SHEDDING RISK WITH INTRACEREBRAL INOCULATION OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS: INFORMING A RISK ASSESSMENT

A case study in building a "win-win-win" scenario

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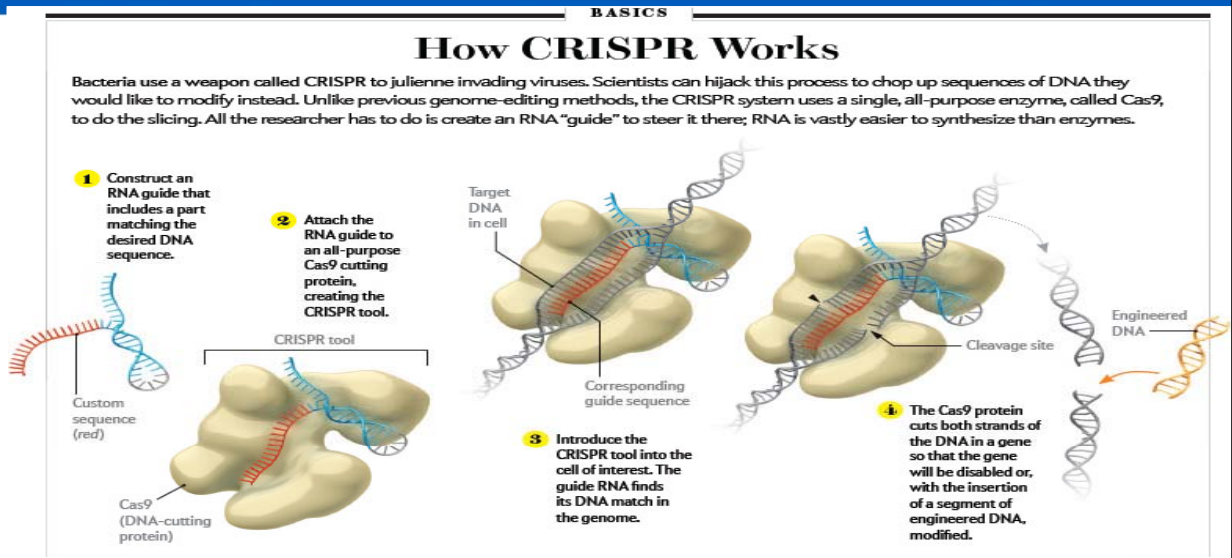
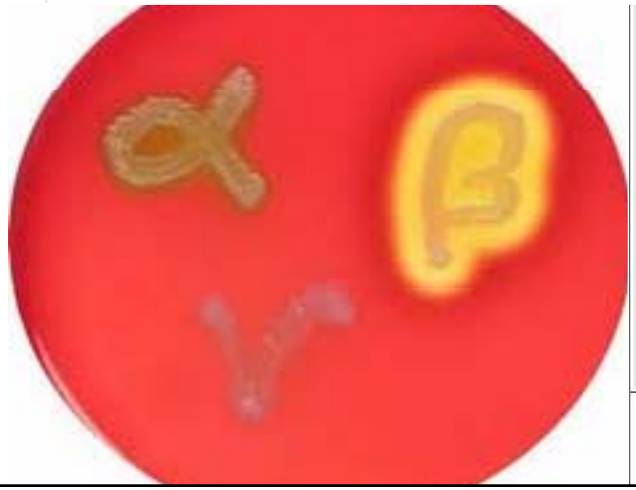
Biosafety is risk assessment driven

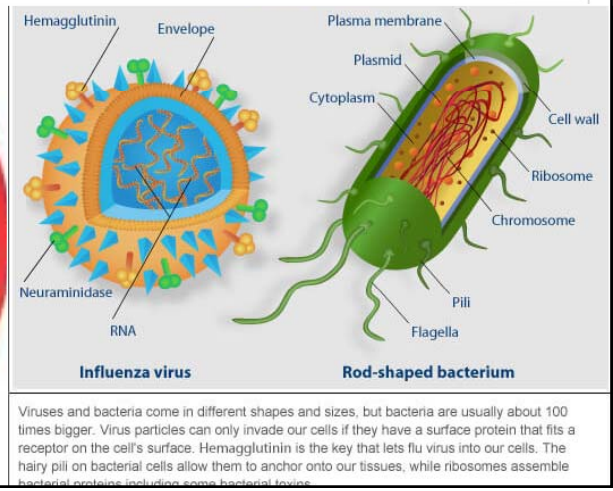
Each organism presents different risks

Each recombinant Nucleic Acid technique or study presents different risks

Not everyone is comfortable with this!







Viruses and bacteria come in different shapes and sizes, but bacteria are usually about 100 times bigger. Virus particles can only invade our cells if they have a surface protein that fits a receptor on the cell's surface. Hemagglutinin is the key that lets flu virus into our cells. The hairy pili on bacterial cells allow them to anchor onto our tissues, while ribosomes assemble bacterial proteins including some bacterial toxins.

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Most people like projects to fit into boxes

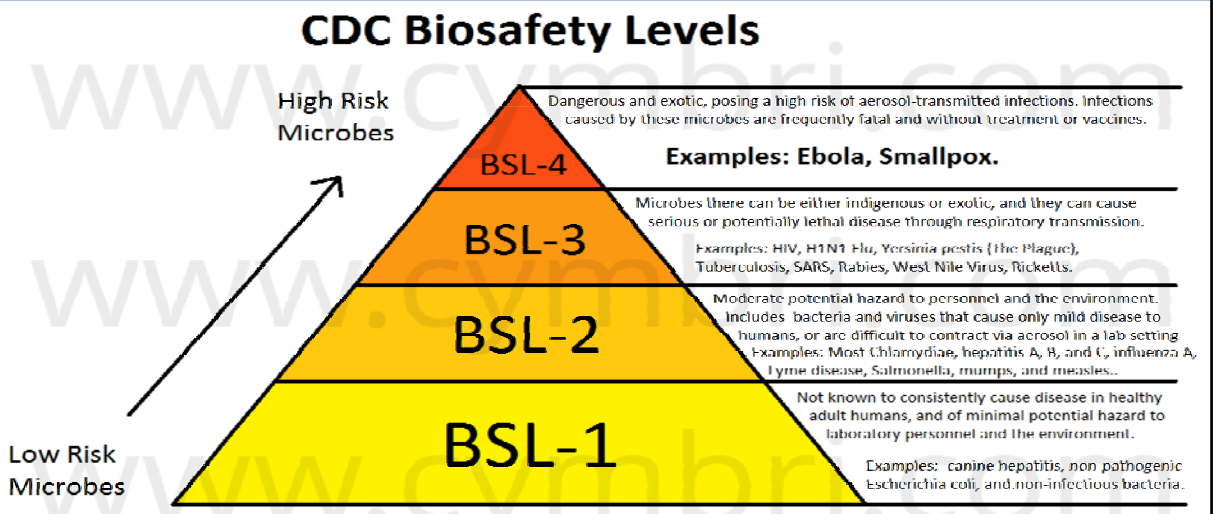
Guidance documents and experience lead to classification of experiments into one size fits all boxes.

Risk Groups

Biosafety Levels

BBP rules/Universal Precautions

Often, this works but some projects deserve more scrutiny



Risk Group (RG)	Agent Risk Description	Examples
RG-1	Agents that are not associated with disease in healthy adult humans	*Bacillus subtilis, Escherichia coli* K12, adeno-associated virus (AAV)
RG-2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available	*Staphylococcus aureus, Salmonella* sp, Herpes simplex viruses, Adenovirus
RG-3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may *be* available	*Mycobacterium tuberculosis, Bacillus anthracis*, HIV
RG-4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available	Ebola virus, Marburg virus, Lassa virus

Relation of risk groups to biosafety levels, practices and equipment

Risk group	Biosafety level (BSL)	Laboratory type	Lab. practice	Safety equipment
1	Basic BSL- 1	Basic teaching and research	Good microbiol. techniques (GMT)	None, open bench work
2	Basic BSL- 2	Diagnostic services and research	GMT + protective clothing biohazard sign	Open bench plus bio – safety cabinet (BSC) for potential aerosols
3	Containment BSL- 3	Special diagnostic services and research	As BSL-2 plus special clothing controlled access directional airflow	Biosafety cabinet and/or other primary devices for all activities
4	Maximum Containment BSL- 4	Dangerous pathogen units	As BSL-3 plus airlock entry, shower exit and special waist disposal	Class-3 BSC or positive pressure suites in conjunction with class-2 BSCs, double ended autoclave trough the wall and filtered air

This Particular Case: Theiler's Murine Encephalomyelitis Virus (TMEV)

Is a non-enveloped, +stranded, RNA picornaviridae

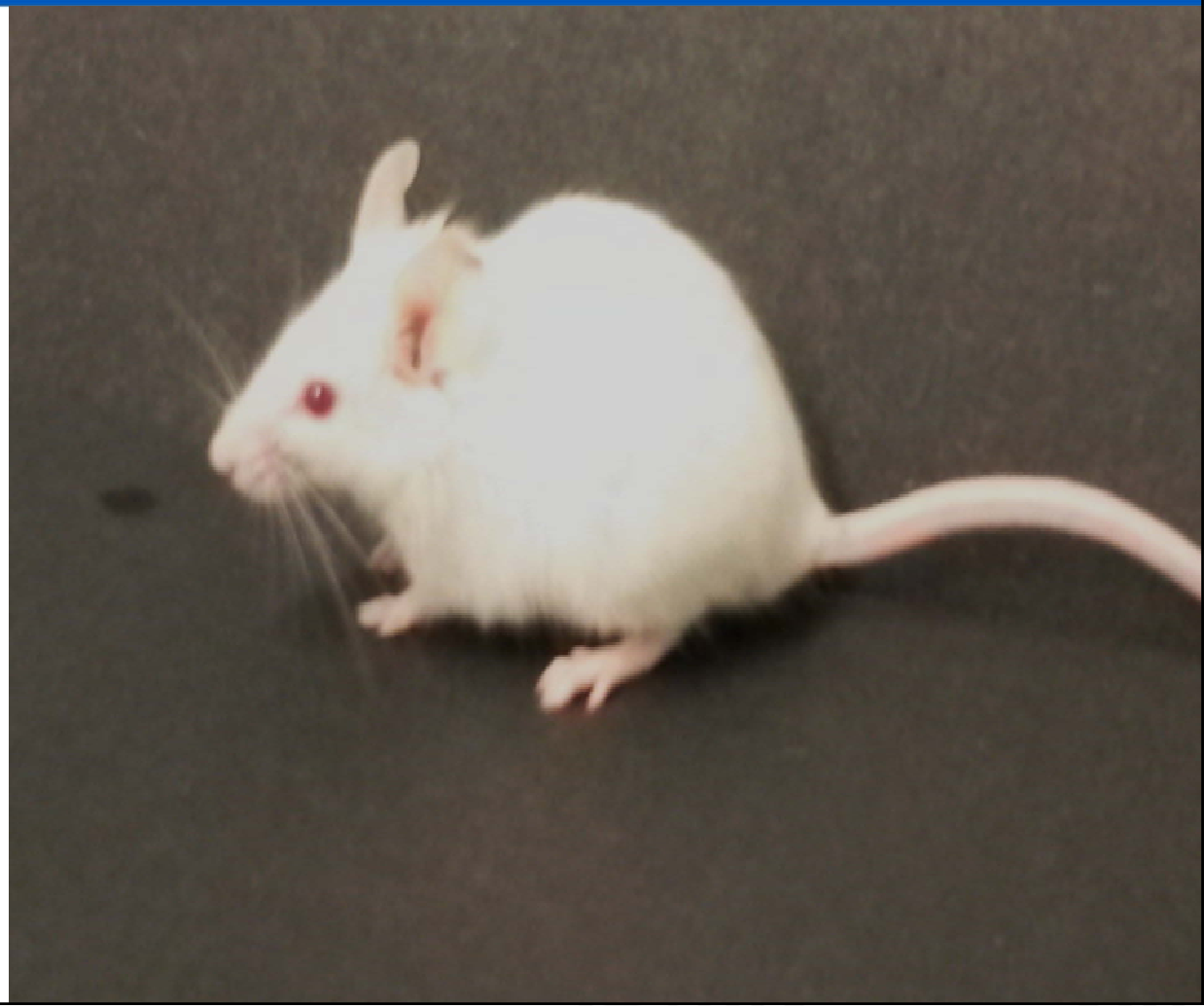
Is normally an enteric virus (stomach bug)

Is very virulent – runs rampant throughout animal facilities if it gets in

Stable on fomites

outbreaks can have serious consequences for research projects

Does NOT infect humans



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TMEV injected into the brain of a mouse creates a disease analogous to Multiple Sclerosis (MS)

Meaning, mice with intracerebral (IC) inoculated TMEV are model organisms for the study of MS!!!!

So, what's the problem?

Animal Facility POV

1) Consequences of a TMEV outbreak are incalculable
2) Dedicated space
3) ABSL-2 only
4) Strict PPE
5) Chlorine dioxide decon of everything

PI POV

1) IC inoculation is not the same as enteric infection
2) Dedicated space and ABSL-2 procedures are too costly
3) PPE guidance does not make sense (the virus does not infect people)
4) Decon is costly and cannot even occur in some cases (sensitive equipment)

BSO POV

1) Stuck in the middle, I see both sides of the debate
2) Biosecurity – biocontainment issue

What happens when people disagree on containment levels & practices?

An angry professor

BSO must scrutinize the experiment:

In depth risk assessments assist in determining the safety and containment requirements for each experiment.

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Biological Risk Assessment & Mitigation Tool

Primary Investigator:	**Department:**
Building:	**Room Number:**

Agent: _____

Key Characteristics:

The risk assessment shows that we didn't have enough information

What specific info are we missing for a complete risk assessment?

Do IC inoculated mice shed the TMEV virus?

Can IC inoculated mice infect other mice?

Do IC inoculated mice actually create a biological barrier to TMEV spread?

Filling in the risk assessment gaps

Conversation with the graduate student in charge of the project

1) are you willing to do some extra work to make your life easier in the end?
2) Potential $ to pay for supplies
3) Potential publication

Conversation with the PI in charge of the project

1) are you willing to let your grad student do some extra work?
2) Split the cost?
3) Potential publication
4) Relaxed LAF rules
5) CHEAPER overall

Conversation with the Lab Animal Facility Management

1) Will you accept the results of our work?
2) If so, will you relax the rules?
3) What else can I do for you…

Do some science!

BSO and Grad student designed a study to obtain the missing data for the risk assessment!

The BSO actually received a small grant to help fund the project!!

UUP Professional Development Award ***889303-68 - FY13/14***

Sat back and provided guidance to the Grad Student ☺

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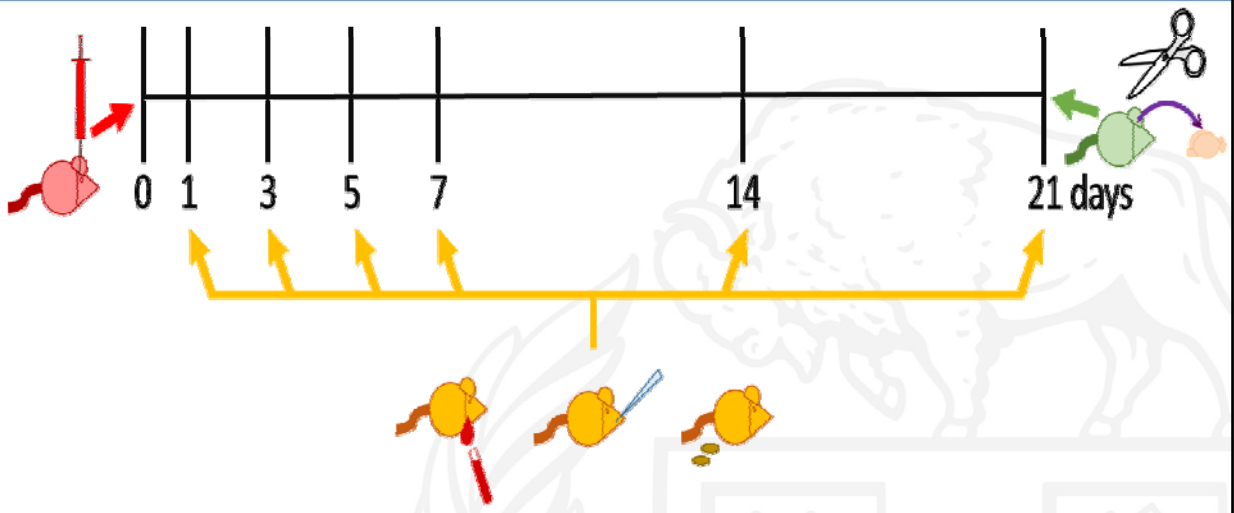
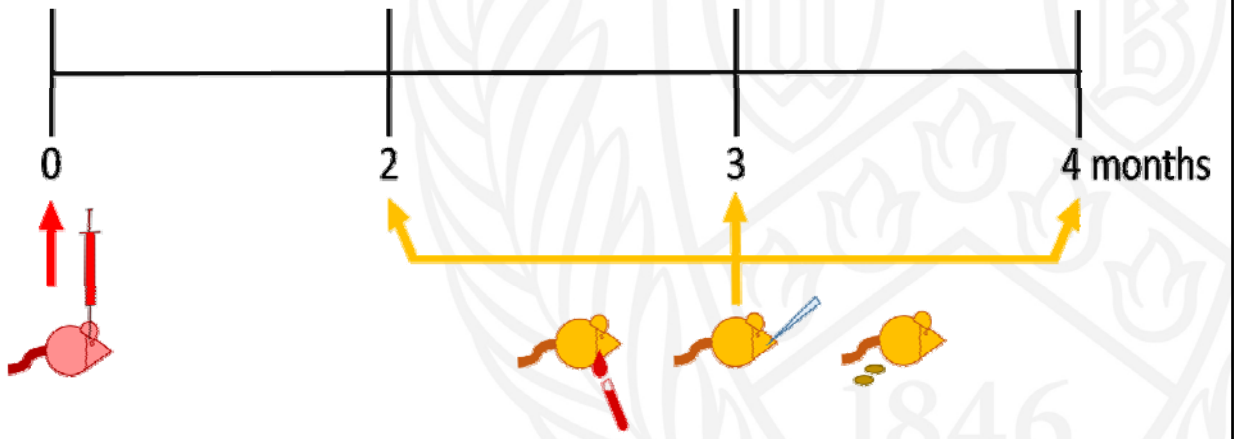
Study Design

Acute Phase of TMEV infection



Chronic Phase of TMEV infection



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Acute Phase

TMEV is found only in the CNS of IC inoculated mice during acute infection stage

Meaning they are NON-infectious!!!

Table 1. Ct for TMEV-infected and saline-injected biomaterials during acute infection

			TMEV-infected				Saline-injected			Positive Virus Contro
			TMEV1	TMEV2	TMEV3	TMEV4	Saline1	Saline2	Saline3	
Days	Blood	1	NA	NA	NA	NA	NA	NA	NA	10.85
		3	NA	NA	NA	NA	NA	NA	NA	
		5	NA	NA	NA	NA	NA	NA	NA	
		7	NA	NA	NA	NA	NA	NA	NA	
		14	NA	NA	NA	NA	NA	NA	NA	
		21	NA	NA	NA	NA	NA	NA	NA	
	Saliva	1	NA	NA	NA	NA	NA	NA	NA	10.58
		3	NA	NA	NA	NA	NA	NA	NA	
		5	NA	NA	NA	NA	NA	NA	NA	
		7	NA	NA	NA	NA	NA	NA	NA	
		14	NA	NA	NA	NA	NA	NA	NA	
		21	NA	NA	NA	NA	NA	NA	NA	
	Feces	1	NA	NA	NA	NA	NA	NA	NA	10.72
		3	NA	NA	NA	NA	NA	NA	NA	
		5	NA	NA*	NA	NA	NA	NA	NA	
		7	NA	NA	NA	NA	NA	NA	NA	
		14	NA	NA	NA	NA	NA	NA	NA	
		21	NA	NA	NA	NA	NA	NA	NA	
Brain			32.12	34.59	28.99	29.83	NA	NA	NA	10.35
Spinal cord			27.66	NA	27.33	36.75	NA	NA	NA	

Each column represents a subject or the viral supernatant. *Amplification past Ct 39 was detected, but melt curve data indicated it was an unrelated product.

Viral load in CNS

TMEV concentration appeared to be from 100 pfu – 4000 pfu/mL of tissue.

Table 4. Ct and viral titer

	Ct	Conc (pfu/mL)
Virus 10^0	12.44	$100x10^6$
Virus 10^{-1}	16.09	$100x10^5$
Virus 10^{-2}	19.93	$100x10^4$
Virus 10^{-3}	23.92	$100x10^3$
Virus 10^{-4}	27.87	$100x10^2$
Virus 10^{-5}	31.66	$100x10^1$

Conc: concentration, in PFU/mL.

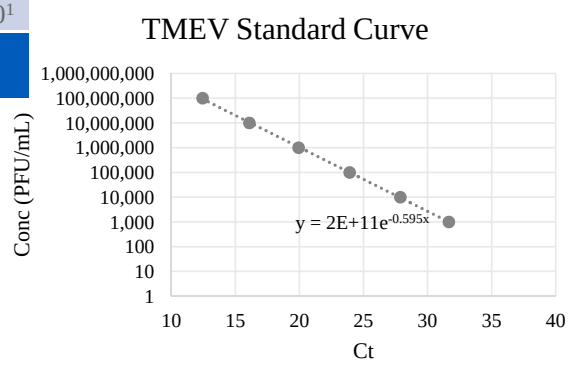

Table 5. Ct and viral concentration in CNS

	Brain		Spinal cord	
	Ct	Conc (pfu/mL)	Ct	Conc (pfu/mL)
TMEV1	33.73	385.76	29.73	4155.69
TMEV2	36.22	87.68	NA	NA
TMEV3	31.41	1533.96	29.70	4243.14
TMEV4	31.69	1298.56	38.99	16.82

Rows represent infected subjects. Conc: concentration in PFU/mL.

Chronic Phase

TMEV is found only in the CNS of IC inoculated mice during acute infection stage

Meaning they are NON-infectious!!!

Table 3. Ct for TMEV-infected biomaterials during chronic infection

			TMEV-infected					Positive Virus Control
		Month	TMEV5	TMEV6	TMEV7	TMEV8	TMEV9	
Months	Blood	2	NA	NA	NA	.	.	11.14
		3	NA	NA	NA	NA	NA	
		4	NA	NA	NA	.	.	
	Saliva	2	NA	NA	NA	.	.	
		3	.	.	.	NA	NA	
		4	NA	NA	NA	.	.	
	Feces	2	NA	NA	NA	.	.	
		3	.	.	.	NA	NA	
		4	NA	NA	NA	.	.	

Each column represents a subject or the viral supernatant.

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Applied Biosafety publication

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Shedding Risk with Intracerebral Inoculation of Theiler's Murine Encephalomyelitis Virus: Informing a Risk Assessment

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Abstract

Theiler's murine encephalomyelitis virus (TMEV) is a naturally occurring enteric infection, easily passed from mouse to mouse in communal housing. However, TMEV is often inoculated intracerebrally (IC) to produce a mouse model of multiple sclerosis (MS). It has long been accepted that maintaining colonies of IC-infected mice within laboratory animal facilities poses a risk of spreading infection from mouse to mouse via the fecal-oral route as well as contaminated equipment or personnel. Interestingly, the extent of virus shedding from IC-inoculated mice has not been investigated, although several publications have remarked on the lack of virus in the peripheral body of this MS mouse model. Viral shedding, thus infectivity, would require that TMEV escape the central nervous system (CNS) and be found in bodily secretions. We hypothesized that if the virus can escape the CNS, it would be found circulating within blood or other secretions postinjection (PI), after the blood-brain barrier has been experimentally breached. The data presented show no TMEV RNA was found in the serum, saliva, or feces during the acute and chronic infection stages, although all subjects were positive for TMEV RNA in the CNS. These results, in conjunction with published anecdotal evidence, suggest that mice IC-inoculated with TMEV are not contagious, and thus a relaxation of containment methods is warranted. This report is an example of a collaborative effort between biosafety and research professionals to identify and collect scientifically relevant data to inform a risk assessment.

Keywords

Theiler's murine encephalomyelitis virus, TMEV, risk assessment, viral shedding, qPCR

In the End

WIN

PI & Grad student

1) An extra publication
2) Decreased cost
3) Decreased stringency of PPE
4) Decreased stringency of decontamination procedures

WIN

Lab Animal Facility Management?

1) Peace of mind
2) Relaxation of oversight

WIN

EHS

1) Publication
2) Viewed as a Facilitator
3) Good will

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