Update on *In Vitro* Leptospira Vaccine Potency Test

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SACATM Meeting

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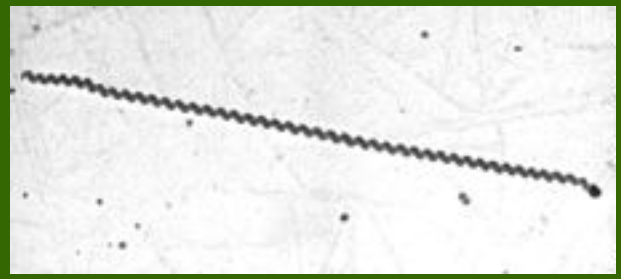
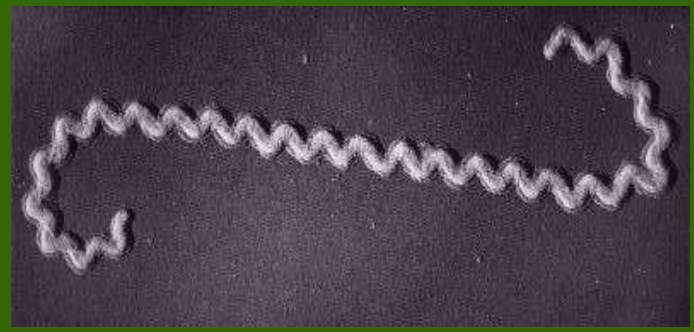
Taxonomy

- Order: Spirochetales
- Family: Leptospiraceae
- Genus: *Leptospira* and *Leptonema*
- Leptospira Species: *interrogans & biflexa*
  - *L. interrogans*: pathogenic
  - *L. biflexa*: saprophytic, not generally pathogenic, found in pond water
- Basic Taxon is the serovar (> 220 pathogenic serovars) based on cross-reacting antigens
- Serovars with common surface antigens grouped into Serogroups (> 20-30 Serogroups)

Taxonomy-continued

- Pathogenic leptospires are not readily distinguishable on the basis of morphology, biochemical, or cultural characteristics.
- They do have distinctive antigenic properties that can be demonstrated serologically using the microscopic agglutination test (MAT).
  - Important for serological diagnosis and provides a basis for classification





L. interrogans Zoonosis

- Leptospires occur naturally in a wide variety of feral and domestic mammals.
- **Natural (maintenance) hosts:** kidneys are colonized and shed in urine. Definitive hosts include:
  - Rat = icterohaemorrhagiae
  - Raccoon = grippotyphosa
  - Dog = canicola
  - Cattle/swine = pomona
  - Cattle/sheep = hardjo
  - Sheep/swine/hedgehog = bratislava

Zoonosis

- **Incidental hosts** (i.e., humans): infections are related to occupational/recreational activities involving direct contact with infected urine, or contact with water/soil contaminated with infected urine

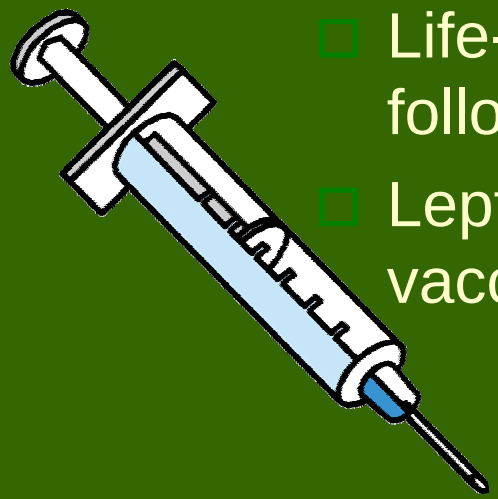
Clinical Manifestations

- **Are variable and depend on whether the animal is a natural definitive (maintenance) host or an incidental host**
  - Other factors include the exposure dose, route of exposure, immune & hormonal status of the animal, pathogenicity of the inocula, and previous exposure (e.g., antibody titers)

- **Symptoms range from:**
  - Inapparent infections
  - Acute phase (Incidental Host): flu-like illnesses, hemolytic anemia, hemoglobinuria, jaundice
  - Chronic phase (Definitive Host): kidney and liver damage, abortion and stillbirths (usually the first and only sign of a herd infection)

Immunity

- Generally humoral, but there is a cell-mediated immunity component
- Measured by a strong and rapid antibody (agglutination) response
- Life-long but serovar-specific immunity following an active infection
- Leptospirosis in animals is controlled by vaccination

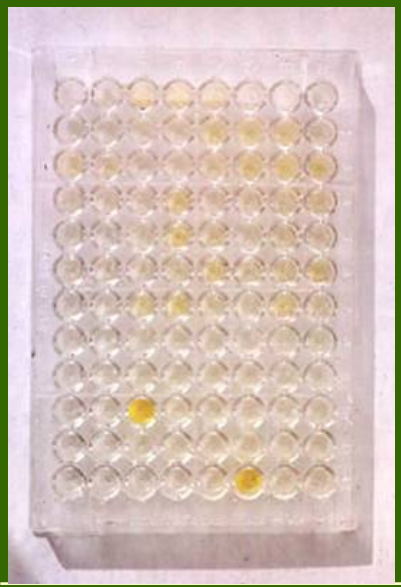


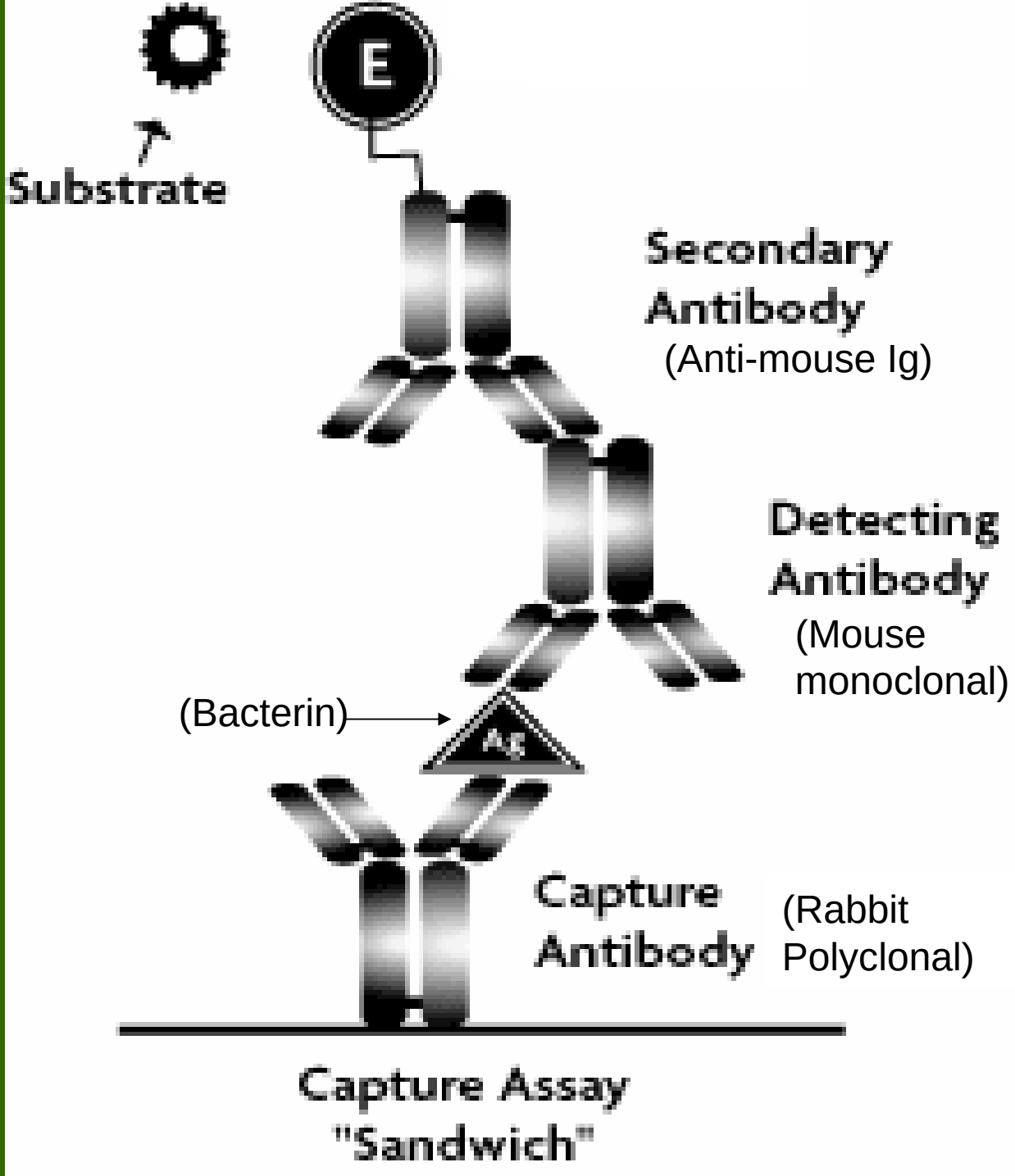
Potency Test

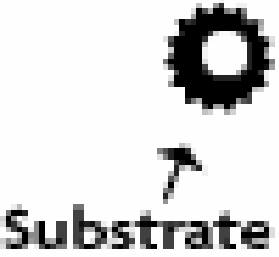
- **Hamster vaccination-challenge assay (9 CFR)**
  - Hamsters vaccinated with a specified dilution of bacterin
  - Exposed to virulent challenge with appropriate serovar 14 days later
  - After 14 days, the number of live/dead determined
  - A minimum of 80% of vaccinates must survive
  - A minimum of 80% of controls must die
- **Disadvantages:**
  - Large numbers of hamsters required (expensive)
  - Time consuming (5+ weeks per test) and labor intensive
  - Exposes personnel to viable pathogenic organisms

Proposed Potency Test

- **ELISA**
  - Utilizes monoclonal antibodies prepared against host animal virulent cultures
  - “Sandwich” ELISA format:
    - Polyclonal rabbit sera coated to wells of microtiter plate
    - Add serial bacterin and reference bacterin
    - Appropriate MAb added, washed, anti-mouse immunoglobulin conjugate added
    - Color substrate added




E
Substrate
Secondary
Antibody
(Anti-mouse Ig)
Detecting
Antibody
(Mouse
monoclonal)
(Bacterin)
Ag
Capture
Antibody
(Rabbit
Polyclonal)
Capture Assay
"Sandwich"



Capture Assay
"Sandwich"

Advantages of the ELISA

- Measures a relevant antigen
- No hamsters involved (few cultures to maintain)
  - Promoting the 3 R's
- Less expensive
  - ~$64,000 for 5% of serials using hamster test (FY01); $640/test
  - ~$1,100 for 600 serials using ELISA test; $2/test
- Personnel are not exposed to a human pathogen

Issues

- Reference bacterin must be correlated to host animal efficacy (dogs, pigs, cattle)
  - Contract with Michigan State University
- Studies require qualified challenge cultures
- Limited supply of MAbs
  - Do have bioreactor fluids for each
  - Not evaluated for equivalency to ascites fluids

Issues

MSU Contract (August 2002)

Background:

- Host animal efficacy studies in dogs by MSU
  - Swine to follow
- 50 L *canicola/icterohaemorrhagiae* bulk fluids produced as national/international ELISA references

Current Status:

- On hold until qualified challenge cultures are obtained
- An additional $500,000 allocated in Sept 2003 for efficacy testing of all four serovars in dogs and swine
- Total of $750,000

Issues

Qualified Challenge Cultures

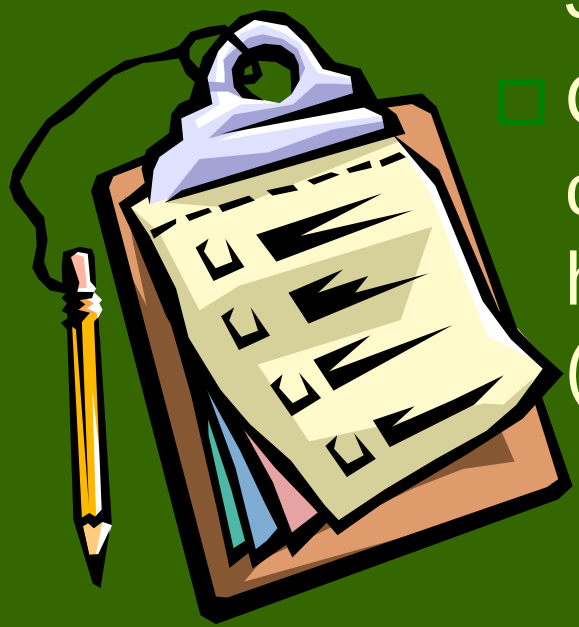
Background:

- January 2003: challenge cultures exceeded 100 hamster passages

Current Status:

- We have no qualified challenge cultures
- Have initiated development

Strategy



- Media studies (completed January 2004)
- Qualify pathogenicity of challenge culture in host animals and hamsters
(*L. pomona* in swine is done)

Strategy

- Qualify bioreactor fluid monoclonal antibodies (hamster passive protection)
- Host animal passive protection
- Reference bacterin validation in hamsters, ELISA
- Reference bacterin validation in host animals (MSU efficacy study)

Summary

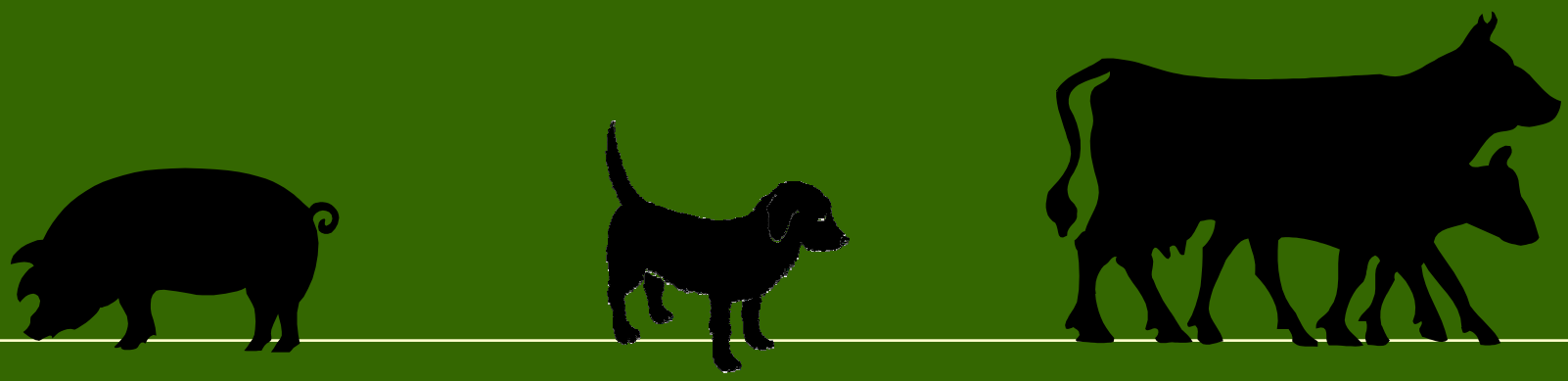
	Canicola	Grippo	Ictero	Pomona
Qualified? -**Swine** -**Dog** -**Calves**	 NO NO NO	 NO NO NO	 NO NO NO	 **YES** **In Process** NO
Target Passive Protection -**Swine** -**Dog** -**Calves**	 NO NO NO	 NO NO NO	 NO NO NO	 NO NO NO
Bid for Reference Bacterin?	NO	NO	NO	**In Process**
Contract Work by CVB?	NO	NO	NO	NO

L. Pomona Timeline

- Dog pathogenicity: by June 2004
- Calf pathogenicity: by August 2004
- Hamster passive protection: July 2004
- Host animal passive protection: September 2004
- Reference bacterin validation: July 2004
- Host animal validation to begin August 2004; will take approximately 18 months to complete

Timeline for other Serovars

- August 2004: Swine pathogenicity (*canicola, grippo, ictero* serovar cultures)
- October 2004: Dog pathogenicity (*canicola, grippo, ictero* serovar cultures)
- December 2004: Calf pathogenicity (*canicola, grippo, ictero* serovar cultures)



Questions?

http://www.aphis.usda.gov/vs/cvb/

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