

Bluetongue virus bio safety by Geoff Letchworth

The unfortunate situation of one of the world's preeminent vaccine companies being precluded from producing a bluetongue vaccine to protect animals against an ongoing epidemic prompts me to weigh in on the subject of bluetongue biosafety.

I am just a private citizen at this point, but I have some experience with this virus from my research at Plum Island in the early 1980's and my recent work at USDA's bluetongue lab in Laramie. Based on that experience, I helped prepare the section on bluetongue for the 5th edition of the NIH-CDC manual "Biosafety in Microbiological and Biomedical Laboratories" (BMBL) (<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>). You will find bluetongue described in Appendix D. However, my comments here are only my own personal opinion and do not represent the BMBL, USDA, or any other organization. Other sources of information would be Dr. James MacLachlan at UC-Davis (Email: njmaclachlan@ucdavis.edu, Phone: 1 530 752 1163, or 1 530 754 8125) and Dr. William Wilson (Email: William.Wilson@ars.usda.gov, Phone: 1 307 766-3622).

My view is that all biosafety has to flow logically from the basic biology of the pathogen, the vector, and the host. So what do we know about bluetongue?

Pathogen: The bluetongue virus (BTV) has amazing environmental stability. It survives essentially forever when frozen. It can survive for years in wet blood kept in a refrigerator. And it survives as long as 50 days in circulation after infection of a ruminant. It apparently survives freezing winters, presumably in *Culicoides* larvae. It is likely that much of the meat frozen during a bluetongue outbreak contains small amounts of virus and becomes widely distributed in the human food chain, but this is of no practical consequence.

Vectors: BTV has two known vectors, *Culicoides* species and humans. Adult *Culicoides* slash the skin and lap blood, becoming infected if the blood carries virus. After a short incubation period, the infected *Culicoides* produces enough virus to infect a second animal during another feeding. Blood-fed *Culicoides* lay their eggs in an environment such as mud or manure, as appropriate for their own species. Eggs hatch into larvae, which progress through stages to pupae, and finally emerge as adults. Adult *Culicoides* disappear when environmental temperatures fall below freezing. Reappearance of the same serotype of BTV in endemic areas following a freezing winter suggests transovarial transmission in *Culicoides* although this has never been documented despite considerable effort.

Humans can transfer BTV by injecting blood or tissues from an infected animal directly under the skin of a naive animal.

Hosts: We know that BTV infects domestic and wild ruminants, neonatal mice, dogs, and chick embryos, but the full range of susceptible species has never been investigated. Ruminants produce relatively low viremias, but enough to infect *Culicoides*. This is the only known mechanism for infecting *Culicoides* in the wild. Ruminants quickly develop antibodies that neutralize BTV, but the virus survives in erythrocytes for about 50 days despite the antibodies bathing those erythrocytes. BTV may be present in semen from infected cattle. The only proven direct animal-to-animal route of BTV transmission is via the semen of infected bulls.

So the facts support the conclusion that BTV is maintained in nature by a ruminant-*Culicoides*-ruminant cycle. Occasional animal-to-animal transmission by either natural or artificial insemination is possible but certainly not frequent enough to maintain the virus in nature. And humans can transmit virus with needles, but this is controllable.

What hazards are created by vaccine manufacture?

1. Obviously, vaccine manufacture involves the growth of large amounts of virus, but this is really a minor hazard. In order for this virus to initiate an epidemic outside the vaccine facility, it would have to escape from the facility and be parenterally inoculated into ruminants, which then would have to serve as hosts for *Culicoides*, which in turn would have to infect other ruminants. Although the accidental release from vaccine production tanks is conceivable, the requisite chain of escape -> parenteral inoculation into ruminants -> *Culicoides* transmission seems most improbable. Of course it is impossible in the winter when adult *Culicoides* are absent.

2. Incomplete inactivation of live BTV in a vaccine is a possible route for escape, but this "problem" is universally recognized by the vaccine industry and precluded by rigorous production methods and testing procedures. Even in the very unlikely event that some minute percentage of the virus escaped inactivation, it seems likely that a killed virus vaccine would engender protective antibodies about the same time that the residual virus began to appear in the blood, so the window for transmitting BTV would be a few days at most. In my opinion, this is not a significant hazard.

3. Safety and efficacy testing of vaccines requires the immunization of animals and subsequent challenge. Some animals, particularly unvaccinated controls, will develop viremia and could be a source of infection for *Culicoides*, and therefore a source for spread outside the facility. These animals should be protected against *Culicoides* bites for two months after challenge infection. Because *Culicoides* are so small and can be present in such high numbers, only BSL-3 containment with rigorous insect control can preclude escape during animal inoculation studies during *Culicoides* season. However, I feel that animal experiments can be done quite safely with minimal containment in the winter.

And quite obviously, when the virus is endemic, rigorous biocontainment requirements would be counterproductive if they delayed or prevented the use of vaccine.

On balance, it seems to me that manufacturing a bluetongue vaccine creates a very small and mostly theoretical risk, but the past year's experience has shown that not having a vaccine is a major risk.