Title: Lausannevirus seroprevalence among asymptomatic young adults.
Authors: Mueller L, Baud D, Bertelli C, Greub G
Journal: Intervirology
Year: 2013
Volume: 56
Issue: 6
Pages: 430-3
DOI: 10.1159/000354565

Lausannevirus seroprevalence among asymptomatic young adults

Authors:

Linda Mueller[1], David Baud[1, 2], Claire Bertelli[1,3] and Gilbert Greub[1, 4]

[1] Institute of Microbiology, University Hospital Centre and University of Lausanne, Lausanne, Switzerland

[2] Materno-fetal and Obstetrics Research Unit, Department of Obstetrics and Gynaecology, Maternity, University Hospital, Lausanne, Switzerland

[3] SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

[4] Service of Infectious Disease, University Hospital Centre and University of Lausanne, Lausanne, Switzerland

Corresponding author: Gilbert Greub

Center for Research on Intracellular Bacteria (CRIB)

Institute of Microbiology

University Hospital Center and University of Lausanne

Bugnon 48

1011 Lausanne

SWITZERLAND

Phone: (00) 41 21 314 49 79

Fax: (00) 41 21 314 40 60

Email: gilbert.greub@chuv.ch

Keywords: Megavirales, Marseilleviridae, Lausannevirus, serology, prevalence, Chlamydia, Immunofluorescence, cross-reactivity

Word count = 1393

Abstract word count= 207

ABSTRACT

Objectives: The giant Lausannevirus was recently identified as a parasite of amoeba that replicates rapidly in these professional phagocytes. This study aimed at assessing Lausannevirus seroprevalence among asymptomatic young men in Switzerland and hopefully identifying possible sources of contact with this giant virus.

Methods: The presence of anti-Lausannevirus antibodies was assessed in sera from 517 asymptomatic volunteers that filled a detailed questionnaire. The co-reactivity between Lausannevirus and amoeba-resisting bacteria was assessed.

Results: Lausannevirus prevalence ranged from 1.74 to 2.51%. Sporadic condom use or multiple sexual partners, although frequent (respectively 53.97% and 60.35%), were not associated with anti-Lausannevirus antibodies. On the contrary, frequent outdoor sport practice as well as milk consumption were significantly associated with positive Lausannevirus serologies (p-value=0.0066 and p-value=0.028, respectively). Co-reactivity analyses revealed an association between *Criblamydia sequanensis* (an amoeba-resisting bacterium present in water environments) and Lausannevirus seropositivity (p-value=0.001).

Conclusions: Lausannevirus seroprevalence is low in asymptomatic Swiss men. However, the association between virus seropositivity and frequent sport practice suggests that this member of the *Megavirales* may be transmitted by aerosols and/or exposure to specific outdoor environments. Milk intake was also associated with seropositivity. Whereas the co-reactivity observed for *Criblamydia sequanensis* and Lausannevirus reflects a common mode of acquisition or some unexpected cross-reactivity remains to be determined.

INTRODUCTION

Lausannevirus is a new member of the *Marseilleviridae* family, which is part of the *Megavirales* [1]. It has been discovered by amoebal co-culture in 2005 from a sample collected in the Seine river (Paris, France) [2]. Lausannevirus is rapidly growing in amoebal co-culture using *Acanthamoeba* as cell background, with a 3 log increase in 16 hours and complete amoebal lysis after about 24h [2].

The *Marseilleviridae* family contains two more members: Marseillevirus [3] and Senegalvirus [4]. The latter strain was recovered from the stools of an asymptomatic individual in Senegal, demonstrating that humans may be exposed to these large viruses. It is thus important to study the ecology and the possible medical importance of Lausannevirus and other *Marseilleviridae* in addition to understanding their biology.

The genome of Lausannevirus is 346,754 bp long and contains 450 genes. It exhibits a strong synteny with the 2 distal thirds of the genome of Marseillevirus [1,2]. Interestingly, this icosahedral virus has a size of about 200 nm, allowing its observation by optical microscope [2]. This allowed the straightforward development of a micro-immunofluorescence assay using whole Lausannevirus viral particle as antigen.

This serological assay was then applied to 517 asymptomatic young adults, in order to determine the seroprevalence and ideally identify some specific source of exposure to Lausannevirus.

METHODS

Patients and Statistical analyses: We took advantage of the availability of sera from previous seroprevalence studies on *Chlamydia trachomatis* and *Chlamydia*-like organisms [5,6]. All Swiss young men who presented at the medical entry examination at the Army recruitment Centre of Lausanne in winter 2006-2007 were enrolled in this study. A questionnaire was filled to collect demographic data, sexual and behavioural risk as well as animal exposure. Sera were analyzed by immunofluorescence (see below), using as antigen Lausannevirus particles grown in amoebae and purified by ultracentrifugation (see below).

Statistical analyses were performed using R [7].

Growth of Lausannevirus: Lausannevirus was grown in *Acanthamoeba castellanii* ATCC 30010 as described previously [2]. Briefly, we filtered at 5µm a one week infected *A. castellanii* flask grown in peptone yeast-extract glucose (PYG) at 32°C and we re-infected a new flask with the filtrate. Five days later, the virus was harvested and flask supernatant was centrifuged at 5000 *g* for 15 min. The supernatant was then collected and filtered at 5µm to remove residual amoebal cells. The filtrate was then centrifuged at 35'000 *g* for 1h and the virus pellet was resuspended in 1 ml of PBS.

Immunofluorescence: Sera were investigated as described previously [8], by micro-immunofluorescence (MIF), using formaldehyde-inactivated viral particles as antigen. Briefly, sera were screened for Lausannevirus antibodies at a dilution of 1:64 with FluolineH. Mice polyclonal anti-Lausannevirus antibodies were used as positive control and PBS was used as negative control. IgG and IgM reactivity were tested for sera exhibiting a total Ig titre ≥1:64, as previously described [9]. IgG and IgM positivity cut-offs were ≥1:32. Blind lecture of each micro-immunofluorescence was performed by two independent observers. A targeted lecture of 11 doubtful samples was performed by a third reader.

RESULTS

According to the first reader, among the 517 volunteers tested, aged from 18 to 26 years, 13 exhibited antibody reactivity against Lausannevirus, corresponding to a Lausannevirus seroprevalence of 2.51%. Among the 13 seropositive patients identified by the first reader, 9 were confirmed by at least one of the additional readers, reducing the seroprevalence rate to 1.74%. Considering all 13 positive patients, demographic, social and behavioural characteristics as well as animal contact are reported in Table 1. Among the seropositive volunteers, 84.6% were Swiss, 61.5% lived in cities with more than 10'000 inhabitants and 61.5% were students. A total of 84.5% of participants reported to be sexually-active and 60.35% of them admitted to have had more than two previous partners. However, no correlation was observed between Lausannevirus seropositivity and sexual behaviour (Table 1). Among the 517 volunteers 58.79% had contact with animals but, again, no association has been observed (Table 1). Other behavioural risks have also been analysed, showing no correlation between virus infection and smoking, alcohol intake or drug consumption (Table 1). On the contrary, frequent sport practice and milk consumption were associated with Lausannevirus seropositivity (p-value=0.0066 and p-value=0.028 respectively). Analysis on the 9 patients showing anti-Lausannevirus antibodies reported that the correlation between Lausannevirus seropositivity and milk consumption was maintained (p-value=0.02). On the contrary the correlation with a frequent sport activity was no longer statistically significant (p-value=0.06).

DISCUSSION

This first Lausannevirus sero-epidemiological study performed among 517 young asymptomatic Swiss adults demonstrated that humans might be exposed to Lausannevirus and/or other related cross-reactive viruses (Table 1). The relatively low seroprevalence observed (1.74-2.51%) may be explained by the characteristics of the target population which is composed exclusively of asymptomatic young Swiss men. Since this is the first study investigating Lausannevirus seroprevalence, no data from others countries or from different target populations are available to draw any comparison.

Further investigations revealed no correlation between seropositivity and sexual behaviour or animal contact, excluding sexual and zoonotic exposure to Lausannevirus (Table 1). Frequent sport practice has been shown to be strongly associated with seropositivity (p-value=0.0066). Sportspeople have an active social life, allowing meeting and socializing with many persons sharing the same exercise environments, as example, gyms or swimming pool. Also, most of the sports are practiced outdoor (as example mountain or forests), which may represent another exposure route. Taken together, these observations suggest that human get in contact with Lausannevirus by exposure to water or soil during sportive activities. Milk consumption has also been shown to be associated with Lausannevirus seropositivity (p-value=0.028). It is possible that cows may shed Lausannevirus particles in the milk, however this remains to be studied. Since previous studies focused on *C. trachomatis* and *Chlamydia*-like prevalence on the same target population [5,9], we investigated for a potential co-reactivity between these bacterial species and Lausannevirus. Unexpectedly, we observed a strong association between Lausannevirus seropositivity and antibodies directed against *C. sequanensis* (p-value=0.001) (Table 2). Conversely, we observed no co-reactivity between Lausannevirus and *W. chondrophila*, which has been identified in bovine abortion and which is considered to be a possible zoonotic agent [6,8].

Co-reactivity with *C. sequanensis* may be due to a common mode of exposure because *C. sequanensis* is a *Chlamydia*-related bacterium generally associated with water and free-living amoebae and Lausannevirus has been isolated from water by amoebal co-culture. Thus, amoebae might act as a reservoir of Lausannevirus and human might get infected following exposure to contaminated water, similarly to what is known for *Legionella* [10]. Whether amoebae act as a Trojan horse by increasing the viral infectivity remains to be determined. Nevertheless, the resistance to amoebal microbicidal effectors may be associated with resistance to microbicidal effectors of human macrophages [11]. Conversely, the absence of co-reactivity with *C. trachomatis* (p-value>0.05), the most common sexually transmitted bacteria, suggests that Lausannevirus is not sexually transmitted and is congruent with our observed absence of association between seroprevalence and risky sexual behavior. However, due to the limited number of patients exhibiting anti-*C. trachomatis* antibodies, we were not able to definitely rule out a sexual transmission.

Co-reactivity with *C. sequanensis* may also arise as a result of cross-reactivity with a shared epitope. However, this latter hypothesis is unlikely, since Lausannevirus is distantly related to *C. sequanensis*. BLASTP analysis for small highly conserved peptides between these two microorganisms revealed that only one peptide of 10 amino acids in the deoxyuridine 5'-triphosphate nucleotidohydrolase is identical (100%). Thus, the recognition of a linear epitope is unlikely. However we are not able to exclude a possible recognition of conformational epitopes. Although the described co-reactivity between Lausannevirus and an amoeba-resisting bacterium suggests a seroconversion following infection by Lausannevirus-containing amoebae, we cannot exclude the possibility that Lausannevirus is able to directly infect humans, without using amoebae as a Trojan horse. In fact, recent studies demonstrated the presence of viruses of *Marseilleviridae* and *Mimiviridae* families in human samples [1,12].

For the future, we suggest to expand the seroprevalence studies to other countries, as for example France and Senegal, from where Lausannevirus, Marseillevirus and Senegalvirus have been isolated. Furthermore, the sampling population should be increased and include persons with various infectious diseases of unknown etiology, people from different ages, as well as women, since seroprevalence may be gender dependent. Moreover, it will be interesting to evaluate whether Lausannevirus is circulating in the blood of asymptomatic subjects by using a specific PCR, which exhibits a high sensitivity.

REFERENCES

1 Colson P, Fancello L, Gimenez G, Armougom F, Desnues C, Fournous G, Yoosuf N, Million M, La Scola B, Raoult D: Evidence of the megavirome in humans. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2013;57:191-200.

2 Thomas V, Bertelli C, Collyn F, Casson N, Telenti A, Goesmann A, Croxatto A, Greub G: Lausannevirus, a giant amoebal virus encoding histone doublets. Environmental microbiology 2011;13:1454-1466.

3 Boyer M, Yutin N, Pagnier I, Barrassi L, Fournous G, Espinosa L, Robert C, Azza S, Sun S, Rossmann MG, Suzan-Monti M, La Scola B, Koonin EV, Raoult D: Giant marseillevirus highlights the role of amoebae as a melting pot in emergence of chimeric microorganisms. Proceedings of the National Academy of Sciences of the United States of America 2009;106:21848-21853.

4 Lagier JC, El Karkouri K, Nguyen TT, Armougom F, Raoult D, Fournier PE: Non-contiguous finished genome sequence and description of anaerococcus senegalensis sp. Nov. Standards in genomic sciences 2012;6:116-125.

5 Baud D JK, Bertelli C, Kulling JP, Greub G: Low prevalence of chlamydia trachomatis infection in asymptomatic young swiss men. BMC infectious diseases 2008;8

6 Baud D GG, Osterheld MC, Borel N, Vial Y, Pospischil A and Greub G: Waddlia chondrophila: From bovine abortion to human miscarriage. Clinical Infectious Diseases 2011;52:1469-1471.

7 Team RDC: R: A language and environment for statistical computing. 2008

8 Baud D TV, Arafa A, Regan L and Greub G: Waddlia chondrophila, a potential agent of human fetal death. Emerging Infectious Diseases 2007;13:1239-1243.

9 Baud D KC, Kuelling JP and Greub G: Seroprevalence of different chlamydia-like organisms in an asymptomatic population. The Authors Journal Compilation 2009;15:213-215.

10 Paola Borella MTM, Vincenzo Romano-Spica, Serena Stampi, Giovanna Stancanelli, Maria Triassi, Rachele Neglia, Isabella Marchesi, Guglielmina Fantuzzi, Daniela Tatò, Christian Napoli, Gianluigi Quaranta, Patrizia Laurenti, Erica Leoni, Giovanna De Luca, Cristina Ossi, Matteo Moro and Gabriella Ribera D'Alcalà: Legionella infection risk from domestic hot water. Emerging Infectious Diseases 2004;10

11 Greub G RD: Microorganisms resistant to free-living amoebae. Clinical Microbiology 2004;17:413-433.

12 Saadi H PI, Colson P, Cherif J, Beji M, Azza S, Armstrong N, Robert C, Fo, urnous G LS, Raoult D: First isolation of mimivirus in a patient with pneumonia. Clinical Infectious Diseases 2013

Table 1 . Lausannevirus prevalence according to demographic, social and behavioural factors among 517 young Swiss male

Characteristics	Lausannevirus positive n=13 (2.51%)	Lausannevirus negative n=504 (97.49%)	p-value*
Nationality at birth			
Switzerland	11 (84.62%)	424 (84.13%)	1
Other	2 (15.38%)	80 (15.87%)	
Place of residence**			
>10'000 inhabitants	8 (61.54%)	209 (41.47%)	0.26
<10'000 inhabitants	5 (38.46%)	279 (55.36%)	
Main occupation**			
Studies	8 (61.54%)	199 (39.48%)	0.26
Work	5 (38.46%)	269(53.37%)	
Contact with animals			
Animal contact	5 (38.46%)	260 (51.6%)	0.41
No animal contact	8 (61.54%)	244 (48.41%)	
Smoke/drugs			
Cigarettes	4 (30.77%)	242 (48.02%)	0.26
Cannabis	3 (23.08%)	137 (27.18%)	1.00
Drugs	0 (0%)	23 (4.56%)	1.00
Alcohol ≥ 2x/week	10 (76.92%)	341 (67.66%)	0.37
Any reported disease			
Asthma	0 (0%)	35 (6.94%)	1.00
Sport practice**			
≥ 3x/ week	5 (38.46%)	83 (16.46%)	0.0066
≤ 3x/week	1 (7.69%)	235 (46.62%)	
Milk consumption**			
Milk consumption	11 (84.62%)	494 (98.01%)	0.028
No milk consumption	2 (15.38%)	9 (1.79%)	
Sexual orientation**			
Heterosexual	8 (61.54%)	382 (75.79%)	0.17
Homo/bisexual	1 (7.69%)	8 (1.59%)	
Condom use**			
Always	5 (38.46%)	158 (31.34%)	0.55
Sometimes	7 (53.84%)	313 (62.1%)	

*Fisher's exact chi^2

**Some patients declined to answer to this question and were excluded from statistical analysis

Table 2. Co-reactivity between Lausannevirus and *Chlamydia* as well as *Chlamydia*-related organisms

Bacteria	Bacteria positive	Lausannevirus positive n=13 (2.51%)	Lausannevirus negative n=504 (97.49%)	p-value*
Waddlia chondrophila	167 (32.3%)	5 (38.46%)	162 (32.14%)	0.67
Criblamydia sequanensis	252 (48.74%)	9 (69.23%)	243 (48.21%)	0.001
Parachlamydia acanthamoebae	17 (3.29%)	1 (7.69%)	16 (3.17%)	0.37
Protochlamydia naegleriophila	4 (0.77%)	1 (7.69%)	3 (0.60%)	0.1
Chlamydia trachomatis	6 (1.16%)	1 (7.69%)	5 (1.00%)	0.14

*Fisher's exact chi^2