

Some Basic Information on PRV

The virus:

The formal taxonomic name of Pseudorabies Virus (PRV) is Suid herpesvirus 1. It is classified as a member of the subfamily of Alphaherpesvirinae of the family Herpesviridae. It is a CDC/NIH Class 2 agent and when we work with samples of virus larger than a few milliliters, we work with it under Biosafety Level 2 conditions. The standards for BSL2 relative to PRV work have been reviewed by Card and Enquist (1999), Strick and Card (1992), Card et al. (1990), and Pomeranz et al (2005).

The diseases caused by PRV.

PRV causes a natural, economically important disease (Aujeszky's Disease) in swine. It also causes the same disease in other members of the Suidae family. PRV causes fatal "mad itch" in cattle, dogs, and some wild animals.

Wild type PRV has a broad host range infecting many classes of mammals and avian embryos, but horses, reptiles, insects and higher primates including humans, are resistant. The pathogenesis and molecular biology of the virus have been studied in some detail.

There are no documented cases of accidental lab infections of humans. PRV is not a human pathogen despite enormous exposure of farmers, veterinarians, meat processors, abattoir workers to PRV. In the heyday of PRV epidemics around the world, literally millions of pigs were affected, as well as cows, and other animals on affected farms. PRV's ability to cause human disease is minimal. Remarkably, no data exists suggesting that people even have antibodies to PRV, despite this massive exposure. One paper reported one individual had a titer of 1:12 - this titer is really very low. The assertion that the individual was infected with PRV was refuted subsequently as follows: We know that people infected with HSV have very low titer cross-reacting antibody to PRV (the cross reacting protein is the conserved membrane protein gB). Thus, any report of a low titer PRV response, must prove that it isn't an anti-HSV response. Most humans have antibody to HSV and this poor individual probably had something else other than PRV.

Among laboratory animals, the rabbit is the most sensitive to infection. Rodents are also highly susceptible. Some lower primates are susceptible to experimental infection (direct injection) including rhesus monkeys, macaques and Grivet monkeys.

Laboratory infections

In general, infection of laboratory animals can happen only by injection, scarification or biting, and not by aerosol or inadvertent or casual contact. We have infected animal cages adjacent to uninfected animal cages and there has never been any cross infection. On occasion, a student has left an uninfected animal in a cage with an infected animal with no cross infection noted.

Under the conditions used for BSL2, PRV presents no danger to pet dogs, cats, and rodents. HOWEVER, dogs and other pets should NOT be allowed in lab areas or lab animal quarters. For pets to be infected, they would need to be directly injected with virus or eat an infected mouse or rodent carcass. As noted above, PRV is not spread in the lab by aerosols, and it is not secreted in infected animal feces or urine.

When small volumes of virus are used, as they are in microscope or electrophysiology experiments, the probability of inadvertent infection of nearby susceptible animals is vanishingly small. Certainly, the probability of infecting humans approaches zero.

Tracing strains of PRV are attenuated vaccine strains

For most neural tracing studies with PRV, attenuated vaccine strains are used. These strains induced mild to no symptoms in infected animals despite replicating and spreading in neural circuits. Infected animals live almost twice as long as those infected with wild type virus. Wild type PRV strains are rarely used for tracing because they are too virulent and kill animals before there is extensive spread in the nervous system. Attenuated viruses may require more virions to initiate an infection and cause milder disease compared to wild type virus.

The need for a biohood when working with PRV

Biohoods are NOT needed every time one works with PRV. The need for a biosafety hood is two fold: first, to keep contaminants from getting into virus cultures (when virus stocks are produced), and second, to keep potential aerosols from large volumes of infected liquid from escaping. Aerosols can be produced during vigorous pipetting when handling culture supernatants. Pipettes should be plugged and filtered and fluid transfers should be done gently with no foaming. Biohoods have relevance to PRV safe handling only when growing the virus in quantity (e.g., more than 10ml of liquid). Since animal infections involve very small quantities of infectious virus (usually less than 1ml of liquid in a stock vial and usually less than 10 microliters of liquid being injected), the biohood is superfluous. In fact, doing animal injections or analyses in the confines of a biohood often creates more hazards to the animal and the investigator than is inherent in the virus and the situation.

PRV is easily inactivated

The PRV virion has a lipid membrane that is absolutely required for infectivity. The virion is incredibly sensitive to extreme variations in temperature, ice-formation, and a number of chemical agents. It is inactivated at 60C within 30-60 minutes, killed at 70C within 10-15 minutes, inactivated at 90C within 3 minutes, and destroyed at 100C within 1 min. Additionally, it is inactivated within 12 weeks at temperatures from minus 18C to minus 25C due to ice formation that ruptures the membrane. Do NOT store PRV stocks in the minus 20C freezer! Multiple cycles of freezing and thawing will inactivate the virus. Aldehydes effectively inactivate the virus at low concentrations: 3% glutaraldehyde inactivates PRV within 10 minutes and 4% formaldehyde reduces infectivity by 60% within 5 minutes, and completely after 3 hr. Alcohols, chloroform and Clorox effectively sterilize the virus at room temperature.

References:

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- Strick and Card. 1992. in *Experimental Neuroanatomy. A Practical Approach* (J. P. Bolam, ed), pg. 81. IRL Press, Oxford.
- Card, J.P. and L.W. Enquist. 1999 Transneuronal circuit analysis with pseudorabies viruses. *Curr. Prot. Neurosci. Supp.* 9, Unit 1:1-27.
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