

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







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





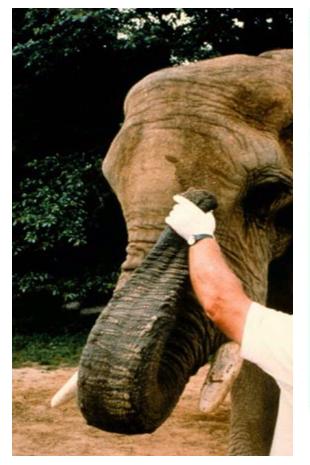


14" French feeding tube





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





Safeguarding Ani



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 cmpx, 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, LNRTP

✤ NIM

≻ TW-

Safeguarding Animal Health

✤ NIM







Submission of TW samples

- Quality of paper work/packaging generally high.
- 3 TW prefer daily consecutive collections.
- Freezing at -20C not as good as -70C, so only freeze if can not get samples to lab by day 4.
- Freezing at -70 and shipping on dry ice is probably as good as shipping each sample next day

		ing the data needed, and	completing	and revie	wing the collecto	n of information		I	PAGE	1212	
UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE NATIONAL VETERINARY SERVICES LABORATORIES P.O. BOX 844, 1920 DAYTON AVENUE, AMES, IA 60010 (516) 337-314				SPECIMEN SUBMISSION							
NSTRUCTIONS: Use a separa 1. SUBMITTER NAME (Including		nd each owner/broker.			s for Completing		-4" for definition		f widife (no	owner)	
								_			
EMAIL ADDRESS						OWNER	CITY		STATE/CO	UNTRY	
PHONE NO.	FAX NO						TION OF ANI				
HONE NO.						PREMIS		MALS			
						COUNTY	r		STATE/CO	UNTRY	
5. PAYMENT METHOD											
USER FEE ACCOUNT NO.		CHECK/MONEY (Enclosed, payable		In US di	ollars)	CREE					
6. HERD/FLOCK SIZE	9. EXAMINATIONS REC	UESTED				CAR	D Exp. Date: 10. COLLECTED BY				
							11. DATE C				
7. NO. IN HERD/FLOCK AFFECTED											
8. NO. IN HERD/FLOCK DEAD		12. AUTHORIZED BY									
13. PURPOSE OF SUBMISS					_		14. COUNT	RY OF ORIGIN	DESTINATI	ON	
Interstate Movement Export	FAD/EP Diagnos	tic General	Discourt		Reagent E		15. REFER	RAL NUMBER			
Pre-Import	Surveillance	Develop	-								
16. PRESERVATION							1				
None Cice Pack Do 17. SPECIMENS SUBMITTER			Other (a	specity)				18. TOTAL N	UMBER OF		
Blood Feces Parasite Serum Tissue (specify)						s 🗆 08	SPECIMENS	SUBMITTED			
	_	specify) 🔲 Water			DNARNA						
19. SPECIES OR SOURCE (20. NUMBER SAMPLED	ER OF ANIMALS				
	Goat Chicken Bison Fish Other (specify)										
Sheep Dor		_		_	Reagent						
21. IDENTIFICAT	ION (See instructions S	250 samples per form	0	_			IDENTIFIC	ATION			
Sample ID	Animal ID	Age	Sex	Sample ID	Animal ID		Breed	Age	Sex		
		1									
		-								-	
		-								-	
		+									
1		1				tions. Use ad					

	UBMITTER AND DAT	E		NV8L USE ONLY
x				
		NV8L U8E ONLY		
CONDITION	PRIORITY	DISTRIBUTION	RECEIVED BY	







What could be improved?

 Make sure phone numbers are current and we can reach someone.

- Give us a little history
 - Routine surveillance
 - MAPIA/Stat-PaK results
 - Name of the elephant or permanent ID

According to the Pa a valid OMB control is estimated to aver instructions, search	ccording to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, an valid OMB control number. The valid OMB control number for this information collection is for estimated to avenge. Shows per response for COTA-0000, 1 hour per response for COTA-01 structions, searching existing data sources, gethering and maintaining the data needed, and					d a person is not required to respond to, a collection of information unless it displays 579-0090, 0101, and 0212. The time required to complete this information collection (0), and .333 hours per response for 0579-0212, including the time for reviewing completing and reviewing the collection of information.						OMB A 0579-0090, and 057
ANIM	UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE NATIONAL VETERINARY SERVICES LABORATORIES P.O. BOX 544, 1920 DAYTON AVENUE, AMES, IA 60010					SPECIMEN SUBMISSION						
INSTRUCTIONS		(616) 337-71				Case Name		for Council allow	100 5000 40	and the state of the		
1. SUBMITTER	NAME (Includi	ng Business	ach species an Name)	a each owns	er/broker.	2. NVS	UCDONS	ITTER ID	3. NAME	E OF OWNER	Check	t if wildlife (
EMAIL ADDRES	s								OWNER	CITY		STATES
PHONE NO.	-		FAX NO.						4. LOCA	TION OF ANI	MALS	.
MAILING ADDRESS (Street, City, State, Z/P Code)								PREMIS	ES ID			
									COUNT	r		STATE/
6. PAYMENT M	ETHOD											
USER FEE A	CCOUNT NO.			CHECK (Enclosed			n 115 de	(mm)	CRED	Number		
6. HERD/FLO	OK OTT		NATIONS REQ		, payable				CAR	Exp. Da		
6. HERDIFLO	NA GIZE	5. EAM	WATIONS REG	DEGTED						IU. COLLE	CIED BT	
 NO. IN HEP AFFECTED 	RD/FLOCK	1								11. DATE C	OLLECTED	
8. NO. IN HEP DEAD	RD/FLOCK	1								12. AUTHO	RIZED BY	
13. PURPOSE	OF SUBMISS	NON (See Ins	tructions for de	finitions)						14. COUN	TRY OF ORIGI	N/DESTIN
Interstate M	lovement	🗖 İm		_	тв			Reagent E			RAL NUMBER	
Export			D/EP Diagnost			Diagnostic		NVSL Intra	lab	IS. REPER	RAL NUMBER	
Pre-Import 16. PRESERV	ATION		rvellance		Develop	mental Re:	earch					
None k	ce Pack 🗖 🛛			rax 🗖 Ak	cohol 🗖	Other (Sp	ecity)				18. TOTAL	
		Paraste	Serum			(specify)		Whole Anima		S SUBMIT		
		Plant	Sol		Urine			Fetus	_			
Extract	MIK	Semen	Swab (s	pecity)	Water			DNARNA				
19. SPECIES	OR SOURCE ((% ONLY or	e)								20. NUMBE	R OF AN
Cattle	Go		Chicken		Bison Rsh Other (specify)				SAMPLED			
Swine	Ho		Turkey		Deer (specify)	_	Environment				
Sheep	Do Do		Other bird (sp	ecity)	EK			Reagent				
21. Sample ID	IDENTIFICAT	TION (See In Animal ID	structions <2	50 camplec Bre		Age	Sex	Sample ID	A1	IDENTIFIC imai ID	ATION	1 1
												_
											_	
											_	
22. ADDITIONAL	. DATA (Histor	y, clinical sig	ns, post morten	i findings, re	marks, te	ntative dia	gnosis,	special instruc	tions. Use ad	iditional sheets	I necessary).	
-												
										h	IVSL USE ONI	LY
23. SIGNATURE	OF SUBMITT	ER AND DAT	E									
23. SIGNATURE X	OF SUBMITT		NVSL US			EIVED B						







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

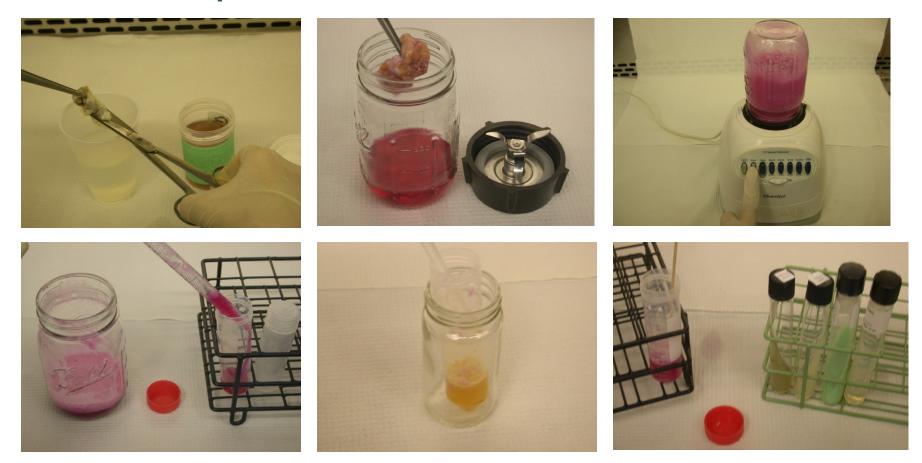
- Non-lesioned elephants
 - Thoracic nodes, examine all, pool representative sample from each node.
 - Lung, pool two, 2cm samples from both caudal and ventral areas of each lung- total of 4 samples. (4, 2cm pieces of lung tissue is maximum we can culture).
 - If head nodes can be recovered, collect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







Culture procedure









Culture procedure













Machines signal positive or have suspicious colonies



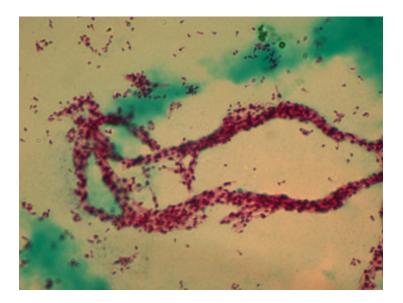






All suspicious media- Acid fast stained



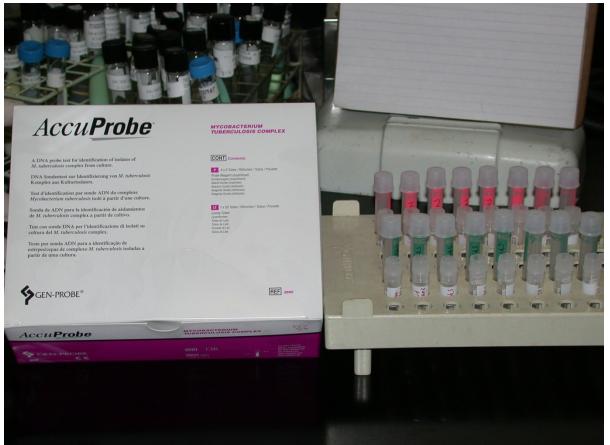








All Acid Fast positive media- tested DNA probes

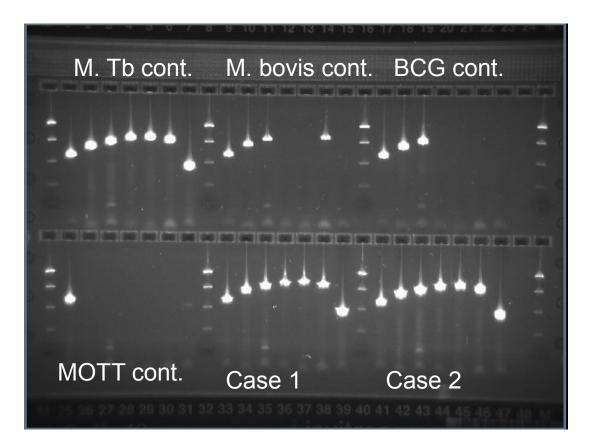








All TB cmplx probe positive- TB differential PCR and/or Spoligotype









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



Veterinary Services



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?

