Lausannevirus Seroprevalence among Asymptomatic Young Adults

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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 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. © 2013 S. Karger AG, Basel
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 x 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.

**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 the table below:
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 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*

### 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 other 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*

| Bacteria                  | Bacteria positive | Lausannevirus positive (n = 13; 2.51%) | Lausannevirus negative (n = 504; 97.49%) | p value¹ |
|---------------------------|------------------|----------------------------------------|------------------------------------------|----------|
| *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     |

Values in parentheses are percentages. ¹ Determined by Fisher’s exact χ² test.
Although the described coreactivity 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 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.

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