Animal models for pre-clinical testing of antibiotics against gonorrhea: Established and new models under development

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Disclaimers

The Jerse laboratory has or is currently conducting research under an Interagency Agreement between USU and NIAID/NIH that is designed to accelerate pre-clinical testing of products against gonorrhea, and directly with the following companies under cooperative research and development agreements (CRADAs) or subcontracts to NIH grants.

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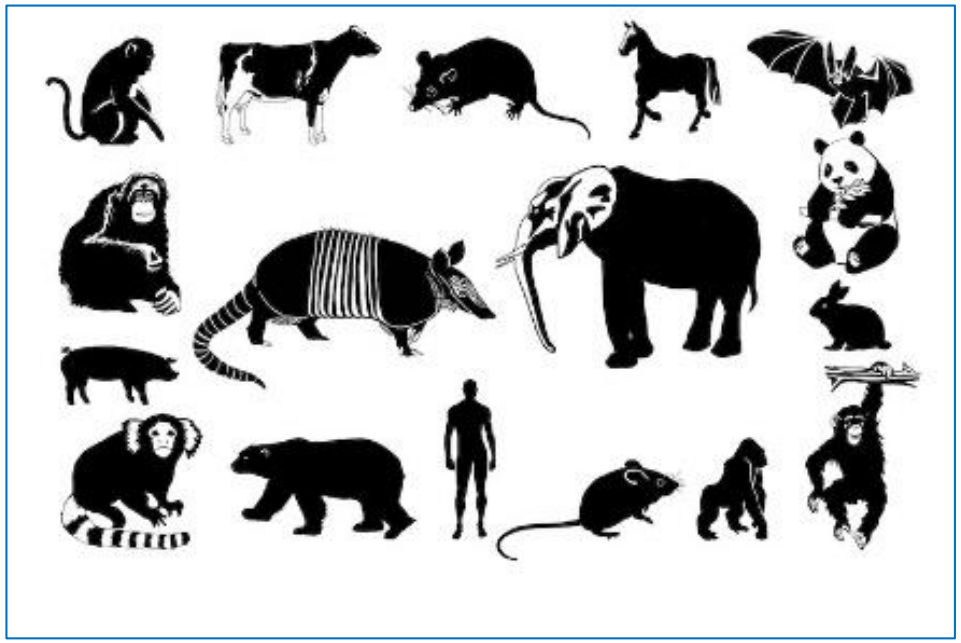
Animal Modeling of *Neisseria gonorrhoeae* Infections

Neisseria gonorrhoeae (*Ng*)

- Human-specific pathogen, no outside animal or environmental reservoir
- Well-adapted to human mucosae
- Numerous host-restricted factors
  - Colonization receptors (pilus receptor, CEACAMs, C3b/C3R receptor)
  - Iron-binding glycoproteins (transferrin, lactoferrin)
  - Calprotectin
  - Soluble regulators of the complement cascade
    (factor H, C4b-binding protein)

(Reviewed in Jerse 2011; Jean 2016)



http://mol-evol.org/publications/heterogeneous-models-place-the-root-of-the-placental-mammal-phylogeny

Published animal models of *Ng* urogenital infection

- Chimpanzees
  - Males: urethritis
  - Females: cervicitis
  - Natural transmission from male to female documented

Lucas 1971, Brown 1972

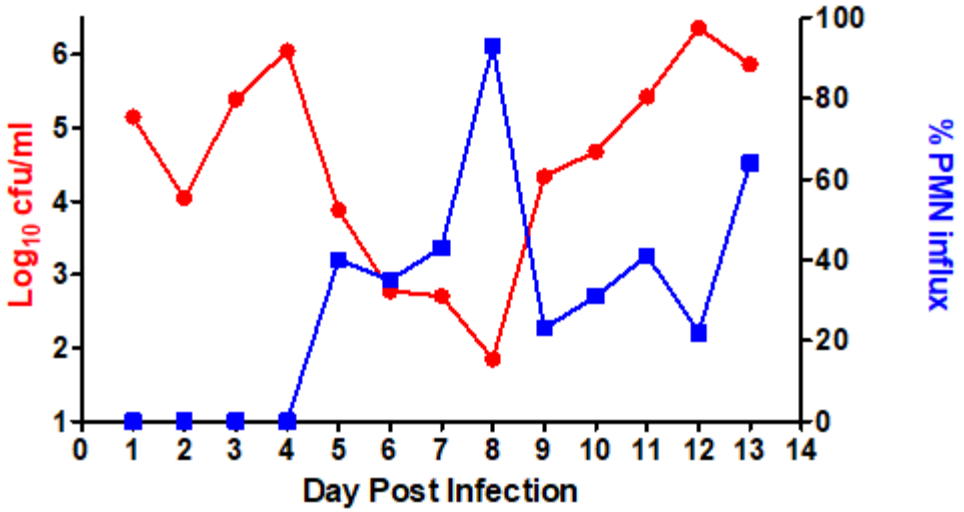
- Estradiol-treated female mice
  Lower reproductive tract (LRT) model
  - cervico-vaginal infection

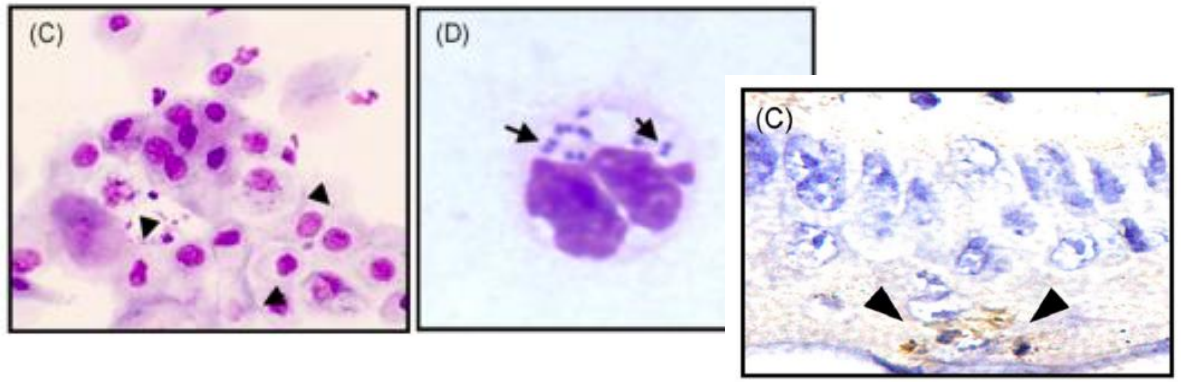
Taylor-Robinson 1990, Jerse 1999, 2011

Murine model of lower reproductive tract (LRT) infection model

- **Treat with estradiol and antibiotics to promote long-term susceptibility; vaginal inoculation**
- **Localization of infection**: Vaginal lumen, associated with nucleated and squamous epithelial cells in cervical and vaginal tissue; seen within PMNs and in the lamina propria
- **Recovery:** $10^2 – 10^5$ CFU/100 µl of a single vaginal swab suspension
- **Cyclical recovery pattern (hormonally driven):** mimics human cervical infections in women of reproductive age

Characteristic Recovery Pattern (vaginal swabs)



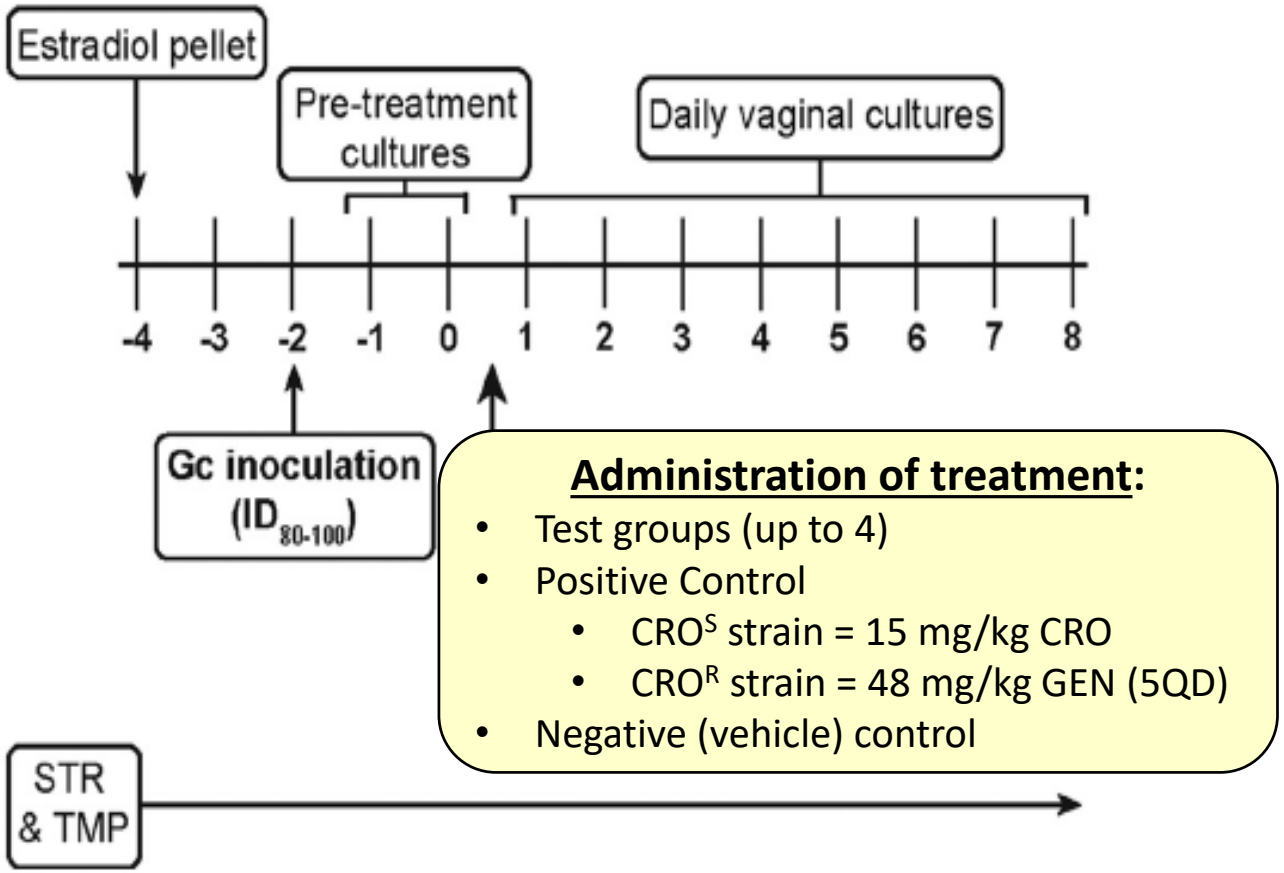


- **Innate responses (BALB/c mice):** Vaginal PMN influx and localized proinflammatory cytokine and chemokine response; induction of cationic antimicrobial peptides (CAMPs)

- **Adaptive response is suppressed and not protective**

(Reviewed in Jerse 2011)

Typical design for *in vivo* efficacy studies (Jerse lab protocol)



Current test strains

Laboratory Strains: FA1090, MS11, FA19, F62

MDR Clinical Isolate: H041

As described in Connolly *et al*. *Antimicrobial Agents and Chemotherapy* 2019; **63(3):**e01644-18.

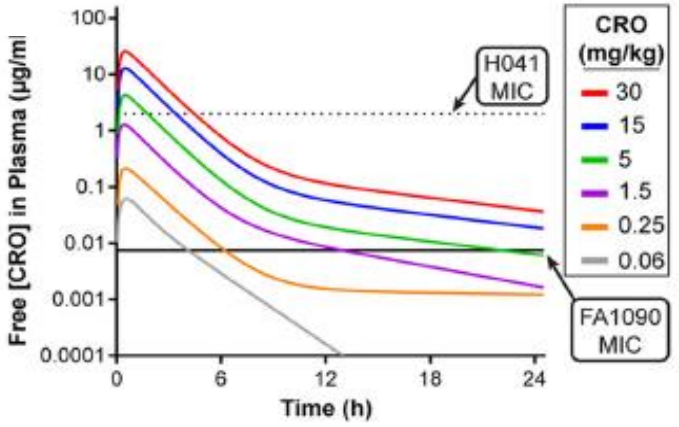
- BALB/c mice are treated with estradiol and antibiotics (STR, TMP).
- Mice are vaginally inoculated with *Ng*; pre-tx cultures taken on days +1 and +2 post-inoculation
- Test compound(s), vehicle control, and positive control [ceftriaxone (CRO) or gentamicin (GEN) **are administered on day 2 of infection**.
- Clearance rate and bioburden are measured **over 8 consecutive days** by quantitative vaginal culture.

National Institute of Allergy and Infectious Diseases

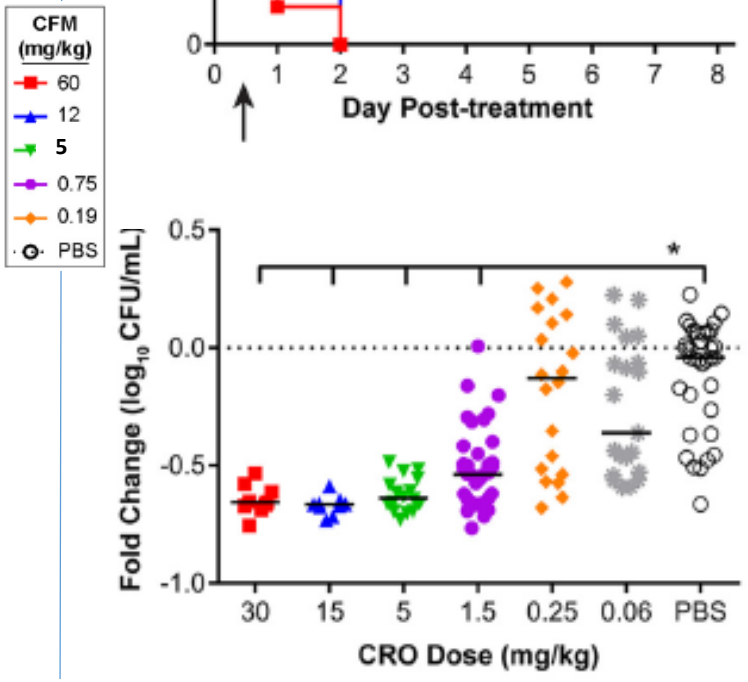
Published pre-clinical efficacy trials using murine models of *Ng* lower reproductive tract infection

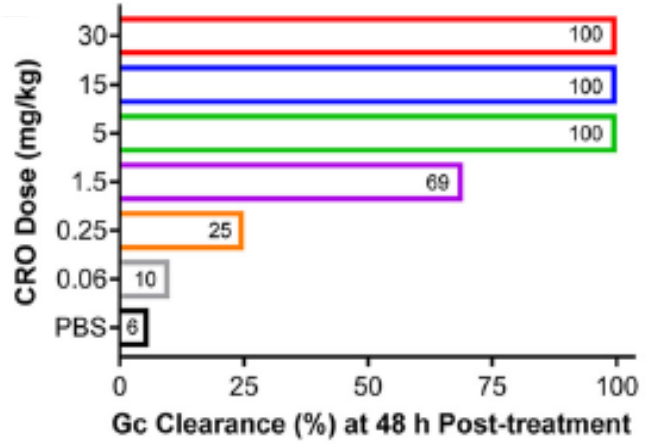
PRODUCT TYPE	MODEL	STUDY OUTCOME	CITATION
Antibiotics			
Aminomethyl spectinomycins and spectinomycin (Microbiotix)	BALB/c mice	All compounds (5QD) significantly cleared infection by a MDR *Ng* strain compared to vehicle; results comparable to GEN.	*Butler 2018*
Resorufin pentyl ether (RPE)	BALB/c mice	5QD significantly reduced the number of *Ng* recovered over time compared to vehicle; a trend for faster clearance of infection was observed.	*Schmitt 2016*
Acylaminooxadiazole (trans-translation inhibitor) (Microbiotix)	BALB/c mice	A single oral dose of MBX-4132 significantly cleared infection by a MDR *Ng* strain compared to vehicle; results comparable to GEN.	*Aron 2021*
Tri-cyclic topoisomerase inhibitor REDX05931	Ovariectomized BALB/c mice	Dose-dependent decrease in *Ng* bioburden at 1 and 24 hr post-tx compared to vehicle. Highest dose resulted in no recovery of *Ng* after 24 hr, and no or low numbers of *Ng* after 7 days.	*Savage 2016*

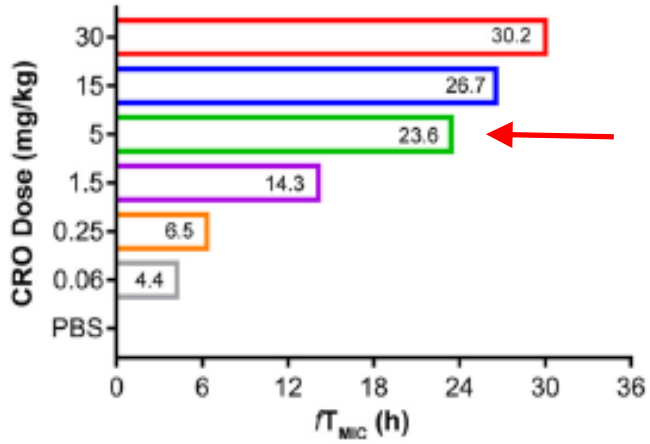
A single 5 mg/kg dose of CRO showed 100% efficacy against a CRO^S strain (FA1090) and produced a therapeutic time of 23.6 hours



Strain FA1090 CRO MIC
0.0075 µg/mL



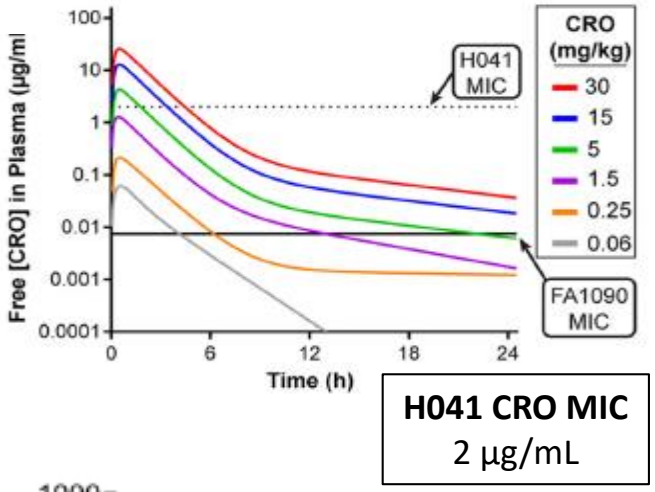




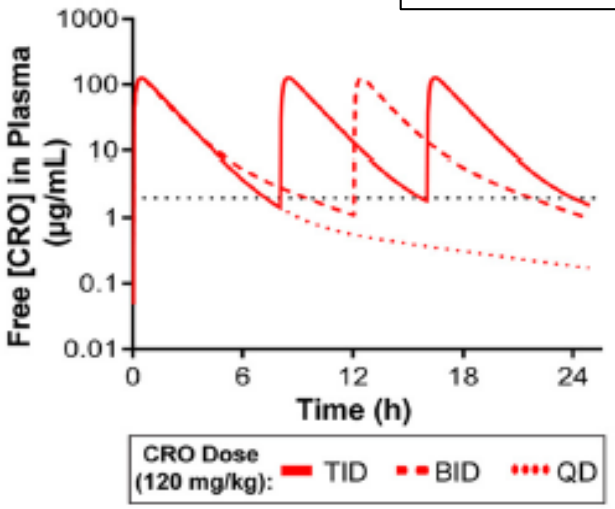
Connolly KL, Eakin AE, Gomez C, Osborn BL, Unemo M and AE Jerse. Antimicrobial Agen Chemoth. 2019

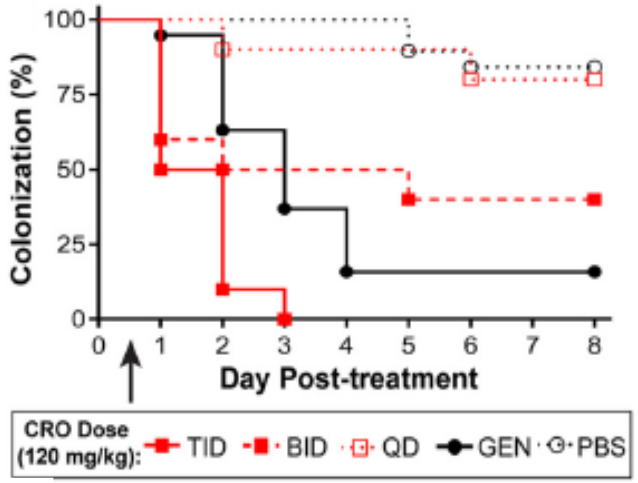
National Institute of Allergy and Infectious Diseases

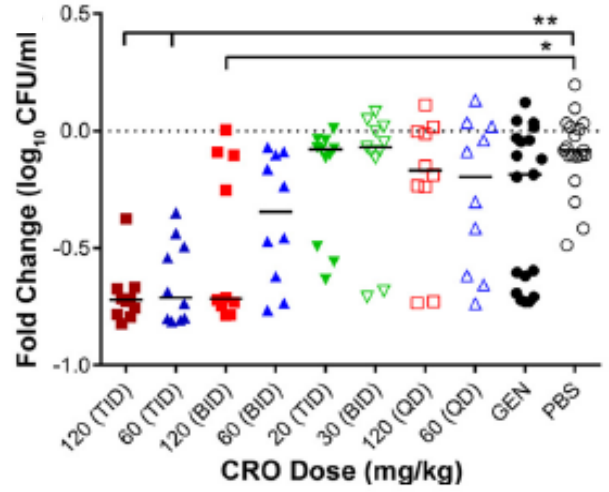
A multiple CRO dosing regimen with a therapeutic time of 22.9 h cleared a CRO^R strain (H041) in 90% of mice at 48 hr

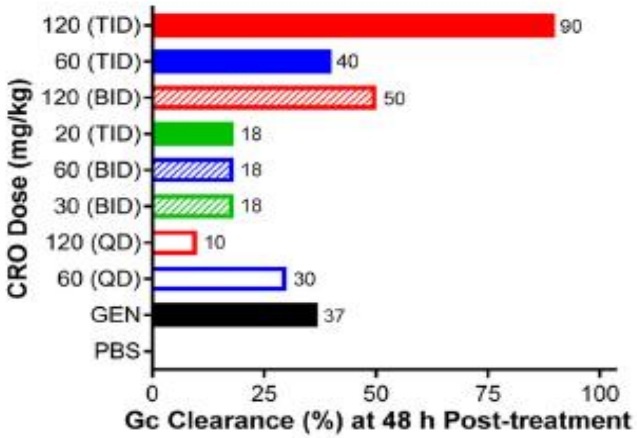


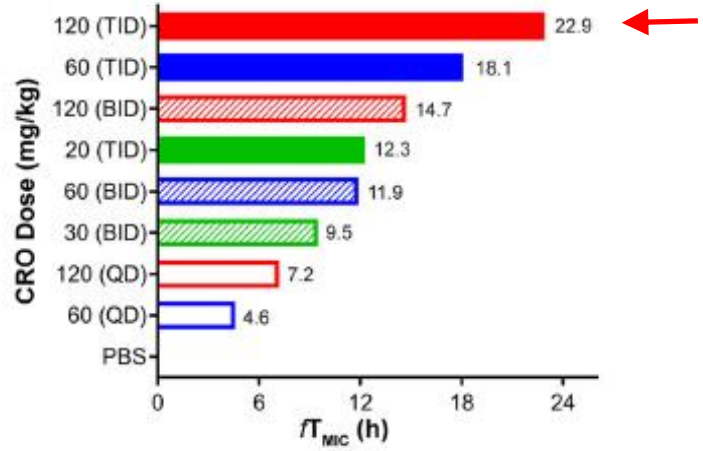
H041 CRO MIC
2 µg/mL







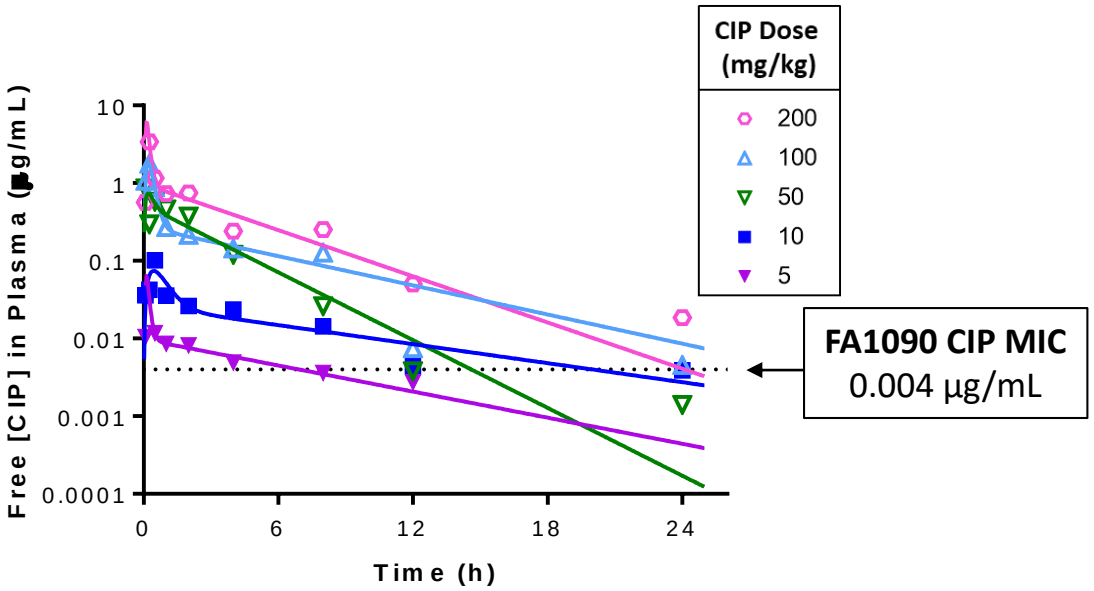


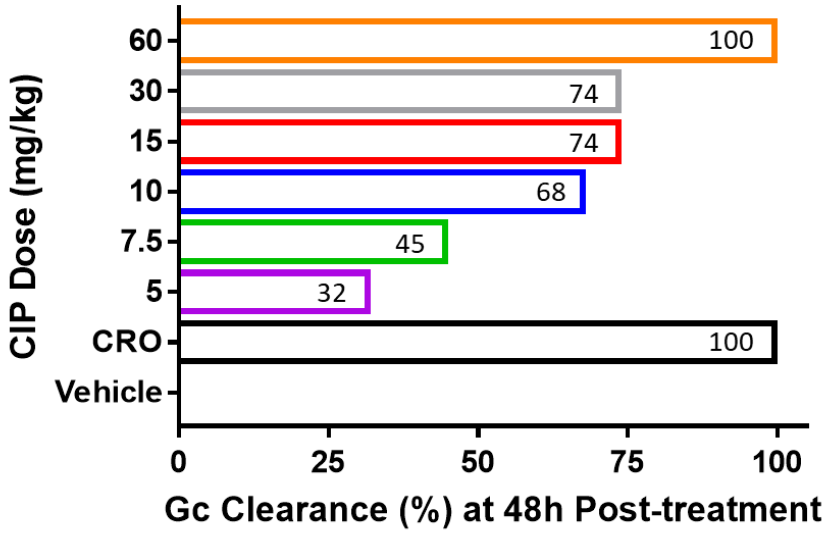


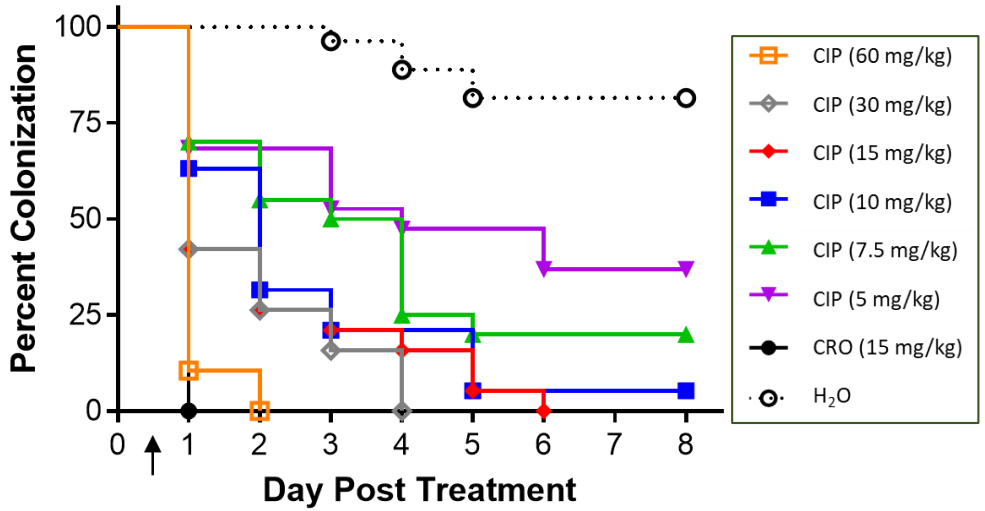
Connolly KL, Eakin AE, Gomez C, Osborn BL, Unemo M and AE Jerse. Antimicrobial Agen Chemoth. 2019.

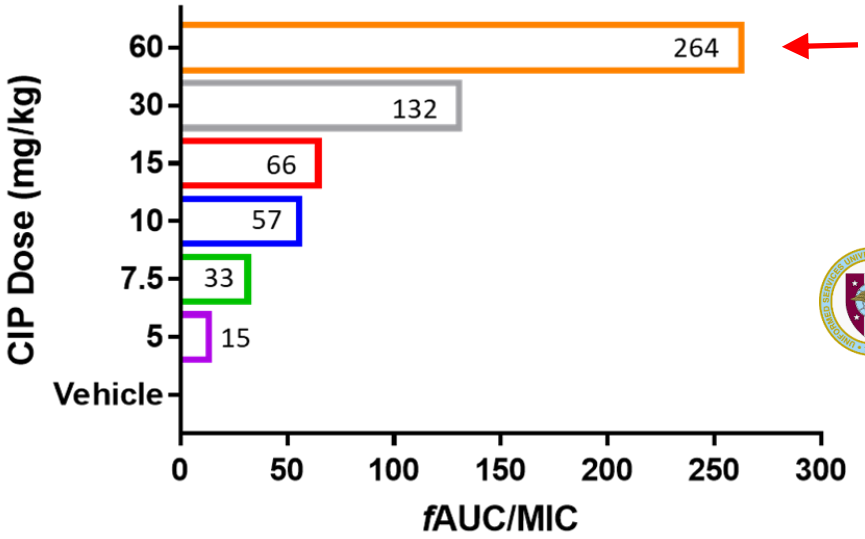
National Institute of Allergy and Infectious Diseases

CIP: The lowest dose that cleared infection by a CIP^S strain (FA1090) in 100% of mice within 48 h has a *f*AUC/MIC of 264









National Institute of Allergy and Infectious Diseases

Need for *Ng* upper reproductive tract infection models

- **Limited number assessments of treatments for clearance of endometrium and fallopian tube infection or for prevention of tubal infertility and ectopic pregnancy** *(Workowski and Bolan, 2015)*

(Walker 1991)

- Efficacy study for cefotetan with doxycycline and cefoxitin with doxycycline in 108 women with acute salpingitis due to *Ct* or *Ng*, with or without anaerobes
  - Clinical cure in 51 of 54 (94%) patients in each group.
  - **All six patients whose treatment failed had positive cultures for *Ng* and facultative/anaerobic bacteria; none had *Ct*.**
  - **Only 92% effective against gonorrhea (66/72 cured)**
  - **Pre-dates emergence of *Ng* strains with reduced cephalosporin susceptibility**

Are PK/PD modeling predictions for clearance of cervical infections the same for endometrial and fallopian tube infections?

Studies on women undergoing prophylactic antibiotic treatment prior to hysterectomy

- Cephalosporins and cephamycin antibiotics may have similar plasma concentrations, but can differ in uterine and fallopian tube tissues and not be high enough for all potential PID pathogens.
- Comparisons of ceforanide and cefazolin:
  - Ceforanide levels in endometrial samples exceeded the MIC90 for *E. coli*
  - Cefazolin levels were below the MIC90 in 50% of myometrial and 67% of endometrial samples

  (Elder 1977; Souney 1988)

Pregnancy

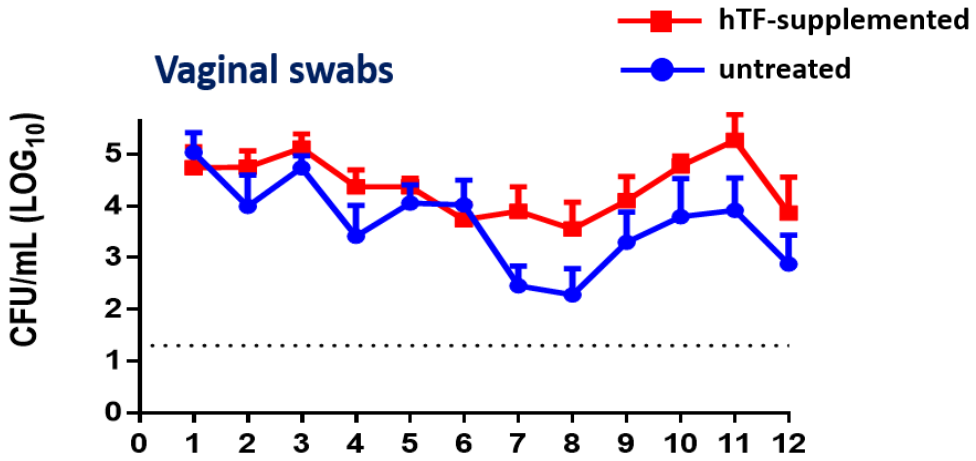
- PK of antibiotics may also differ during pregnancy and for several weeks after pregnancy due to:
  - Changes in renal function
  - Increased uterine weight
  - Physiological changes that may cause poor antibiotic perfusion into the uterus (i.e. changes in in blood volume, extracellular fluid and endometrial blood flow

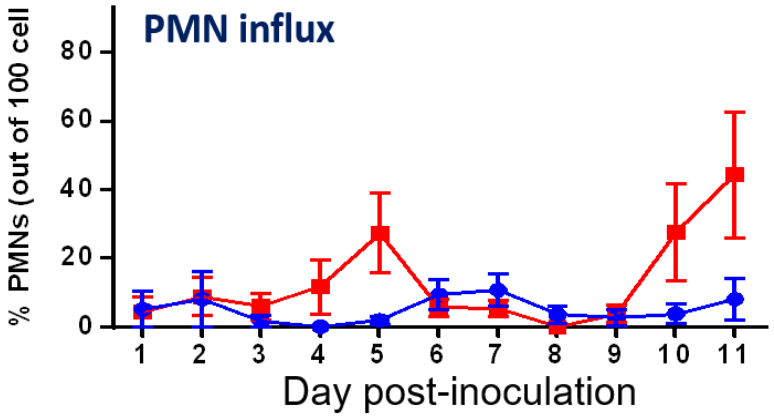
  (Fortunato and Dodson, 1988)

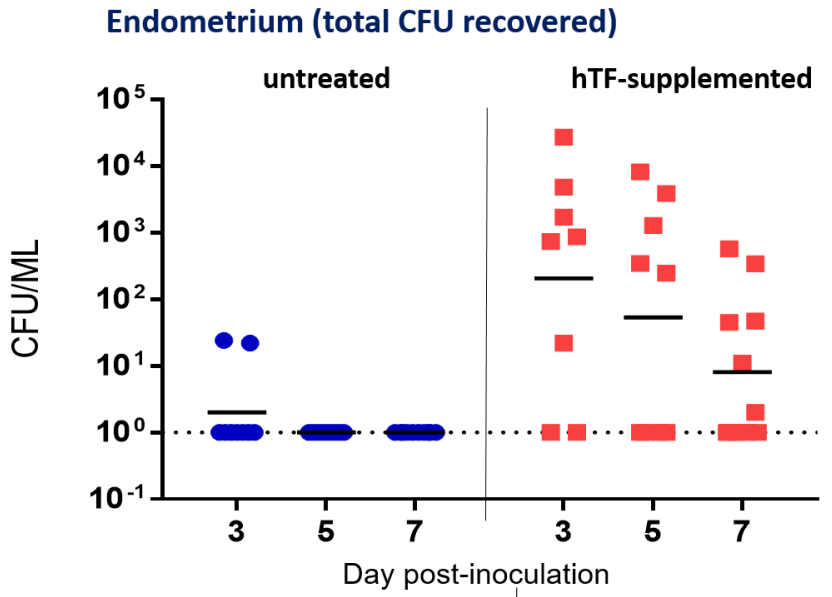
Antibiotic bioavailability with respect to the menstrual cycle – not investigated

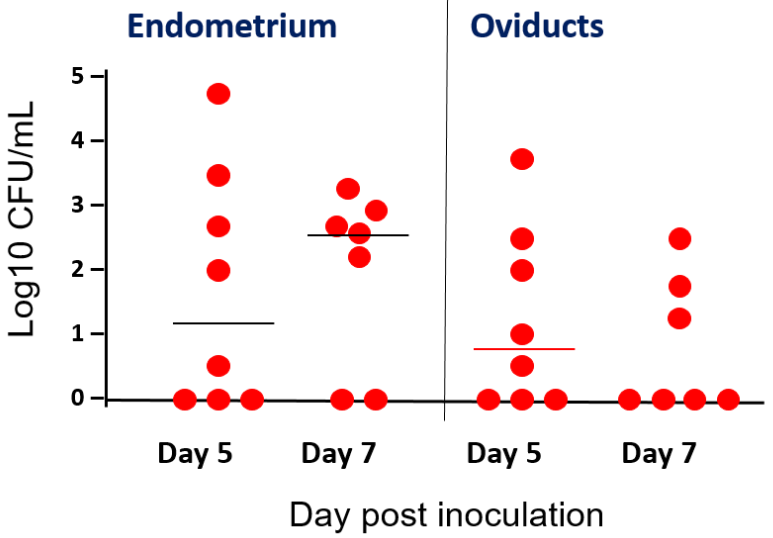
Ng upper reproductive tract (URT) infection model

- Supplement mice with human transferrin (hTF) to relieve the host-restriction in the URT for a usable iron source
- Inoculate vaginally or transcervically with *Ng*









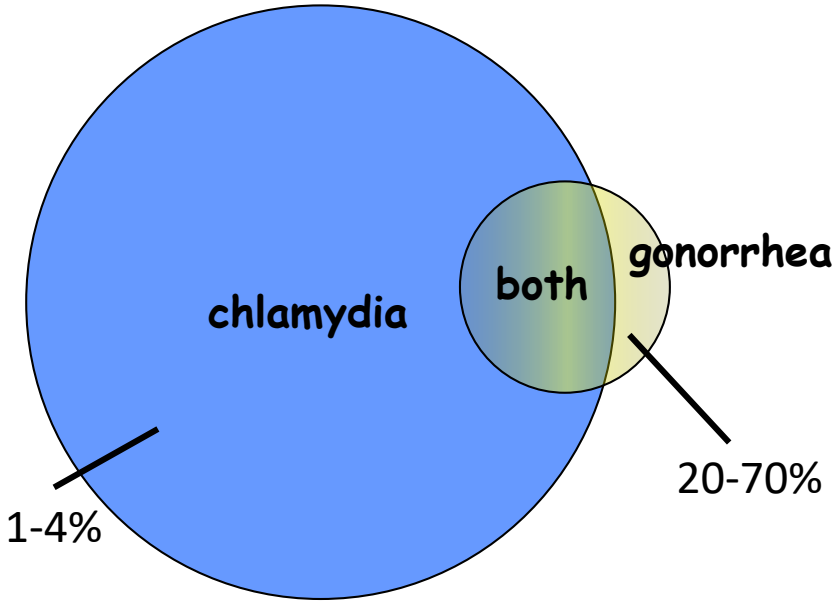
Connolly, Pilligua-Lucas, et al. J Infect Dis, in press

Atlantic Coast STI CRC

Need for *Ng/Chlamydia* coinfection models

- **Gonorrhea/chlamydial coinfections are very common**

- **Question: For dually active new drugs, what is the most effective treatment regimen for both pathogens?**
  - also for co-packaged or fixed-dose combination therapies



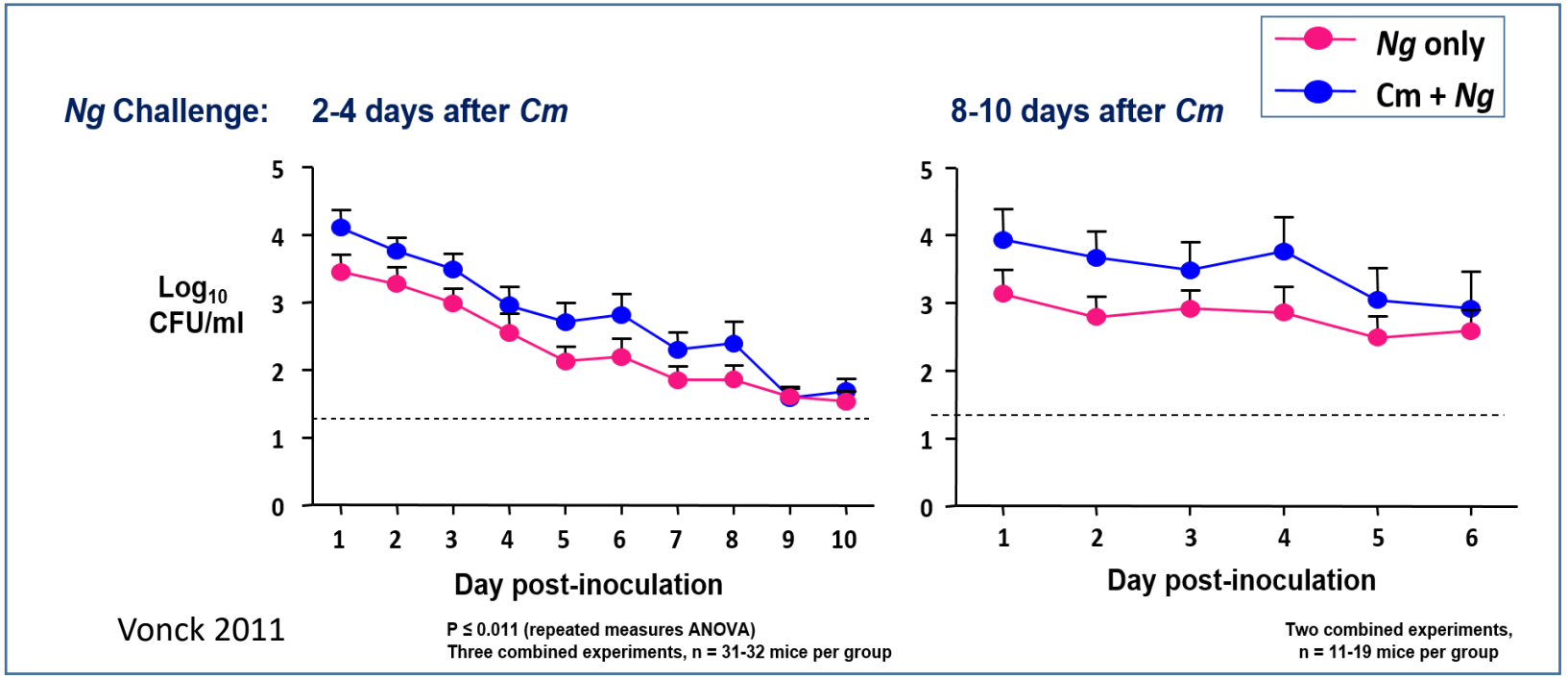
Alirol 2017 Target Product Profile for gonorrhea

Table 1. Consensus Target Product Profile (TPP) developed by the gonorrhea expert group.

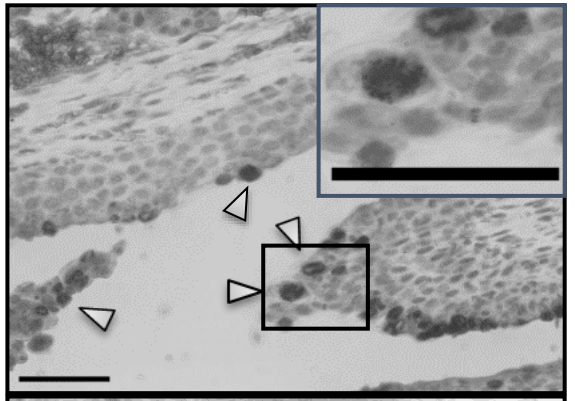
	Short-term (up to 5 years)		Long-term (up to 10 years)	
	Ideal	Acceptable	Ideal	Acceptable
Indication	(First-line) treatment of uncomplicated, urogenital gonorrhea (susceptible and MDR)	(First-line) treatment of uncomplicated, urogenital gonorrhea (susceptible and MDR)	(First-line) treatment of urogenital gonorrhea (susceptible and MDR, complicated and uncomplicated)	(First-line) treatment of urogenital gonorrhea (susceptible and MDR)
	First-line treatment of extra-genital gonorrhea (ano-rectal and oro-pharyngeal)		First-line treatment of extra-genital gonorrhea (ano-rectal and oro-pharyngeal)	
			Treatment of *Chlamydia* infections	
Activity against coinfecting STI pathogens	*Chlamydia trachomatis*		*Chlamydia trachomatis*, *Mycoplasma genitalium*	*Chlamydia trachomatis*

Ng/Chlamydia muridarum (Cm) lower reproductive tract coinfection model

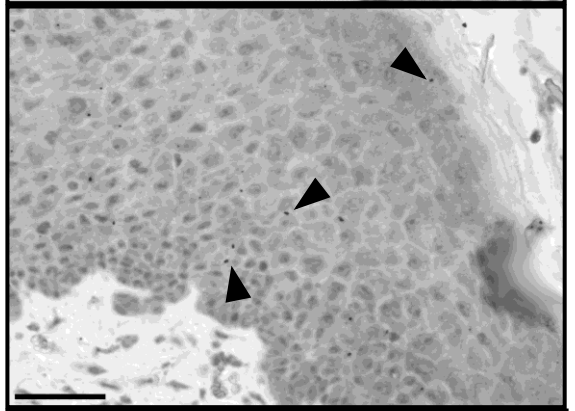
- Inoculate with *Cm* first, followed by *Ng* 2-5 days later
- Higher numbers of *Ng* are isolated from *Cm*-infected mice compared to mice infected with *Ng* alone.



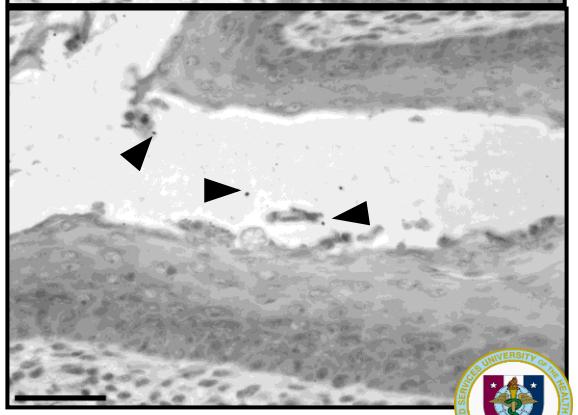
Chlamydial inclusions - cervix



Ng within vaginal tissue



Ng in cervical lumen



- Results are consistent with a human study in which higher numbers of *Ng* were isolated from adolescent girls with concurrent *Chlamydia trachomatis* infection compared to *Ng* infection alone

The Natural History of Incident Gonococcal Infection in Adolescent Women *Stupiansky et al. Sex Trans Dis 2011*

Ng/Chlamydia muridarum endometrial coinfection model

- **An upper reproductive tract co-infection model also has been established**
  - *Ng* and *Cm* are recoverd from vaginal swabs and endometrial tissue for as long as 10 days post transcervical inoculation of both organisms.
  - Have identified time points and a positive control two-drug regimen for drug efficacy studies

Costenoble-Caherty, in preparation

Extragenital tract infection models

- **Pharyngeal infections are more refractory to treatment than lower urogenital tract infections**
  - hTF is not sufficient; appears to be a more host-restricted body site *(Connolly and Jerse, unpublished)*
  - A combination of host-restricted factors is likely needed

- **Rectal infections**
  - Not yet successful in female mice
  - Attempt to infect cotton rats unsuccessful *(Spencer and Jerse, unpublished)*

Disseminated gonococcal infection (DGI) model

- **No models yet that replicate dissemination from a mucosal site to the bloodstream**
  - Older literature reports bacteremia in mice following intraperitoneal (IP) injection of *Ng* *(Kita, 1985)*
  - C1q infant rat model: IP injection of *Ng* +C1q resulted in bacteremia for 6 days *(Nowicki 1995)*

- **Arthritis models**
  - Synovial injection of rats, rabbits *(Goldenberg 1983; Flemming 1986)*

Summary

Animal modeling of *Ng* infections is indeed a work in progress.

- Identification of *in vivo* breakpoints for different antibiotics is ongoing and will facilitate testing combination therapies.

- Alleviating the host restriction for iron has allowed establishment of models of *Ng* endometrial and oviduct infection.
  - PK/PD studies are needed to explore difference in bioavailability in the upper reproductive tract.
  - Characterization of the *Ng* URT model with respect to host immune responses and pathology is ongoing.

- *Ng/C. muridarum* lower and upper reproductive tract coinfection models have been established.
  - The higher bioburden of *Ng* seen in *Chlamydia*-infected mice and adolescent girls supports the need to test antibiotic efficacy in the context of coinfection.
  - We have established a positive control for testing dual therapies in the *Ng/Cm* endometrial coinfection model.
  - Attempts to establish a *Ng/C. trachomatis* coinfection model are underway.

- Extra-genital tract infection models and a DGI model are challenged by more host restrictions.
  - Supplementation of mice with host factors or transgenic mice may solve this problem.

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PK/PD and *in vivo* efficacy studies

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Upper reproductive tract and coinfection models

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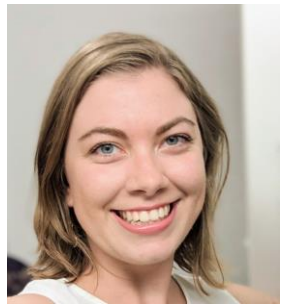

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