
	Door handle 3	+	+	+	1,193
	Wall	-	-	-	na
Lab.4	Main door handle	+	+	+	838
	Entrance keyboard and ring	+	+	+	194
	Freezer handle	+	+	+	620
	Microscope	+	+	+	27
	Incubator handle	+	+	+	539

	Computer keyboard and mouse	+	+	+	112
	Wall	-	-	-	na
City	Streetcar door handle	+	+	+	471
	Ticket machine keyboard 1	+	+	+	182
	Ticket machine keyboard 2	+	+	+	625
	Cash machine keyboard	+	+	+	308
	Drink dispenser keyboard	+	+	+	209
Home1	Home door handle	+	-	-	na
	Refrigerator handle	+	+	+	38
	TV remote control	+	-	-	na
	Wall	-	-	-	na
Home2	Home door handle	+	+	+	1,000
	Refrigerator handle	+	+	+	400
	TV remote control	+	+	+	44
	Wall	-	-	-	na
Home3	Home door handle	+	+	+	7,686

Refrigerator handle	+	+	+	23,000
TV remote control	+	+	+	16,807
Wall	+	+	+	827
Home4 Home door handle	+	+	+	172
Refrigerator handle	+	+	+	28
TV remote control	+	+	+	325
Wall	-	-	-	na
Home5 Home door handle	+	-	+	na
Refrigerator handle	+	-	+	na
TV remote control	+	-	+	na
Wall	-	-	-	na
Home6 Home door handle	+	+	+	7
Refrigerator handle	+	-	-	na
TV remote control	+	-	-	na
Wall	-	-	-	na
na: not applicable				

1 Title

2 **Detection of Merkel cell polyomavirus on environmental surfaces**

4 Shortened title

5 **MCPyV on environmental surfaces**

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16 Key words: MCPyV, environment, surfaces

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Abstract

The Merkel cell polyomavirus (MCPyV) is a human virus identified recently which is associated with the Merkel cell carcinoma. This virus is also detected frequently in the skin of healthy individuals. The presence of MCPyV has been investigated on environmental surfaces in contact with human skin. Various surfaces in 4 laboratories, public places, and individual homes were swabbed. Human DNA and MCPyV DNA were detected in swabs by real-time PCR. MCPyV DNA levels were measured before and after DNase treatment in a set of 12 MCPyV DNA-positive samples. A total of 60 environmental surface samples were collected. Fifty-one (85.0%) were positive for human DNA detection and 45 (75.0%) were positive for MCPyV DNA detection. All samples positive for MCPyV DNA were positive for human DNA detection. After DNase treatment, a 1.3 log decrease in MCPyV DNA level was observed indicating that about 5% of viral DNA is protected from DNase degradation and might be associated with infectious virus. These results indicate that MCPyV DNA may be detected on environmental surfaces in contact with human skin. Detection of viral DNA might reflect the presence of infectious viral particles and transmission from environmental source to humans cannot be ruled out.

1 Introduction

2 The Merkel cell polyomavirus (MCPyV) is a novel human polyomavirus discovered in a rare
3 and aggressive neuroendocrine skin cancer, the Merkel cell carcinoma [Feng et al., 2008].
4 Subsequent studies have confirmed that, unlike the other human polyomaviruses, MCPyV
5 has an oncogenic potential [Shuda et al., 2008; Sastre-Garau et al., 2009; Shuda et al., 2009].
6 However, seroepidemiological studies indicate that MCPyV circulates widely in the human
7 population [Kean et al., 2009], and a widespread distribution of MCPyV in the human
8 population was suggested recently by studies reporting a frequent detection of MCPyV in
9 cutaneous swabs from healthy adults [Foulongne et al., 2010b; Scholwaller et al., 2010;
10 Wieland et al., 2010]. These studies indicate that MCPyV causes a persistent asymptomatic
11 infection of the most superficial layers of the epidermis. The route of transmission of MCPyV
12 remains uncertain. Possible respiratory or fecal-oral routes of transmission have been
13 suggested, supported by the detection of the virus in respiratory secretions, in saliva or in
14 urban sewages [Bialasiewicz et al., 2009; Loyo et al., 2010; Bofill-Mass et al., 2010]. The
15 high rate of detection of MCPyV DNA in the superficial skin might indicate a cutaneous route
16 of transmission through contact with human skin or contaminated surfaces. The aim of the
17 present study was to investigate the contamination of environmental surfaces by MCPyV.

19 Material and methods

20 *Environment samples*

21 Environmental surfaces were swabbed by laboratory workers wearing gloves, using cotton-
22 tipped swabs soaked in phosphate buffer saline (PBS). Samples were collected from various
23 surfaces located in the laboratory involved directly in the study (lab.1), in three other
24 laboratories not involved in MCPyV detection, in public environment within the Montpellier
25 city, and in six individual homes from lab. 1 workers. Swabs were transported at room

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1 temperature and conserved at -20°C until analyzed. Surfaces that were swabbed were chosen
2 depending on the environment, with a particular focus on objects with frequent contact with
3 human skin. They were various handles (doors, refrigerators, freezers, incubators), keyboards
4 (lifts, computers, drink dispensers, cash dispensers, ticket machines) and stair banisters. In
5 each environment, surfaces not exposed to contact with human skin, such as walls sampled
6 around 2 m off the ground, or hood working surfaces in the laboratories, were swabbed as
7 controls (Table 1).

8
9 ***DNA extraction and DNase treatment***

10 Swabs were suspended in 600 µL of PBS and DNA was extracted from 200 µL of the
11 suspension with the EasyMag nucleic acid extractor (BioMérieux, Marcy l'Etoile, France).
12 For DNase treatment, the residual suspension was separated into two 200 µL fractions. The
13 10X DNase buffer was added and 2 IU of DNase (New England Biolabs, Ipswich, MA) was
14 added in one fraction, the other being completed with the corresponding volume of PBS. Both
15 fractions were incubated 1h at 37°C and heated 10 min at 95°C for DNase inactivation prior
16 DNA extraction. The elution volume was 50 µL, and 5 µL of eluate was used for subsequent
17 PCR assays. A non-used swab processed under the same conditions was included in each run
18 as negative control.

19
20 ***MCPyV and human DNA quantitation***

21 MCPyV DNA was detected and quantified by real-time PCR in the large T-antigen coding
22 region as previously described [Foulongne et al., 2010a]. Positive results were confirmed by a
23 second real-time PCR targeting the VP2/3 region [Bialasiewicz et al., 2009], located outside
24 the regions amplified previously in the laboratory.

1 Total human DNA levels in samples were measured using the LightCycler control DNA kit
2 (Roche Diagnostics, Meylan, France) and results were expressed as MCPyV DNA copies/ ng
3 of human DNA or as MCPyV copies/ μ L for sample comparison when DNase treatment was
4 applied.

6 Results

7 A total of 60 surface samples were collected. Fifty-one (85%) were positive for human DNA
8 detection and 45 (75%) were positive for MCPyV DNA detection (Table 1). MCPyV DNA
9 positivity was 81%, 100% and 67% for samples collected in laboratories, city and individual
10 homes, respectively ($P = 0.2$). Human DNA levels in positive samples ranged from 5.0 to
11 2,700 pg/ μ L (median, 25 pg/ μ L). All MCPyV-positive samples were positive for human
12 DNA detection. Among the 11 samples collected on walls, 9 (81.8%) were negative for
13 human DNA and 10 (90.1%) were negative for MCPyV DNA.

14 The median MCPyV DNA level was 2.8 log copies/ng of human DNA (range 0.8-5.2 log
15 copies/ng of human DNA). No correlation was observed between MCPyV DNA and human
16 DNA levels ($R^2 = 0.007$).

17 A set of 12 samples positive for MCPyV DNA were processed in parallel with and without
18 DNase treatment. Median human DNA level in these samples was 97 pg/ μ L (range 10-762
19 pg/ μ L). Median viral DNA load in these samples was 2.45 log copies/ μ L (range, 1.6-4.2 log
20 copies/ μ L). After DNase treatment, MCPyV DNA was still detectable in 10 samples with a
21 median level that decreased to 1.14 log copies/ μ L (range 0.7-3.8 log copies/ μ L), representing
22 a decrease of 1.3 log in MCPyV DNA level and indicating that about 5% of viral DNA was
23 protected from DNase degradation (Fig.1). In contrast, in the same conditions human DNA
24 became undetectable after DNase treatment of the samples.

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Discussion

Polyomaviruses are non-enveloped, double stranded DNA viruses that may persist in various environmental media [Bofill-Mass et al., 2001; Jiang et al., 2009]. Results from the present study indicate that MCPyV DNA is detected with a high frequency on the environmental surfaces.

High viral DNA levels despite low human DNA levels might be explained by the fact that the cutaneous viruses are abundantly present in the most superficial layers of the epidermidis, the stratum corneum, composed mainly of dead and apoptotic cells with genomic DNA fragmented by host cells DNases that might not affect viral DNA. This might also explain why levels of MCPyV DNA, as normalized to human DNA, in environment samples were higher than those reported previously in human skins specimens [Foulongne et al., 2010b].

The relative high level of MCPyV DNA normalized to human DNA on environmental surfaces might be explained also by an accumulation of viral DNA more resistant to degradation than human DNA. This cumulative effect may also explain the highest, although not significant, MCPyV DNA detection rate observed in the public environment, since the objects sampled were more prone to be handled by many people as compared with those sampled in individual homes.

Infectivity of the viruses detected on environmental surfaces could not be investigated since neither animal nor cellular models are available. However, viral MCPyV DNA remained detectable after DNase treatment prior to nucleic acid extraction. A major proportion of the viral DNA was digested by the DNase, suggesting that this viral DNA is not protected by an entire viral capsid. However, the about 5% of viral DNA that remained protected from degradation might represent encapsidated DNA belonging to potentially infectious virions.

This is in accordance with a recent study reporting the cutaneous shedding of MCPyV and novel polyomaviruses in the form of virions [Scholwaller et al., 2010]. Culture-independent

1 methods are widely used to monitor levels of environmental contamination with
2 microorganisms and recent studies have demonstrated that viral genetic markers from both
3 viral particles and naked viral genome persist for a longer time than infectious virions in
4 environment [Walters et al., 2009]. However, the infectious potential of the viral genomic
5 material detected on the environmental surfaces can not be excluded.

6 Numerous environmental media are contaminated with polyomaviruses. This was described
7 for SV40, isolated from waste in monkeys' cage [Bofill-Mass et al., 2004] as well as for the
8 two human JC and BK polyomaviruses that could be easily detected in urban sewage samples
9 [Bofill-Mass et al., 2001; Hundesa et al., 2006]. Recent findings of MCPyV DNA in various
10 samples like saliva [Loyo et al., 2010], respiratory samples [Bialasiewicz et al., 2009] as well
11 as in sewage samples [Bofill-Mass et al., 2010] are consistent with the ubiquitous MCPyV
12 shedding. Results obtained in this study reinforce this hypothesis.

13 In conclusion, the present study indicates that MCPyV DNA may be detected on virtually all
14 the environmental surfaces in contact with human skin. Detection of viral DNA might reflect
15 the presence of infectious viral particles and transmission from environmental source to
16 humans cannot be ruled out.

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Figure 1

Effect of DNase treatment on MCPyV DNA level. Data are presented as box and whiskers plots, where the boxes represent the interquartile range, the lines within the boxes represent the median value, and the whiskers represent 1.5 times the interquartile range.

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