C. botulinum Testing
US Department of Agriculture

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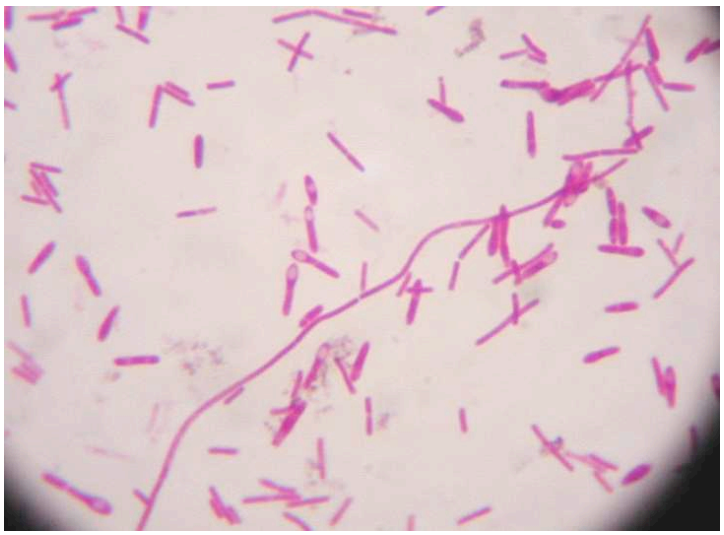
National Veterinary Services Laboratory

- Diagnostics
- Biologics



Diagnostics

- Testing to determine the type of botulinum toxin present
- Usually involves feed or stomach contents from sick horses
- Toxin-Antitoxin neutralization test in mice

Biologics

- 9 CFR 113.110
  Clostridium Botulinum Type C Bacterin-Toxoid
- Product for Mink
- Inadequate storage of wet feed
- ~18 million doses made in 2005

Mink Assay

- Five (5) SubQ vaccinates; three (3) controls
- Challenged IP with *C. botulinum* type C toxin 21 to 28 days later
- All controls must die of botulism
- 80% of vaccinates must remain free of signs of botulism

Mouse Assay for Monitoring Toxin

- *Clostridium botulinum* Type C reference is maintained by CVB
- Four dilution levels used to determine mouse lethal dose (LD_{100})
- Ten (10) mice each
- Usually 9 of 10 mice will die within seven days at a middle dilution; 9 of 10 at highest dilution will survive for seven days

Biologics

- Clostridium Botulinum Type B Antitoxin
- Administered to horses with probable or definite exposure to *Clostridium botulinum* Type B toxin or spores (environmental risk)
- Intended to neutralize toxin that has been ingested
- None made in 2005

Mouse Bioassay (Antitoxin in Sera)

- Prepared toxin
- Standardized antitoxin (to WHO); six dilutions
- Unknown (test sera); six dilutions
- Four (4) mice each = 48 mice (0.5 ml IP)
- Mouse deaths recorded for four days
- Calculate LD_{50}
- Minimum protective level needed for release

Biologics

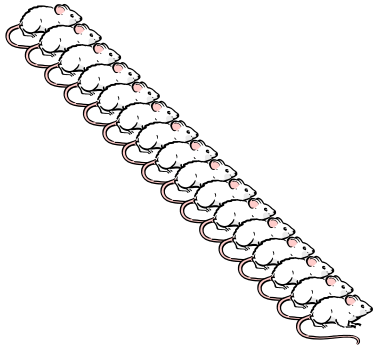
- Clostridium Botulinum Type B Toxoid
- 332,000 doses produced in 2005
- For prevention of botulism due to *Clostridium botulinum* Type B in healthy horses.
- Pregnant mares immunized during the third trimester of gestation, with the third dose (booster) given 2-4 weeks before parturition, respond with antibody. This antibody is concentrated in colostrum and results in significant passively acquired antibody in normal suckling foals.

Guinea Pig and Mouse Assay

- Guinea pigs injected with product
- Produce antitoxin
- Mouse bioassay used to determine if protective level is achieved
- Calculate LD_{50}

"3 Rs" Strategies: Reduction

- Testing required by manufacturer prior to release
- Optional at the federal level



"3 Rs" Strategies: Refinement

April 1, 2004

CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 04-09

Subject: Use of Humane Endpoints in Animal Testing of Biological Products

To: Biologics Licensees, Permittees, and Applicants
Veterinary Services Management Team
Directors, Center for Veterinary Biologics
Deputy Administrator, Animal Care

I. PURPOSE

This notice informs licensees, permittees, and applicants of current Center for Veterinary Biologics (CVB) policy concerning the use of humane endpoints in animal challenge tests.

II. BACKGROUND

Title 9 Code of Federal Regulations (9 CFR) Part 117.4(e) indicates that animals used in testing of biological products may be treated or humanely destroyed if illness has progressed to a point where death is certain to occur. The definition of that point

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