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## The Breadth of Viruses in Human Semen

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DOI: <https://doi.org/10.3201/eid2311.171049>

Zika virus RNA is frequently detected in the semen of men after Zika virus infection. To learn more about persistence of viruses in genital fluids, we searched PubMed for relevant articles. We found evidence that 27 viruses, across a broad range of virus families, can be found in human semen.

The finding by Atkinson et al. that Zika virus RNA is frequently detected in the semen of men after infection (1) highlights our knowledge gaps regarding the persistence of viruses in genital fluids, especially semen. Replicating Zika virus (2), like Ebola and Marburg viruses (3), has been isolated from semen and has been sexually transmitted. However, it is probable that many more viruses capable of causing viremia (presence of virus in the blood) can be found in semen. Seeding to the male reproductive tract may frequently occur in the context of viremia because the blood–testes/deferens/epididymis barriers are imperfect barriers to viruses, especially in the presence of systemic or local inflammation (4). Virus may persist even if incapable of replicating within the male reproductive tract because the testes are immunologically privileged (4); that is, within the testes, the immune response is restricted to enable the survival of sperm, which are immunogenic. Virus may also be transmitted to semen as a result of survival and replication within the accessory glands (5).

To investigate the breadth of viruses in semen, we performed a PubMed search by using the terms “virus\* AND semen OR sperm\* OR seminal.” We imposed no date or

language restrictions. This search returned 3,818 results. We screened the titles, abstracts, and full text articles for data that described detection of viruses in semen by nucleic acid amplification or detection, antigen detection, replication in cell culture, or replication in an animal system. We restricted the results to viruses capable of causing viremia. Where we found evidence for virus in semen, we then searched PubMed for evidence of sexual transmission by using the terms “(name of virus) AND sex\* AND Transm\*.”

Our search revealed that 27 viruses that can result in viremia have been found in human semen (Table). For many of these, data on sexual transmission are lacking. Of these 27 viruses, many cause chronic or latent infection (e.g., HIV virus, cytomegalovirus). However, several cause acute infections, including Lassa fever, Rift Valley fever, and chikungunya viruses. Of those causing acute infections, only Zika and Ebola viruses have been systematically screened for in semen (i.e., in case series or cohort studies rather than case reports). These 27 viruses come from diverse families, suggesting that the presence of many viruses in semen is unlikely to be exclusively dependent on specific or conserved viral epitopes, ability of virus to replicate within the male reproductive tract, or common mechanisms of immune evasion. Other factors that may also influence whether viruses exist in semen are level of viremia, inflammatory mediators (altering blood-barrier permeability), systemic immunosuppression, male reproductive tract immune responses, presence of sexually transmitted diseases, and virus structural stability. In mammals, numerous viruses are detectable in semen, including viruses that can cause disease in humans, such as Japanese encephalitis virus, foot and mouth disease virus, parainfluenza virus, and paravaccinia virus (6). Several other viruses that result in viremia can cause orchitis and have been detected in human testes, suggesting the possibility that these viruses may also be detectable in semen. These viruses include influenza virus, lymphocytic choriomeningitis virus, phlebotomus fever virus, cocksackie B virus, echovirus, dengue virus, systemic acute respiratory syndrome virus, parvovirus, smallpox virus, vaccinia virus, and rubella virus (7).

Given these findings, the following questions need to be addressed: which viruses are shed and remain viable in semen, for how long, and at what concentrations? The answers to these questions have implications for risks for sexual transmission and, therefore, embryonic infection, congenital disease, miscarriage, and effects on epidemiologic and transmission models. The presence of virus in the male reproductive tract may increase the risk for acquisition of sexually transmitted infections and may reduce male fertility through spermatogonial stem cell infection or local inflammation. Infection of spermatozoa could result in transmission of virus-induced mutations to subsequent

**Table.** Viruses that are capable of causing viremia and found in human semen\*

Virus	Family	Detection in semen, maximum detection time, d	Isolation from semen, maximum detection time, d	Evidence for sexual transmission within same cohort
Adenoviruses	<i>Adenoviridae</i>	AD	RCC	Unknown
Transfusion transmitted virus	<i>Anelloviridae</i>	NAA	No data found	Unknown
Lassa fever virus†	<i>Arenaviridae</i>	NAA, 103	RCC, 20	Unknown
Rift Valley fever virus†	<i>Bunyaviridae</i>	NAA, 117	No data found	Unknown
Ebola virus	<i>Filoviridae</i>	NAA, 531	RCC, 82	Epi + mol + sem
Marburg virus†	<i>Filoviridae</i>	AD, 83	RAS, 83	Epi + sem
GB virus C	<i>Flaviviridae</i>	NAA	No data found	Epi + mol
Hepatitis C virus	<i>Flaviviridae</i>	NAA; AD	No data found	Epi + mol
Zika virus	<i>Flaviviridae</i>	NAA, 188	RCC, 7	Epi + mol + sem
Hepatitis B virus	<i>Hepadnaviridae</i>	NAA; AD	RAS	Epi + mol
Cytomegalovirus	<i>Herpesviridae</i>	NAA	RCC	Epi + mol + sem
Epstein Barr virus	<i>Herpesviridae</i>	NAA	No data found	Epi and semen
Human herpes virus 8	<i>Herpesviridae</i>	NAA	RCC	Epi + mol
Human herpes virus 7	<i>Herpesviridae</i>	NAA	No data found	Unknown
Human herpes virus 6	<i>Herpesviridae</i>	NAA	No data found	Unknown
Human simplex viruses 1 and 2	<i>Herpesviridae</i>	NAA; AD	RCC	Epi + mol + sem
Varicella zoster virus	<i>Herpesviridae</i>	NAA	No data found	Unknown
Mumps virus†	<i>Paramyxoviridae</i>	NAA, 40	RCC, 14	Unknown
Adeno-associated virus	<i>Parvoviridae</i>	NAA	RCC	Unknown
BK virus	<i>Polyomaviridae</i>	NAA	No data found	Unknown
JC virus	<i>Polyomaviridae</i>	NAA	No data found	Unknown
Simian virus 40	<i>Polyomaviridae</i>	NAA	No data found	Unknown
HIV	<i>Retroviridae</i>	NAA; AD	RCC	Epi + mol + sem
Human T-cell lymphoma virus 1†	<i>Retroviridae</i>	No data found	RAS	Epi + mol
Simian foamy virus	<i>Retroviridae</i>	NAA	No data found	Unknown
Chikungunya virus†	<i>Togaviridae</i>	NAA, 30	No data found	Unknown

\*Presence of nucleic acid or antigen in semen does not represent the presence of replication-competent or infection-competent virus, which can generally only be demonstrated by isolation and culture of virus. Maximum detection time refers to time from symptom onset (only in viruses that cause acute only, not chronic, infection). A complete table with references is provided in the online Technical Appendix (<https://wwwnc.cdc.gov/EID/article/23/11/17-1049-Techapp1.pdf>). AD, antigen detection; Epi, epidemiologic evidence of sexual transmission; mol, molecular/phylogenetic evidence of sexual transmission; NAA, nucleic acid amplification or detection; RAS, replication in animal system; RCC, replication in cell culture; sem, isolation from semen.

†Data found only in the context of case reports and not case series, case control, or cohort studies.

generations, thereby elevating risks for cancer and other disorders. Indeed, when virus has been detected in human semen, the extent to which virus existence and replication occurs within spermatozoa is unclear (8). Not all therapeutics will cross the male reproductive tract–blood barriers, and viruses may persist in semen despite systemic clearance of virus, highlighting the need to consider the male reproductive tract–blood barriers when choosing therapeutic agents in clinical trials. Virus within the male reproductive tract can also be genetically distinct from virus in other compartments, including blood (9), which has implications for gene-based vaccines and therapeutics.

The presence of viruses in semen is probably more widespread than currently appreciated, and the absence of virus in genital secretions should not be assumed for traditionally non–sexually transmitted viruses. The investigation of virus detection and persistence in semen across a range of viruses is useful for clinical and public health reasons, in particular for viruses that lead to high mortality or morbidity rates or to epidemics.

Dr. Salam is a clinician and clinical researcher for the United Kingdom Public Health Rapid Support Team. His research interests are clinical trials in epidemic diseases.

Dr. Horby is Professor of Emerging Infectious Diseases and Global Health at the University of Oxford. His research is focused on improving the clinical and public health response to emerging and epidemic-prone infectious diseases in high- and low-income settings.

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## ***Legionella pneumophila* Serogroup 1 in the Water Facilities of a Tertiary Healthcare Center, India**

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DOI: <https://doi.org/10.3201/eid2311.171071>

Proactive environmental surveillance for *Legionella pneumophila* in hospitals that treat immunocompromised patients is a useful strategy for preventing nosocomial Legionnaires' disease. We report the presence of *L. pneumophila* serogroup 1 in 15.2% of the water systems of our tertiary healthcare center, which should prompt health officials to formulate mitigation policies.

*Legionella pneumophila*, the causative agent of Legionnaires' disease (LD), is a bacterium omnipresent in aquatic environments and increasingly recognized as a major cause of community- and hospital-acquired pneumonia. *L. pneumophila* serogroup 1 (*Lp1*), the dominant serogroup, accounts for ≈84% of human infections worldwide (1,2). Hospital-acquired LD has been reported globally, and routine use of environmental cultures is recommended as a useful strategy to prevent infections (3). Although proactive environmental surveillance of *Legionella* and regular

treatment of cooling tower installations are recommended in many countries, these practices are not routine in India, and limited studies have been conducted in this country for monitoring *Legionella* contamination in hospital water systems (4). We conducted a study to detect *L. pneumophila* and to identify *Lp1* in the water systems of a tertiary healthcare center in northern India that has organ transplantation and cancer treatment facilities.

We collected 79 water samples (41 potable, 38 non-potable) from the hospital and general areas of the healthcare center during an 18-month period (May 2015–October 2016). Of 79 samples, 27 were collected from patient areas (wards, intensive care units, outpatient departments, emergency units, and procedure rooms); 14 from residential areas; 15 from cooling towers; and 23 from other buildings (e.g., laboratory divisions, teaching departments, library, and recreational zones). We followed guidelines issued by the US Centers for Disease Control and Prevention regarding isolation of *Legionella* (5). In brief, we concentrated 500 mL of water samples and decontaminated 1 part by using heat treatment (in water bath at 50°C for 30 min) and 1 part by acid (in equal volume of HCl-KCl acid buffer [pH 2.2]). We then inoculated 0.1-mL samples onto buffered charcoal yeast extract agar (Becton Dickinson, Sparks, MD, USA) supplemented with glycine, vancomycin, polymyxin B, and cycloheximide (Oxoid, Basingstoke, UK). We presumptively identified colonies growing only on buffered charcoal yeast extract but not on blood agar as *Legionella* species and confirmed the presence of *L. pneumophila* by amplification of a 375-bp region of the *mip* gene using previously published primers (6). We identified *Lp1* by using a real-time PCR (rPCR) assay targeting the *wzm* gene (7). We used genomic DNA isolated from *L. pneumophila* strain Philadelphia (ATCC 33152) for standardization of PCR and rPCR and *L. pneumophila* strain Knoxville (ATCC 33153) for standardization of culture.

We identified *Legionella* spp. in 21 (26.6%) of 79 water samples (10 potable and 11 nonpotable) by culture. We obtained a collection of 28 isolates from the 79 samples and identified all of them as *L. pneumophila* by PCR. Among these 28 isolates, 18 (64.3%) tested positive for *Lp1* by rPCR, indicating the presence of this pathogenic serogroup in 12 (15.2%) of the 79 water samples (5 potable and 7 nonpotable).

We repeatedly isolated *L. pneumophila* (>4 times) from 2 high-risk sites: a drinking water unit and a cooling tower situated inside the hospital campus. Four water samples collected from patient areas tested positive for *L. pneumophila*, posing a risk for nosocomial infection. We isolated *L. pneumophila* from water bodies with temperatures ranging from 12°C to 57°C but most frequently (11 times) from those with temperatures of 25°C–50°C. We summarized the isolation of *L. pneumophila* with

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## Technical Appendix

**Technical Appendix Table.** Viruses that are capable of causing viremia and found in human semen\*

Viruses	Family	Detection in semen, maximum detection time, d	Isolation from semen maximum detection time, d	Evidence for sexual transmission within same cohort
Adenoviruses	Adenoviridae	AD (1)	RCC	Unknown
Transfusion transmitted virus	Anelloviridae	NAA (2)	No data found	Unknown
Lassa fever virus†	Arenaviridae	NAA, 103 (3)	RCC, 20 (3)	Unknown
Rift Valley fever virus†	Bunyaviridae	NAA, 117 (4)	No data found	Unknown
Ebola virus	Filoviridae	NAA, 531 (5)	RCC, 82 (6)	Epi + mol + sem (7)
Marburg virus†	Filoviridae	AD, 83 (8)	RAS, 83 (8)	Epi + sem (9)
GB virus C	Flaviviridae	NAA (10)	No data found	Epi + mol (11)
Hepatitis C virus	Flaviviridae	NAA (12); AD (13)	No data found	Epi + mol (14)
Zika virus	Flaviviridae	NAA, 188 (15)	RCC, 7 (16)	Epi + mol + sem (17)
Hepatitis B virus	Hepadnaviridae	NAA (18); AD (19)	RAS (20)	Epi + mol (21)
Cytomegalovirus	Herpesviridae	NAA (22)	RCC (23)	Epi + mol + sem (24)
Epstein Barr virus	Herpesviridae	NAA (22)	No data found	Epi and semen (25)
Human herpes virus 8	Herpesviridae	NAA (26)	RCC (27)	Epi + mol (28)
Human herpes virus 7	Herpesviridae	NAA (29)	No data found	Unknown
Human herpes virus 6	Herpesviridae	NAA (22)	No data found	Unknown
Human simplex viruses 1 and 2	Herpesviridae	NAA (22); AD (1)	RCC (1)	Epi + mol + sem (30)
Varicella zoster virus	Herpesviridae	NAA (22)	No data found	Unknown
Mumps virus†	Paramyxoviridae	NAA, 40 (31)	RCC, 14 (31)	Unknown
Adeno-associated virus	Parvoviridae	NAA (32)	RCC (33)	Unknown
BK virus	Polyomaviridae	NAA (34)	No data found	Unknown
JC virus	Polyomaviridae	NAA (34)	No data found	Unknown
Simian virus 40	Polyomaviridae	NAA (35)	No data found	Unknown
HIV	Retroviridae	NAA (36), AD (37)	RCC (38)	Epi + mol + sem (39)
Human T-cell lymphoma virus 1†	Retroviridae	No data found	RAS (40)	Epi + mol (41)
Simian foamy virus	Retroviridae	NAA (42)	No data found	Unknown
Chikungunya virus†	Togaviridae	NAA, 30 (43)	No data found	Unknown

\*Presence of nucleic acid or antigen in semen does not represent the presence of replication-competent or infection-competent virus, which can generally only be demonstrated by isolation and culture of virus. Maximum detection time refers to time from symptom onset (only in viruses that cause acute only, not chronic, infection). AD, antigen detection; Epi, Epidemiological evidence of sexual transmission; mol, molecular/phylogenetic evidence of sexual transmission; NAA, nucleic acid amplification or detection; RAS, replication in animal system; RCC, replication in cell culture; sem, isolation from semen. †Data found only in the context of case reports, and not case series, case control, or cohort studies.

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