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Effects of the presence of other pathogens on the transmission of viruses by mosquitoes

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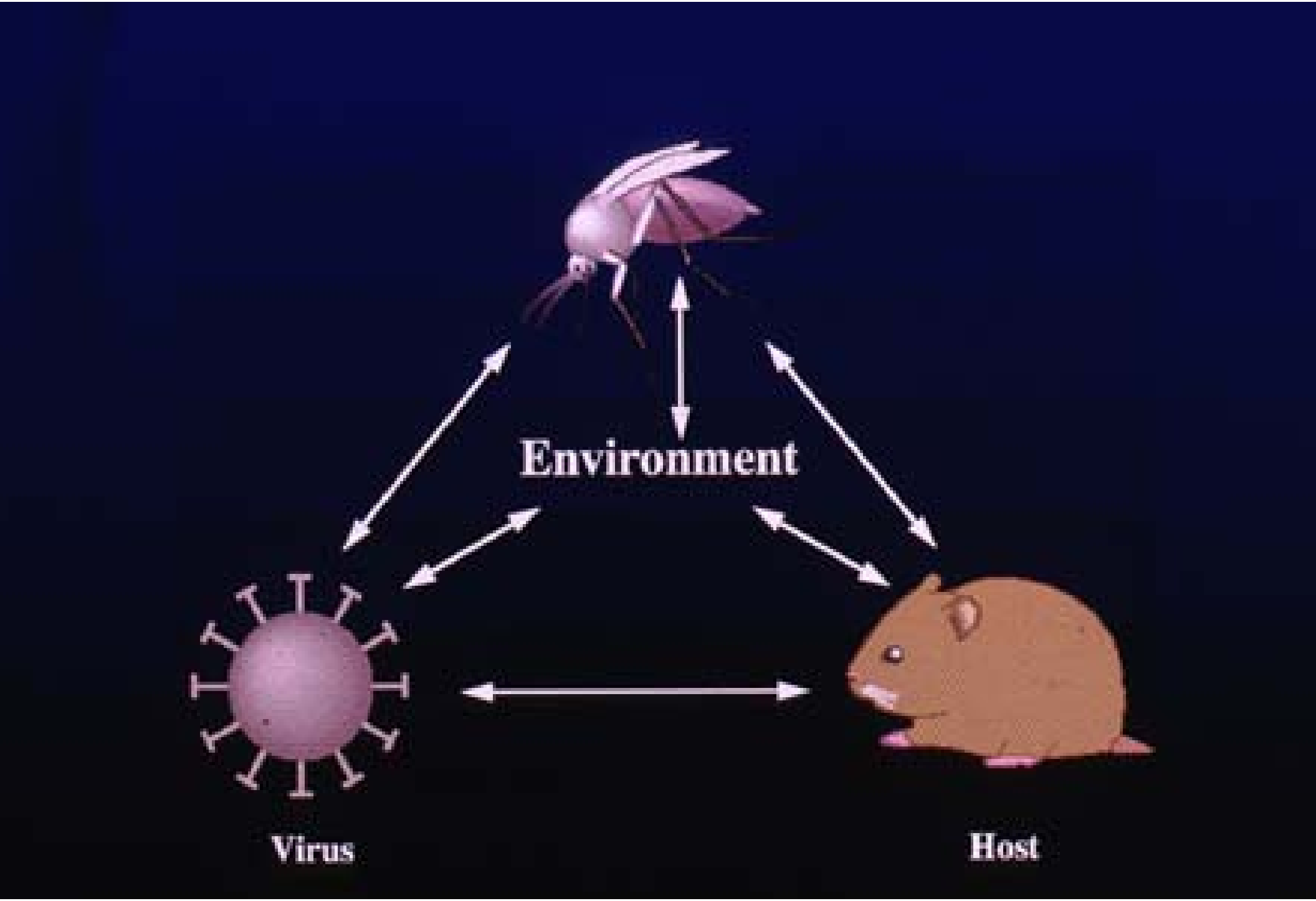
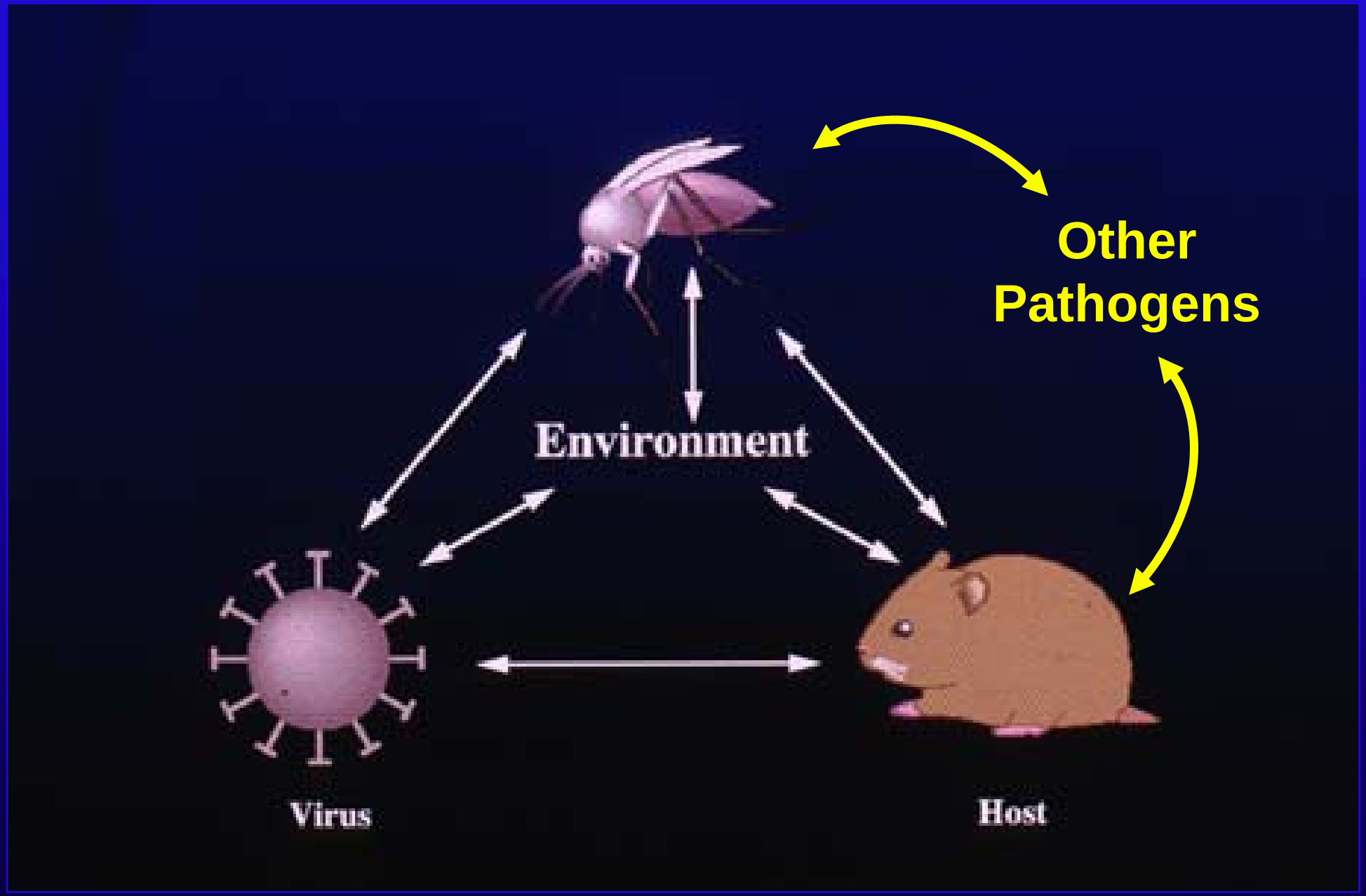
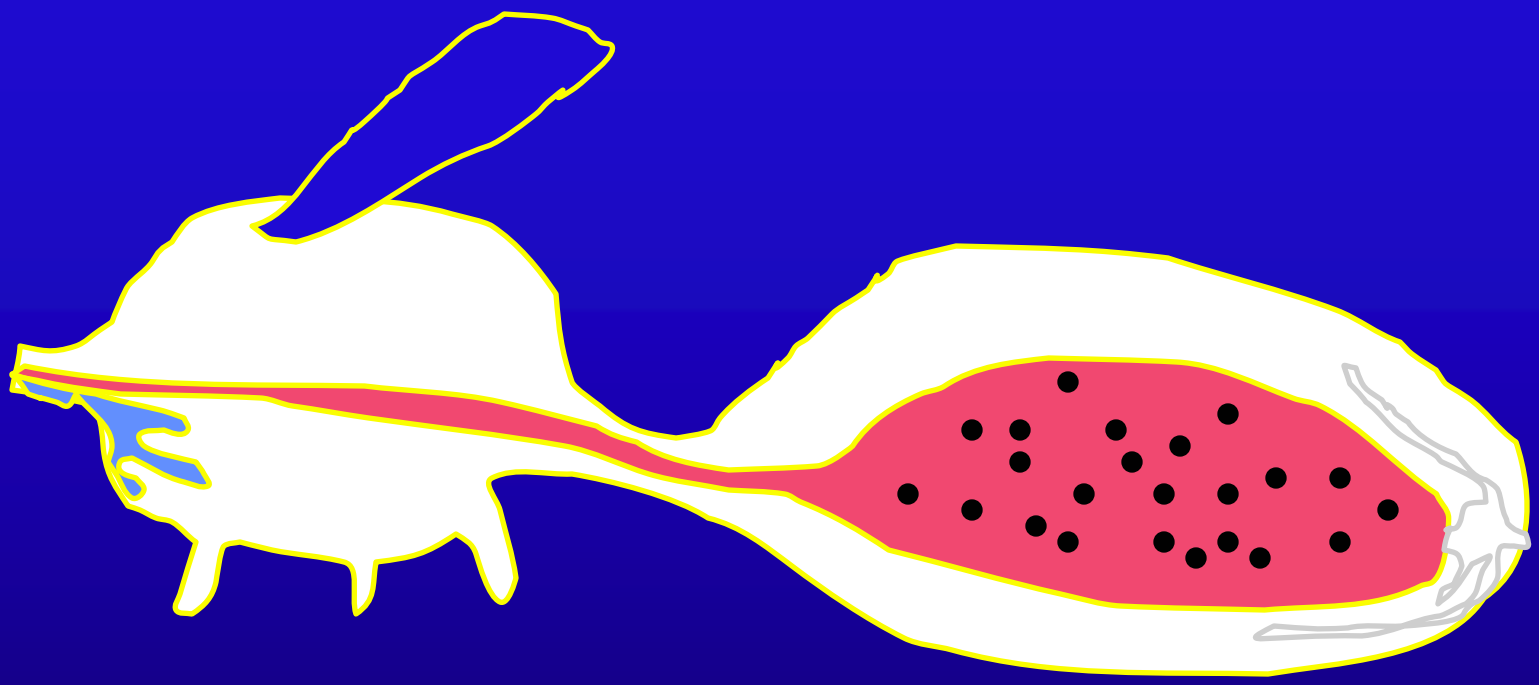
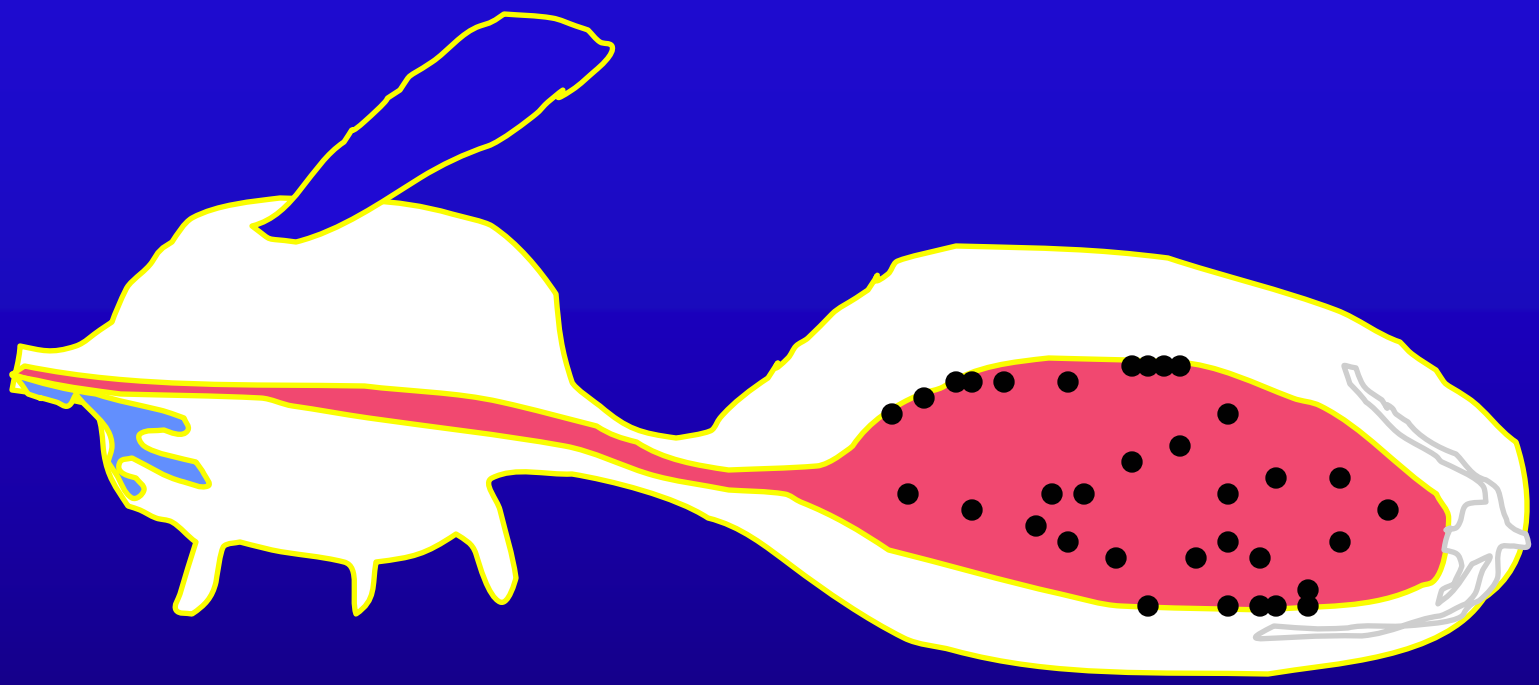

Environment
Virus
Host

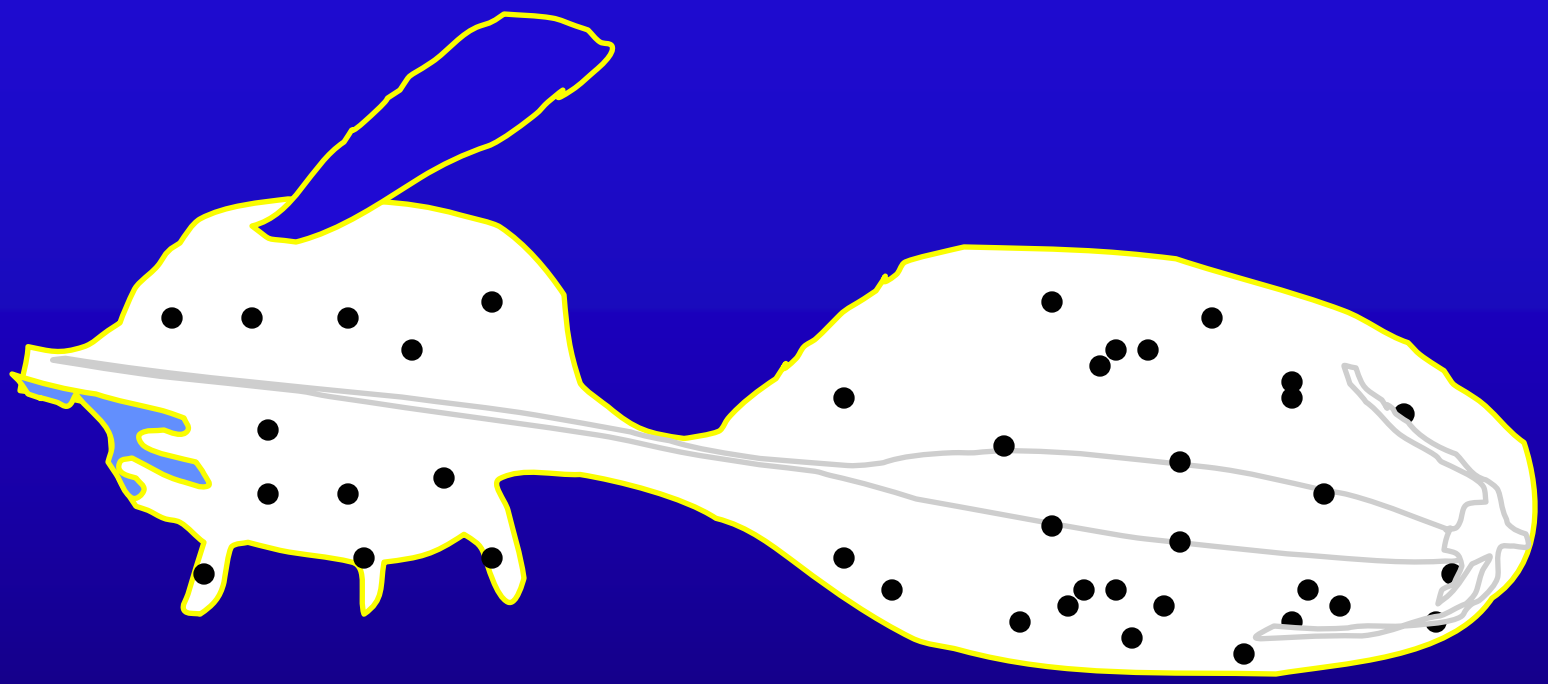

Other
Pathogens
Environment
Virus
Host



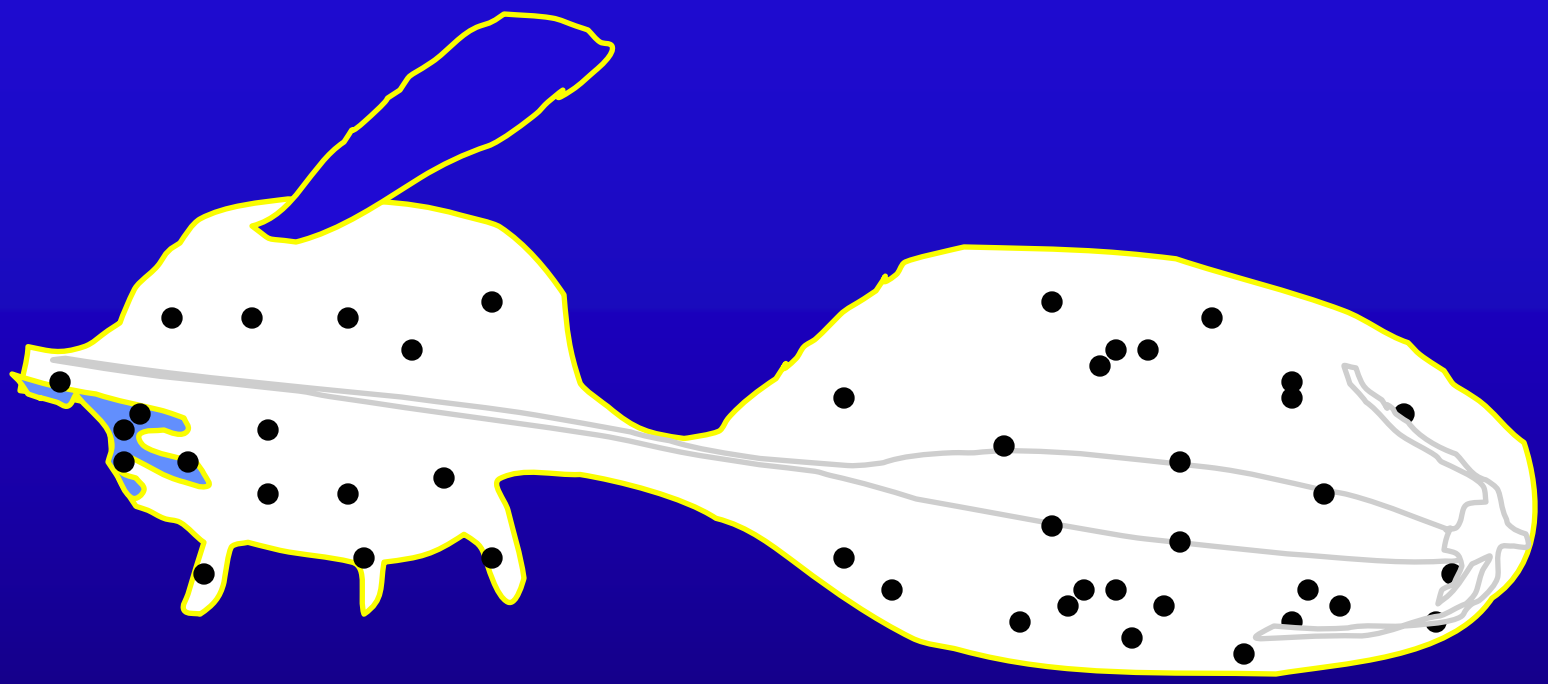
Virus in the blood meal, but mosquito not infected



Mosquito infected, but limited to midgut



Virus disseminated to hemocoel,
but salivary glands not infected



Salivary glands infected, ready to transmit by bite

"BARRIERS" TO VIRUS TRANSMISSION

1. **MIDGUT INFECTION**
2. **MIDGUT ESCAPE**
3. **SALIVARY GLAND INFECTION**
4. **SALIVARY GLAND ESCAPE**

OTHER PATHOGENS

1. Filarial worms (MICROFILARIAE)

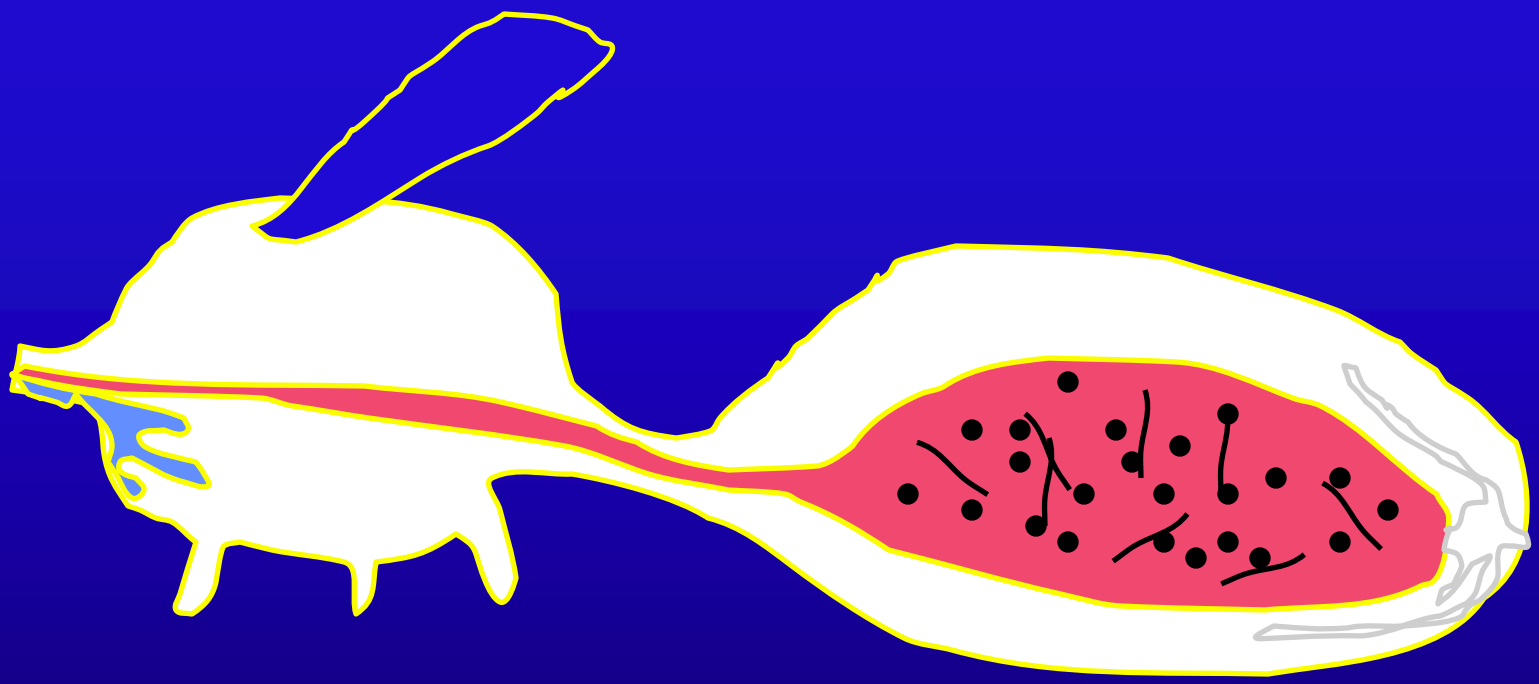
   MIDGUT INFECTION

   MIDGUT ESCAPE

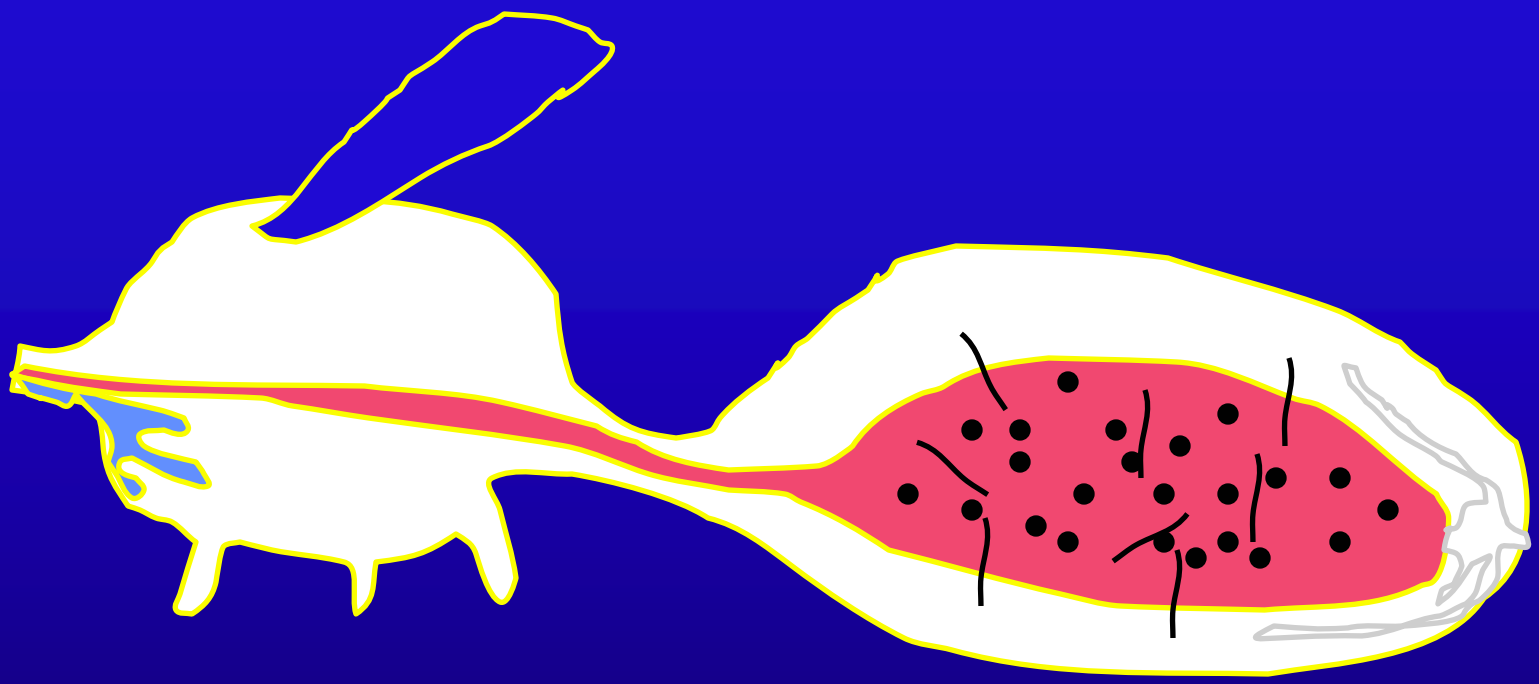
2. Malaria (SPOROZOITES)

   SALIVARY GLAND

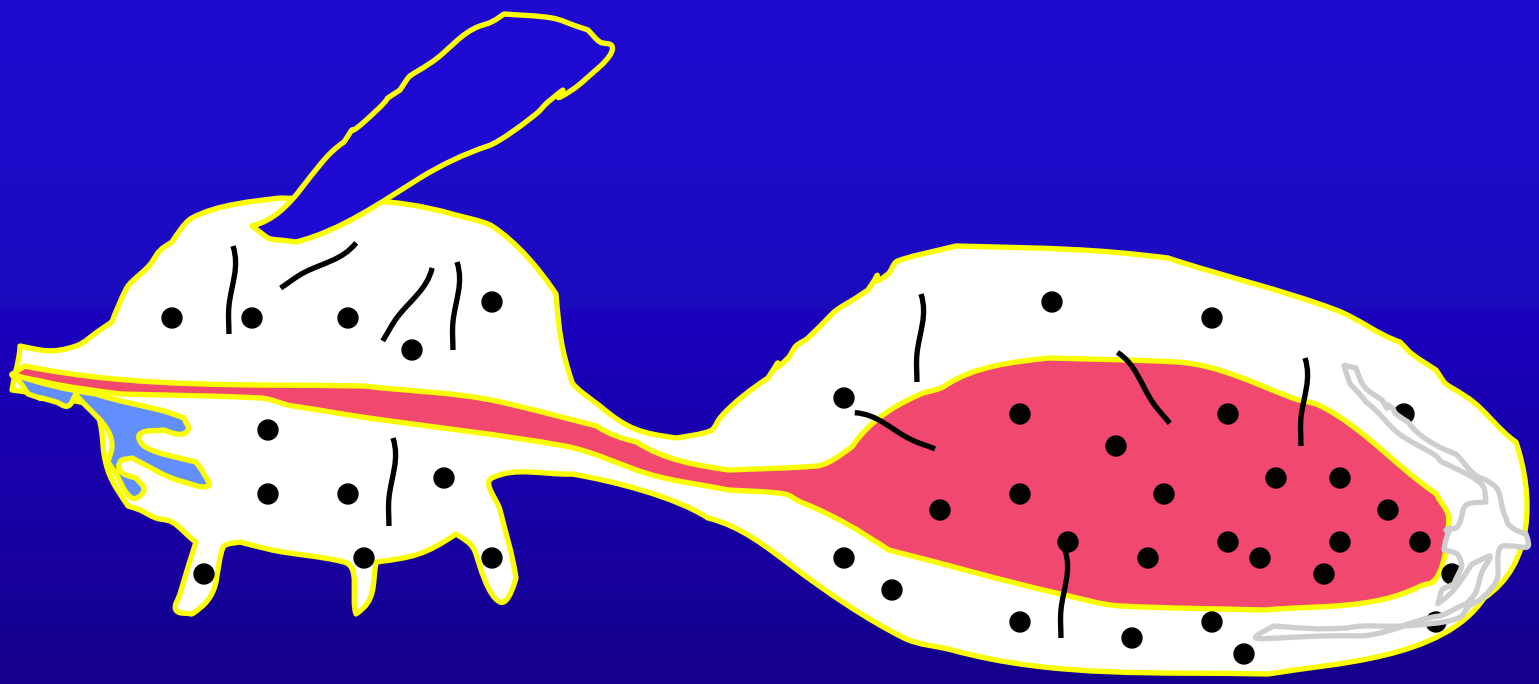
CONCURRENT INFECTIONS WITH MICROFILARIAE AND VIRUSES



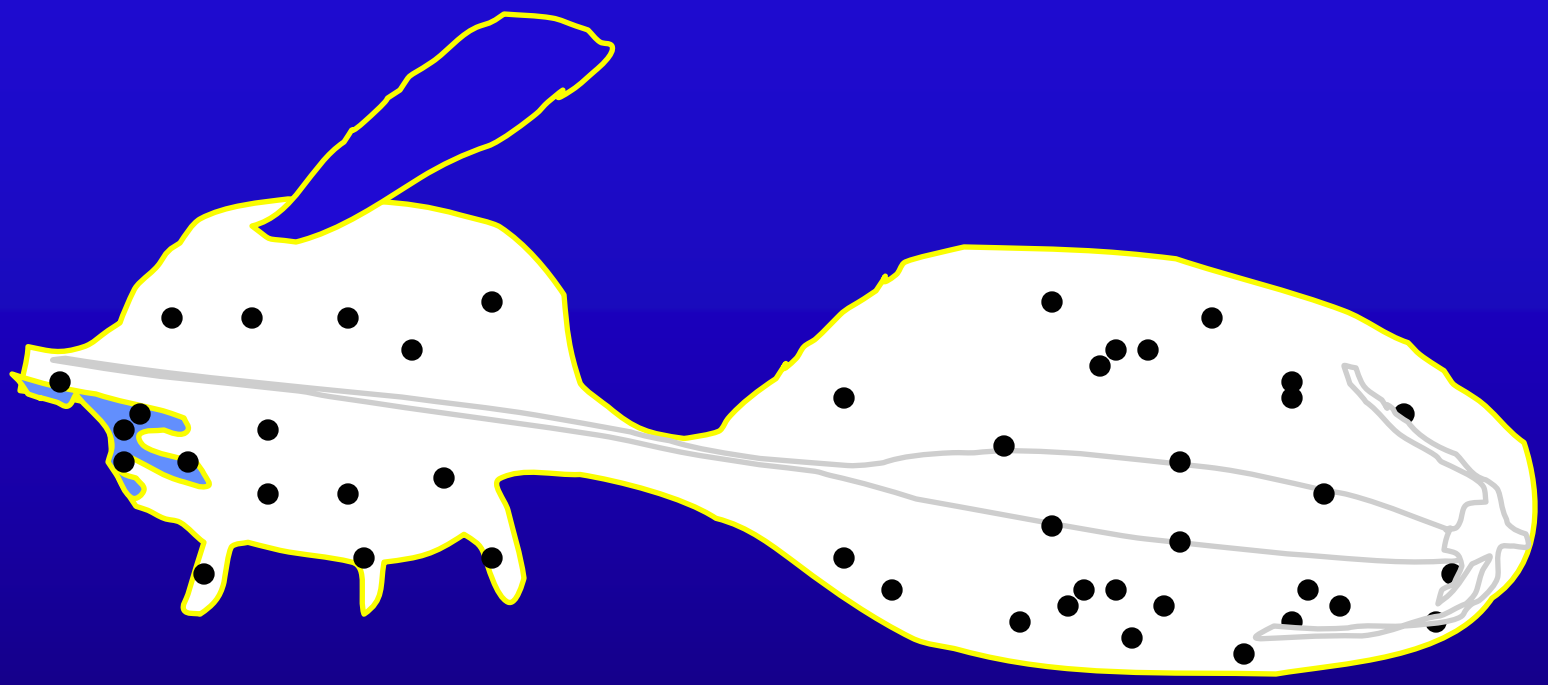
Blood meal with both virus and microfilariae,
but mosquito not yet infected



Microfilariae escaping from the midgut



Virus and microfilariae disseminated to hemocoel



Virus disseminated, but worms are all gone

STUDY PROCEDURE

Infect gerbils with virus

AMICROFILAREMIC

Engorged

Sample at selected time intervals

TRITURATE (Legs + Bodies)

Unengorged (Discard)

MICROFILAREMIC

Engorged

Sample at selected time intervals

TRITURATE (Legs + Bodies)

Unengorged (Discard)

ASSAY FOR VIRUS

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Effect of concurrent ingestion of microfilariae of *Brugia malayi* and Rift Valley fever virus on virus replication in *Oc. taeniorhynchus*

	Amicrofilaremic	Microfilaremic
Number tested	**62**	**50**
Infection Rate	**55**	**88**

Effect of concurrent ingestion of microfilariae of *Brugia malayi* and Rift Valley fever virus on virus replication in *Oc. taeniorhynchus*

	Amicrofilaremic	Microfilaremic
Number tested	**62**	**50**
Infection Rate	**55**	**88**
Dissemination Rate	**15**	**62**

Effect of concurrent ingestion of microfilariae of *Brugia malayi* and Rift Valley fever virus on virus replication in *Oc. taeniorhynchus*

	Amicrofilaremic	Microfilaremic
Number tested	**62**	**50**
Infection Rate	**55**	**88**
Dissemination Rate	**15**	**62**
Transmission Rate	**5** (43)	**31** (29)

Effect of concurrent ingestion of microfilariae of *Brugia malayi* and Dengue 2 virus on virus replication in *Ae. aegypti*

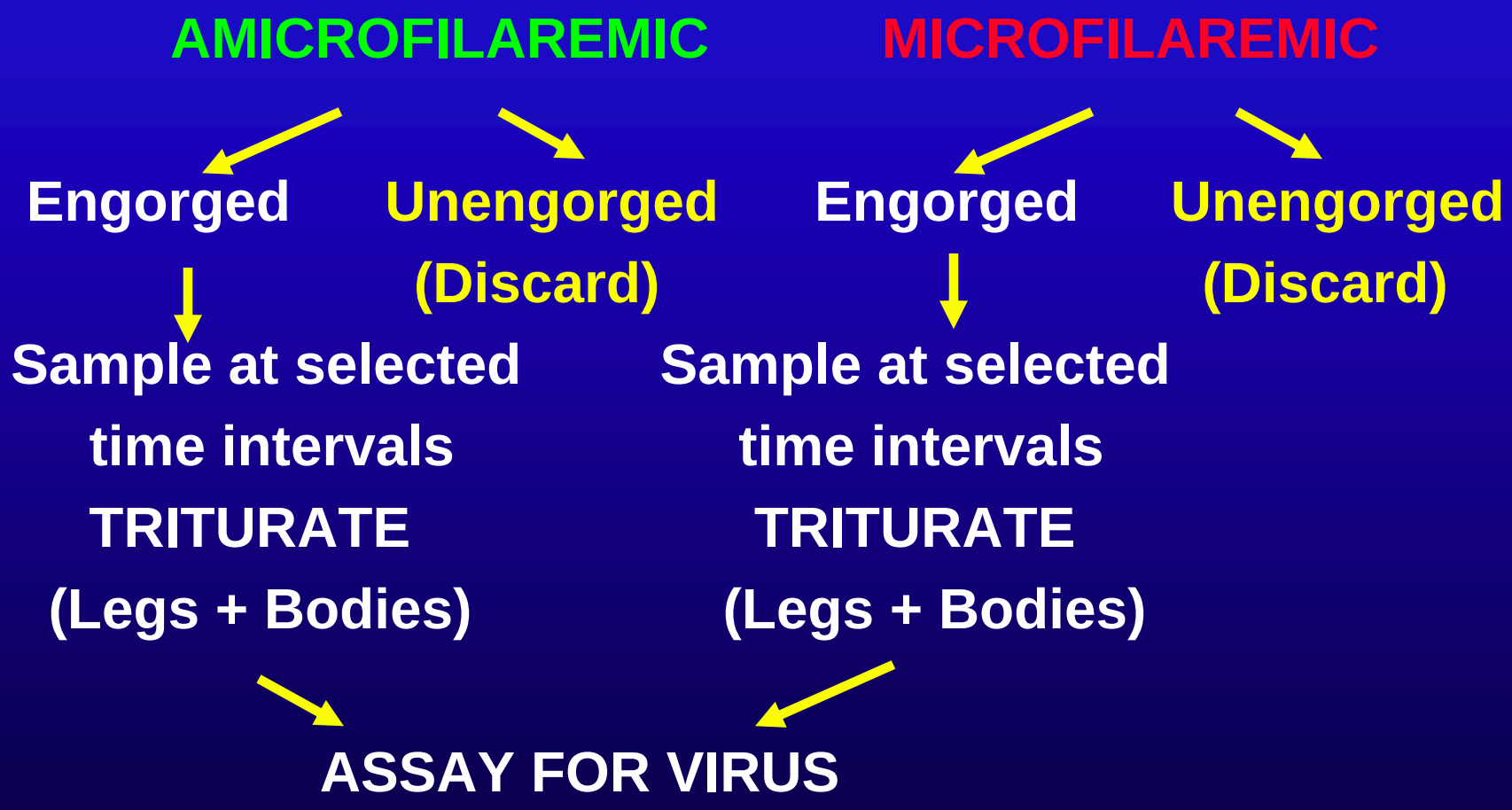
	Amicrofilaremic	Microfilaremic
Number tested	**251**	**160**
Infection Rate	**21**	**16**
Dissemination Rate	**2**	**11**

Effect of concurrent ingestion of microfilariae of *Brugia malayi* and Venezuelan equine encephalitis (subtype IAB) virus on virus replication in *Oc. taeniorhynchus*

	Amicrofilaremic	Microfilaremic
Number tested	**60**	**120**
Infection Rate	**22**	**81**
Dissemination Rate	**2**	**70**

STUDY PROCEDURE

Blood-Virus Suspension and Membrane Feeder



Effect of concurrent ingestion of microfilariae of *Brugia malayi* and Dengue 1 virus on virus replication in *Ae. aegypti*

Days after Blood meal	Microfilaremic			Amicrofilaremic		
	No.	IR	DR	No.	IR	DR
3	20	70	55	10	80	0
5	20	65	65	20	70	0
7	20	60	55	20	65	40
10	not tested			20	70	40

Potential effects of concurrent ingestion of microfilariae and viruses

1. Increase in the Infection/Dissemination Rate - A greater percentage of mosquitoes can transmit virus by bite

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a. More rapid turn around

b. Greater percentage of mosquitoes still alive

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2. Shortening of the extrinsic incubation period – mosquitoes can transmit virus more rapidly
   a. More rapid turn around
   b. Greater percentage of mosquitoes still alive
3. **Have to be balanced with increased mortality due to worm penetration of the midgut and thoracic muscles**

Potential mechanisms for enhancement of viral transmission when concurrently ingested with microfilariae

1. The virus escapes from the midgut through the hole created by the microfilariae.

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2. The virus binds to the microfilariae and is actively transported across the midgut into the hemocoel.

Potential mechanisms for enhancement of viral transmission when concurrently ingested with microfilariae

1. The virus escapes from the midgut through the hole created by the microfilariae.

2. The virions binds to the microfilariae and are actively transported across the midgut into the hemocoel.

3. Virions are ingested by the microfilariae and are actively transported across the midgut into the hemocoel.

Potential mechanisms for enhancement of viral transmission when concurrently ingested with microfilariae

1. The virus escapes from the midgut through the hole created by the microfilariae

2. The virions binds to the microfilariae and are actively transported across the midgut into the hemocoel

3. Virions are ingested by the microfilariae and actively transported across the midgut into the hemocoel

4. Microfilariae are infected with the virus and actively transport virus across the midgut into the hemocoel

CONCURRENT INFECTIONS WITH MALARIA AND VIRUSES

Effect of *P. berghei* sporozoites on RVF virus transmission

Feed *An. stephensi* on mice

infected with *P. berghei*

uninfected mice

Inoculate with RVF virus

Feed on mice or hamsters

Test for the presence of sporozoites

ASSAY FOR VIRUS

Effect of concurrent infection with *Plasmodium berghei* and Rift Valley fever virus on virus transmission by *Anopheles stephensi*

	RVFV only	RVFV + sporozoites
Hamster	0 (19)	40 (10)
Mouse	0 (36)	27 (15)
Total	0 (55)	32 (25)

The question remains:

Is filarial enhancement of viral transmission by mosquitoes a real world phenomenon, or is this a laboratory artifact?

Because filarial infections tend to be life-long in a vertebrate host, the PREVALENCE RATE of filarial infections in vertebrate hosts would be a good estimate of concurrent infection rates.

Infection, dissemination, and estimated transmission rates for mosquitoes that ingested $10^{7.0 \pm 0.5}$ PFU/ml of West Nile virus

Species	Number tested	Infection rate (%)	Dissem. rate (%)	Estimated trans. rate (%)
Cx. quinquefasciatus	78	92	22	21
Cx. pipiens	95	81	23	20
Ae. vexans	13	46	8	8
Oc. sollicitans	50	70	16	11
Oc. taeniorhynchus	75	12	3	3

Department of Vector Assessment, Virology Division, USAMRIID

Where do we go from here:

1. What is the mechanism?

2. How does it affect models such as the dengue simulation model?

3. Can we predict the effects of concurrent ingestion of microfilariae and virus?