Lausannevirus Seroprevalence among Asymptomatic Young Adults

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Key Words

Megavirales · Marseilleviridae · Lausannevirus · Serology · Prevalence · *Chlamydia* · Immunofluorescence · Cross-reactivity

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 who filled a detailed questionnaire. The coreactivity 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 (53.97 and 60.35%, respectively), 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 ($p = 0.0066$ and 0.028 , respectively). Coreactivity analyses revealed an association between *Criblamydia sequanensis* (an

amoeba-resisting bacterium present in water environments) and Lausannevirus seropositivity ($p = 0.001$). **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. Whether the coreactivity observed for *C. sequanensis* and Lausannevirus reflects a common mode of acquisition or some unexpected cross-reactivity remains to be determined.

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Introduction

Lausannevirus is a new member of the Marseilleviridae family, which is part of the Megavirales [1]. It was discovered by amoebal coculture in 2005 from a sample collected in the Seine river (Paris, France) [2]. Lausannevirus grows rapidly in amoebal coculture using *Acanthamoeba* as the cell background, with a 3-log increase in 16 h and complete amoebal lysis after about 24 h [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 microimmunofluorescence assay using as antigen a whole Lausannevirus viral particle. 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 behavioral risk, and also 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 5 µm a 1-week infected *A. castellanii* flask grown in peptone yeast-extract glucose at 32° and we re-infected a new flask with the filtrate. Five days later, the virus was harvested and flask supernatant was centrifuged at 5,000 g for 15 min. The supernatant was then collected and filtered at 5 µm to remove residual amoebal cells. The filtrate was then centrifuged at 35,000 g for 1 h and the virus pellet was resuspended in 1 ml of PBS.

Immunofluorescence

Sera were investigated by microimmunofluorescence as described previously [8], 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 microimmunofluorescence was performed by two independent observers. A targeted lecture of 11 doubtful samples was performed by a third reader.

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

Characteristics	Lausannevirus positive (n = 13; 2.51%)	Lausannevirus negative (n = 504; 97.49%)	p value ¹
Nationality at birth			
Swiss	11 (84.62)	424 (84.13)	1
Other	2 (15.38)	80 (15.87)	
Place of residence ²			
>10,000 inhabitants	8 (61.54)	209 (41.47)	0.26
<10,000 inhabitants	5 (38.46)	279 (55.36)	
Main occupation ²			
Student	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)	
Smoking/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 ≥2×/week	10 (76.92)	341 (67.66)	0.37
Any reported disease			
Asthma	0 (0)	35 (6.94)	1.00
Sport practice ²			
≥3×/week	5 (38.46)	83 (16.46)	0.0066
≤3×/week	1 (7.69)	235 (46.62)	
Milk consumption ²			
Milk consumption	11 (84.62)	494 (98.01)	0.028
No milk consumption	2 (15.38)	9 (1.79)	
Sexual orientation ²			
Heterosexual	8 (61.54)	382 (75.79)	0.17
Homo-/bisexual	1 (7.69)	8 (1.59)	
Condom use ²			
Always	5 (38.46)	158 (31.34)	0.55
Sometimes	7 (53.84)	313 (62.1)	

Values in parentheses are percentages.

¹ Determined by Fisher's exact χ^2 test.

² Some patients declined to answer this question and were excluded from statistical analysis.

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 behavioral characteristics as well as animal contact are reported in

Table 2. Coreactivity 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 ¹
<i>Waddlia chondrophila</i>	167 (32.3)	5 (38.46)	162 (32.14)	0.67
<i>Criblamydia sequanensis</i>	252 (48.74)	9 (69.23)	243 (48.21)	0.001
<i>Parachlamydia acanthamoebae</i>	17 (3.29)	1 (7.69)	16 (3.17)	0.37
<i>Protochlamydia naegleriophila</i>	4 (0.77)	1 (7.69)	3 (0.60)	0.1
<i>Chlamydia trachomatis</i>	6 (1.16)	1 (7.69)	5 (1.00)	0.14

Values in parentheses are percentages. ¹ Determined by Fisher's exact χ^2 test.

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 behavior (table 1). Among the 517 volunteers, 58.79% had contact with animals but, again, no association has been observed (table 1). Other behavioral risks have also been analyzed, showing no correlation between virus infection and smoking, alcohol intake or drug consumption (table 1). On the contrary, frequent sports practice and milk consumption were associated with Lausannevirus seropositivity ($p = 0.0066$ and 0.028 , respectively). Analysis on the 9 patients showing anti-Lausannevirus antibodies reported that the correlation between Lausannevirus seropositivity and milk consumption was maintained ($p = 0.02$), while the correlation with a frequent sport activity was no longer statistically significant ($p = 0.06$).

Discussion

This first Lausannevirus seroepidemiological 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 behavior or animal contact, excluding sexual and zoonotic exposure to Lausannevirus (table 1). Frequent sports practice has been shown to be strongly associated with seropositivity ($p = 0.0066$). Sportspeople have an active social life, allowing meeting and socializing with many people and sharing the same exercise environments, such as gyms or swimming pools. Also, most of the sports are practiced outdoors (for example in mountains or forests), which may represent another exposure route. Taken together, these observations suggest that humans come into contact with Lausannevirus by exposure to water or soil during sports activities. Milk consumption has also been shown to be associated with Lausannevirus seropositivity ($p = 0.028$). It is possible that cows shed Lausannevirus particles in their milk; however, this remains to be determined. Since previous studies focused on *C. trachomatis* and *Chlamydia*-like prevalence on the same target population [5, 9], we investigated a potential coreactivity between these bacterial species and Lausannevirus. Unexpectedly, we observed a strong association between Lausannevirus seropositivity and antibodies directed against *Criblamydia sequanensis* ($p = 0.001$; table 2). Conversely, we observed no coreactivity between Lausannevirus and *Waddlia chondrophila*, which has been identified in bovine abortion and which is considered to be a possible zoonotic agent [6, 8].

Coreactivity 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 coculture. Thus, amoebae might act as a reservoir of Lausannevirus and humans might become 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, 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* ($p > 0.05$), 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.

Coreactivity 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 1 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 coreactivity between Lausanne-

virus 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 have demonstrated the presence of viruses of *Marseilleviridae* and *Mimiviridae* families in human samples [1, 12].

For the future, we suggest expanding the seroprevalence studies to other countries, for example France and Senegal, from where Lausannevirus, *Marseillevirus* and *Senegalvirus* have been isolated. Furthermore, the sampling population should be increased to include persons with various infectious diseases of unknown etiology, people of 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.

Disclosure Statement

The authors have no conflict of interest.

References

- 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. *J Clin Virol* 2013;57: 191–200.
- Thomas V, Bertelli C, Collyn F, Casson N, Telenti A, Goesmann A, Croxatto A, Greub G: Lausannevirus, a giant amoebal virus encoding histone doublets. *Environ Microbiol* 2011;13:1454–1466.
- 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. *Proc Natl Acad Sci USA* 2009;106: 21848–21853.
- 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. *Stand Genomic Sci* 2012;6:116–125.
- Baud D, Jaton K, Bertelli C, Kulling JP, Greub G: Low prevalence of *Chlamydia trachomatis* infection in asymptomatic young Swiss men. *BMC Infect Dis* 2008;8:45.
- Baud D, Goy G, Osterheld MC, Borel N, Vial Y, Pospischil A, Greub G: *Waddlia chondrophila*: from bovine abortion to human miscarriage. *Clin Infect Dis* 2011;52:1469–1471.
- Team RDC: R: A Language and Environment for Statistical Computing: Reference Index. R Foundation for Statistical Computing, 2008.
- Baud D, Thomas V, Arafa A, Regan L, Greub G: *Waddlia chondrophila*, a potential agent of human fetal death. *Emerg Infect Dis* 2007;13: 1239–1243.
- Baud D, Kebbi C, Kuelling JP, Greub G: Sero-prevalence of different *Chlamydia*-like organisms in an asymptomatic population. *Clin Microbiol Infect* 2009;15:213–215.
- Borella P, Montagna MT, Romano-Spica V, Stampi S, Stancanelli G, Triassi M, Neglia R, Marchesi I, Fantuzzi G, Tatò D, Napoli C, Quaranta G, Laurenti P, Leoni E, De Luca G, Ossi C, Moro M, Ribera D'Alcalà G: *Legionella* infection risk from domestic hot water. *Emerg Infect Dis* 2004;10:457–464.
- Greub G, Raoult D: Microorganisms resistant to free-living amoebae. *Clin Microbiol* 2004; 17:413–433.
- Saadi H, Pagnier I, Colson P, Cherif J, Beji M, Azza S, Armstrong N, Robert C, Fournous G, La Scola B, Raoult D: First isolation of *Mimivirus* in a patient with pneumonia. *Clin Infect Dis* 2013;57:e127–e134.