

Isolation and Identification

Tuberculosis in Elephants



NVSL's Laboratory Procedures

Suelee Robbe-Austerman, DVM, PhD
 Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories
 U.S. Department of Agriculture
 Animal and Plant Health Inspection Service
 Veterinary Services
 3/5/2011



Safeguarding Animal Health



Objectives

- NVSL
- Sample submission for organism detection.
 - Surveillance Trunk Washes
 - Postmortem Samples
- Culture procedure
- NVSL Fees
- Future Technologies- direct PCR



NVSL- Mycobacteria culture laboratory

- High volume laboratory, >20,000 cultures/yr
 - Cases include
 - ❖ ~50%- tissue lesions from US inspected cattle at slaughter
 - ❖ ~15%- tissues from antemortem test positive or trace cattle
 - ❖ ~15% -tissues from wildlife surveillance and research groups
 - ❖ ~10%- tissues and biopsies from companion/zoo animals (MOTT)
 - ❖ ~10%- Fluid, aspirates, washes: trunk, gastric, tracheal/bronchial



Trunk Washings



Safeguarding Animal Health



14" French feeding tube



Adding saline to the trunk



Collecting sample from trunk wash into bag

- **Elephant is trained to lower trunk and expel contents into a container**

- **Some have used stainless steel bucket**



Pour contents into leak proof container



Culture of respiratory secretions- what's in the literature....

- Elephants- Research limited, no Se/Sp compared to a gold standard. (Anecdotally ~60%)
 - 8/12 TB+ elephants identified on TW culture (Mikota et. al. J Zoo Wildl Med. 2001 Mar;32(1):1-16.)
 - 15/26TB+ elephants identified on TW culture (Greenwald et. al. Clin Vaccine Immunol. 2009 May; 16(5): 605-612.)



Culture of respiratory secretions- what's in the literature....

- Humans- active expelling of sputum critical
- Passive washing upper resp. tract least desirable
 - (Oberhelman et al Lancet ID 2010) case control study 218 DST +
 - ❖ children- nasopharyngeal wash x2= 12/22 (54%)
 - ❖ gastric washes x2 = 22/22 culture
 - ❖ Stool 4/22
- Some controversy over “best” non-active respiratory secretion collection method: J Trop Pediatr (2010) 56 (5): 291-298.
 - Induced sputum Lancet. 2005 Jan 8-14;365(9454):130-4
 - Bronchoscope, BAL
 - Gastric washes
- Summary: without patient actively expelling sputum, sensitivity is poor for noninvasive techniques
 - However, increase severity of disease, increased sensitivity



Trunk Washings- other issues

- MOTTs common
- Contamination
- Possible ways to improve sensitivity
 - Increased volume of fluid at collection
 - Concentration at laboratory of sample
 - Can BAL of MAPIA positive elephants be considered? (Bronchoscope guided)



Case “story” Elephant

- Trunk Washes submitted

- Nov 2009, Jan 2010, Feb 2010
 - ❖ NIM x3
- Mar 2010
 - ❖ NIM x1, MOTT x1, *M. intracellulare* x1
- Apr 2010
 - ❖ TW1= *M. intracellulare*
 - ❖ TW2= *M. terrae*, *M. fortuitum* cplx, *M. goodii*
 - ❖ TW3= *M. intracellulare*
- May 2010
 - ❖ TW1= *M. fortuitum*
 - ❖ TW2= *M. szulgai*
 - ❖ TW3= *M. terrae*

- Necropsy samples

- Lung
 - ❖ MTB- >11 day Bactec/MGIT
 - ❖ MOTT on solid
- LNPSC, LNRTTP
 - ❖ NIM
- TW-
 - ❖ NIM



Elephant tissue samples

- NVSL routinely does not receive the samples recommended by the elephant TB guidelines.
- Historical data (from cattle tissues) shows for high risk, exposed animals it is worthwhile to culture non-lesioned tissue. (All elephants are high risk)



NVSL recommended tissues

- Lesioned animals:
 - If multiple lesions- send multiple lesioned tissues
 - ❖ TB infected animals can have lesions with different etiology
 - Always submit thoracic lymph nodes
 - Submit formalin fixed tissues
 - ❖ PCR done on FF tissues
 - Give abbreviated necropsy report with samples.
 - ❖ Examples:
 - “Lesions in lung, MesLN, THX LN, all other tissues NGL”
 - “Lungs loaded with lesions”
 - “One small lesion in lung, everything else NGL”

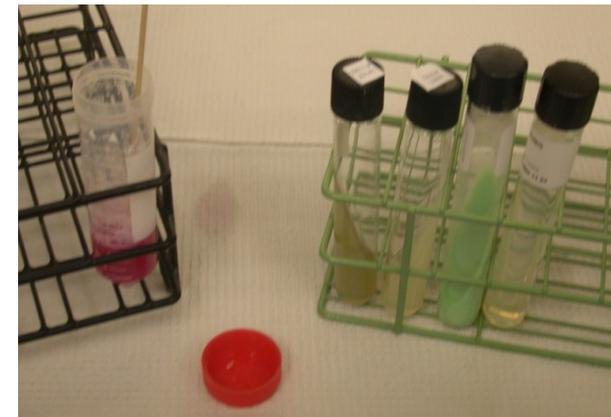
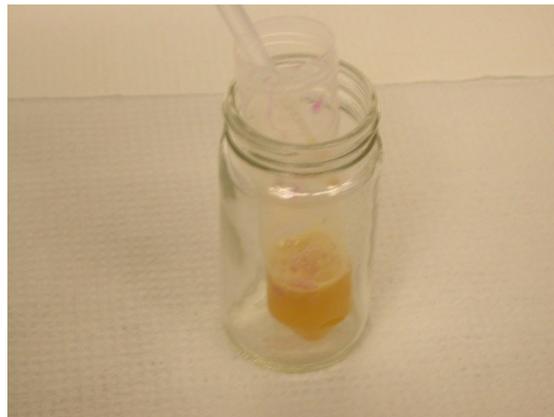
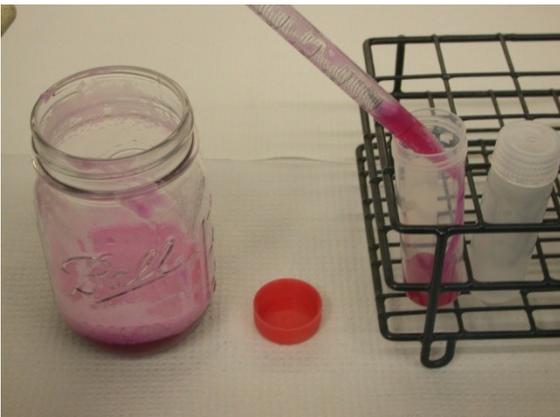
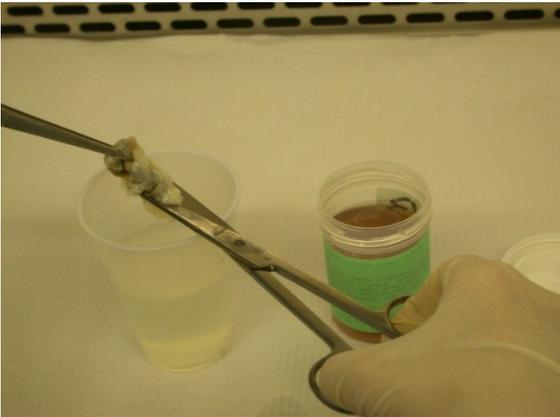


NVSL recommended tissues

- Non-lesioned elephants
 - Thoracic nodes, examine all, pool representative sample from each node.
 - Lung, pool two, 2cm samples from both caudal and ventral areas of each lung- total of 4 samples. (4, 2cm pieces of lung tissue is maximum we can culture).
 - If head nodes can be recovered, collect and pool those.
 - Generally not productive to culture other non-lesioned tissue



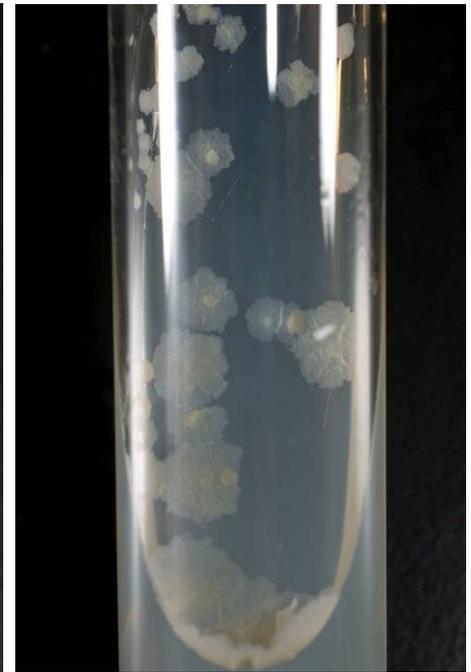
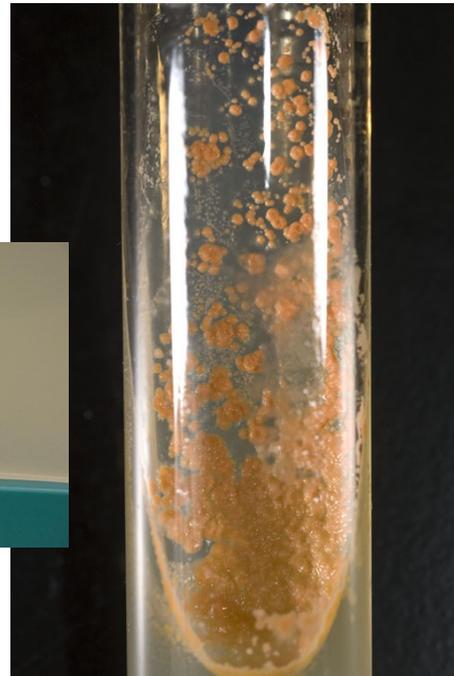
Culture procedure



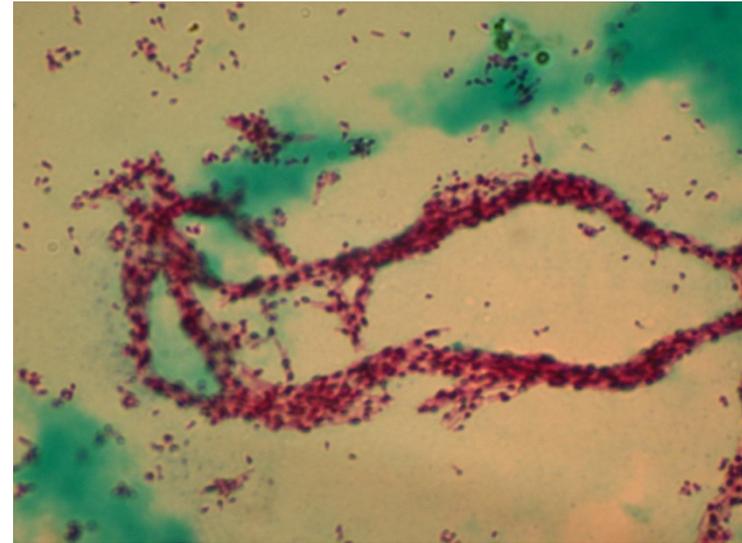
Culture procedure



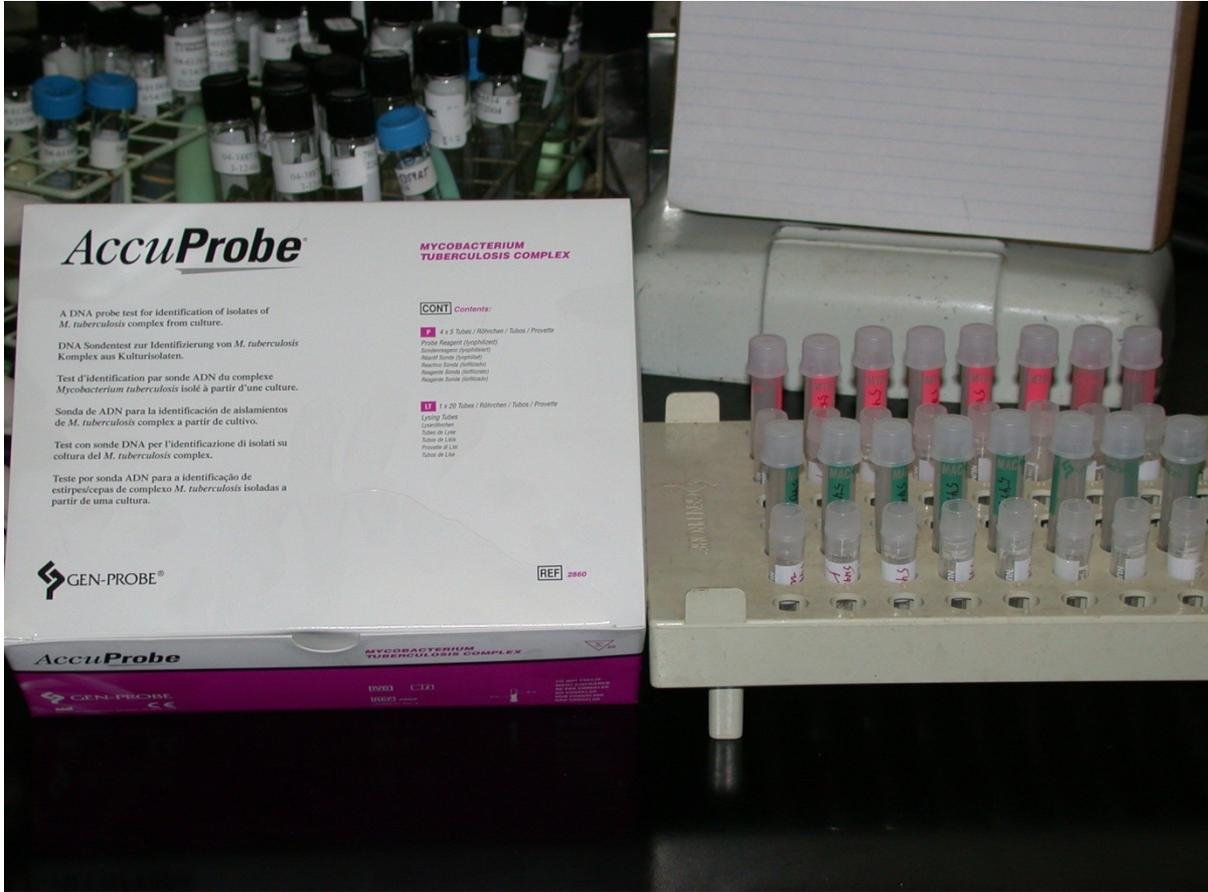
Machines signal positive or have suspicious colonies



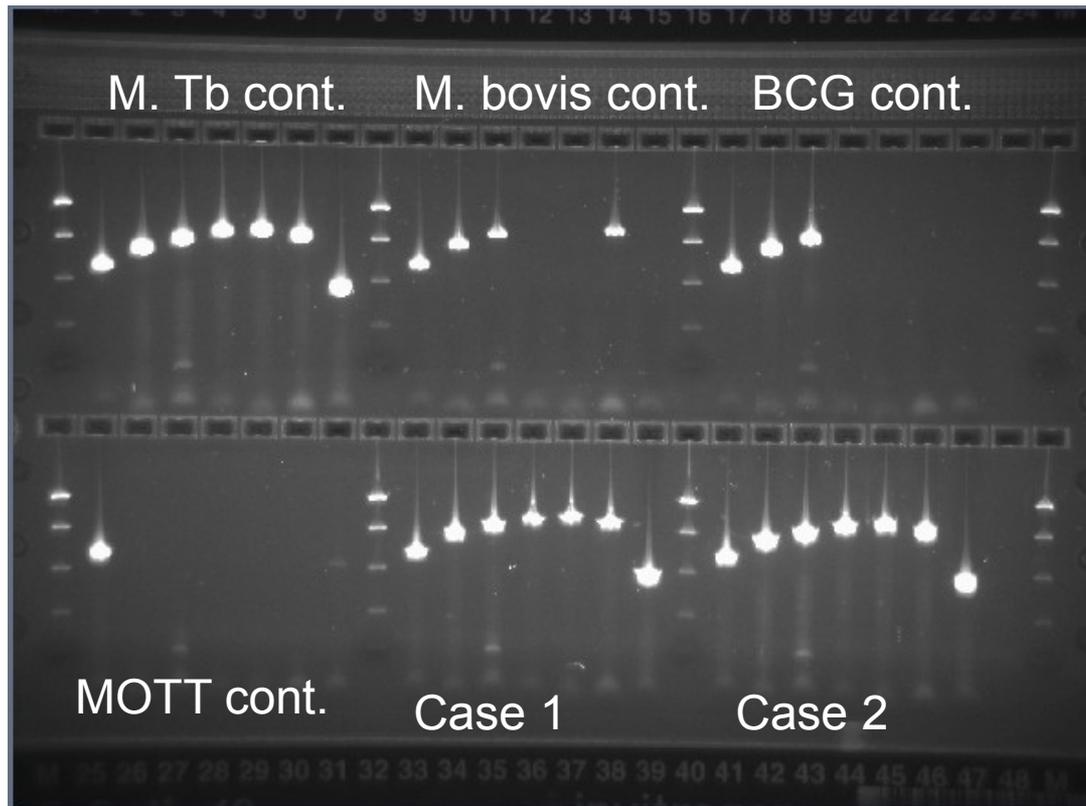
All suspicious media- Acid fast stained



All Acid Fast positive media- tested DNA probes



All TB cmplx probe positive- TB differential PCR and/or Spoligotype



NVSL Charges

- TW culture- \$39.00/culture, All AF isolates will be subject to a \$57.00 identification fee.
- Elephant necropsy samples will not be charged provided the recommended tissues are submitted per elephant guidelines. (at the discretion of NVSL)
- AST \$110.00
- Genotyping- No charge



Future diagnostics

- Direct PCR
 - Typically PCR is not as sensitive as culture.
 - ❖ Mycobacterial cell wall
 - ❖ Only a small amount of material can be tested
 - Problem when granulomas are localized
 - Advantages:
 - ❖ Turn around time
 - ❖ Heavily contaminated samples, samples improperly stored



Questions?

