

Vaccination of White-tailed deer with *Mycobacterium bovis* BCG

Mitchell V. Palmer, Tyler C. Thacker, Pauline Nol, W. Ray Waters

National Animal Disease Center, ARS, USDA, Ames, IA

National Wildlife Research Center, APHIS, USDA, Ft. Collins, CO

2008 Michigan Bovine TB Conference

East Lansing, MI



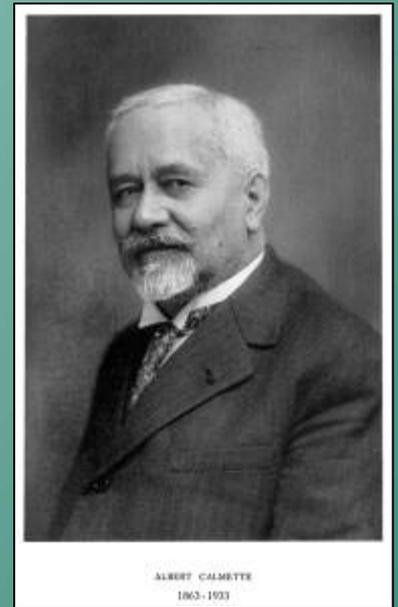
A member of the
National Centers for
Animal Health



United States
Department of Agriculture

Mycobacterium bovis bacille Calmette-Guerin (BCG)

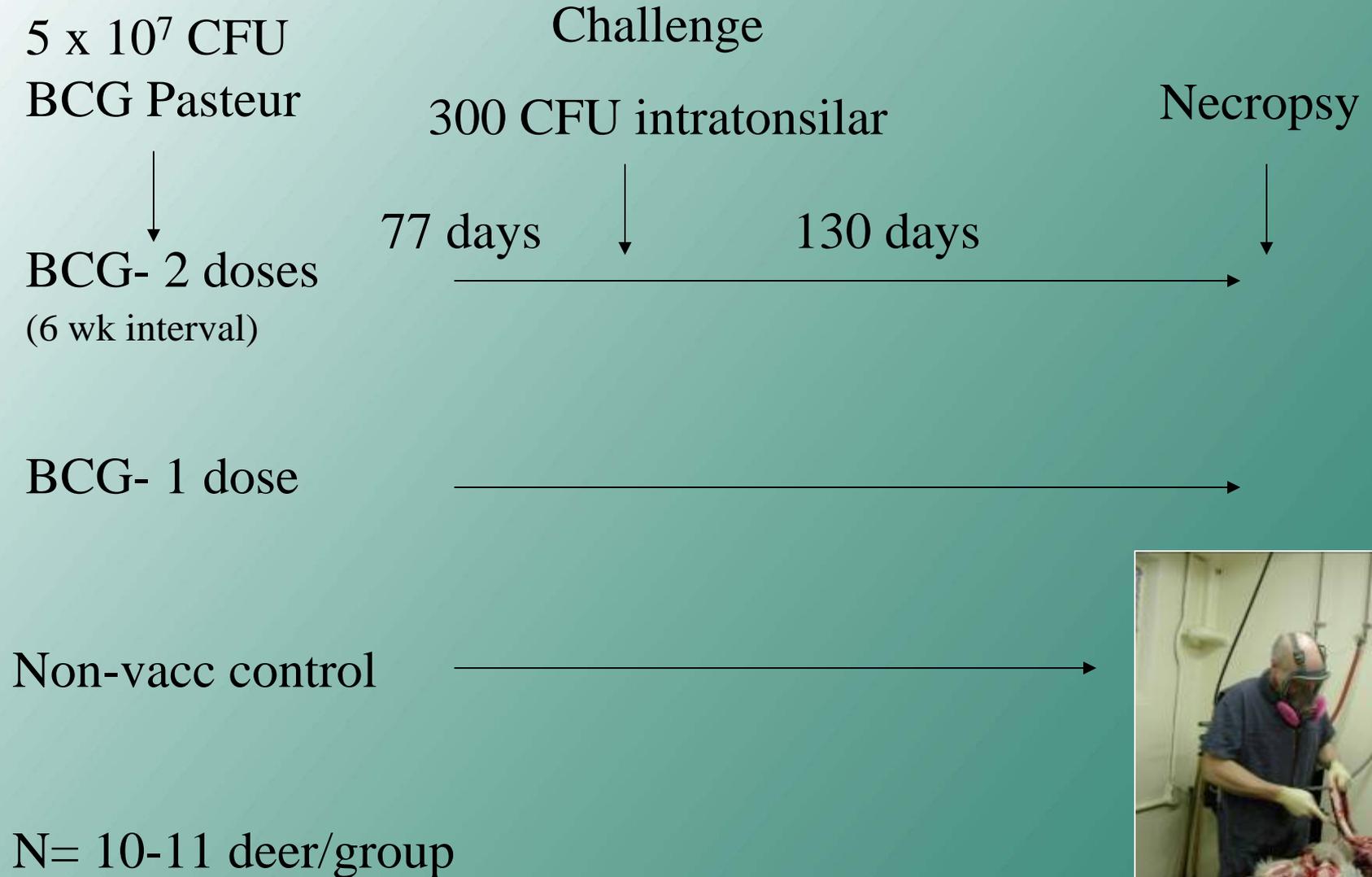
- BCG was originally developed by serial subculture (1906-1919).
- Original isolate of *M. bovis* isolated from a tuberculous cow.
- Attenuation of the original BCG stain resulted from a deletion of a region of the genome termed RD1.
- Continuous passage led to the development of several genetically distinct strains of BCG (i.e. Pasteur, Danish, Tokyo, Trice, etc.).
- Oldest of vaccines in use today.
- World's most widely used vaccine (3 billion doses since 1920).
- Efficacy in humans variable (0-80%).



Mycobacterium bovis BCG

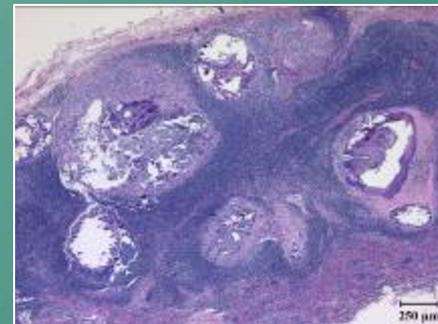
- Red deer- no vaccine-induced lesions, low numbers of BCG in tissues 12 weeks after booster.
- Red deer- 2 doses protected against infection and disease.
1 dose protected against disease but not infection.





Necropsy

- Gross examination with lesion scoring of lymph nodes and lung lobes.
- Microscopic examination with lesion staging.
- Weight of lymph nodes and lung lobes.



Lesion scoring

Lungs: (0-5)

0 = no lesions

1 = no external lesions, but gross lesion(s) seen upon slicing

2 = < 5 gross lesions of < 10 mm in diameter

3 = > 6 gross lesions of < 10 mm in diameter

4 = ≥ 1 gross lesion of > 10 mm in diameter

5 = coalescing lesions

Lymph nodes: (0-3)

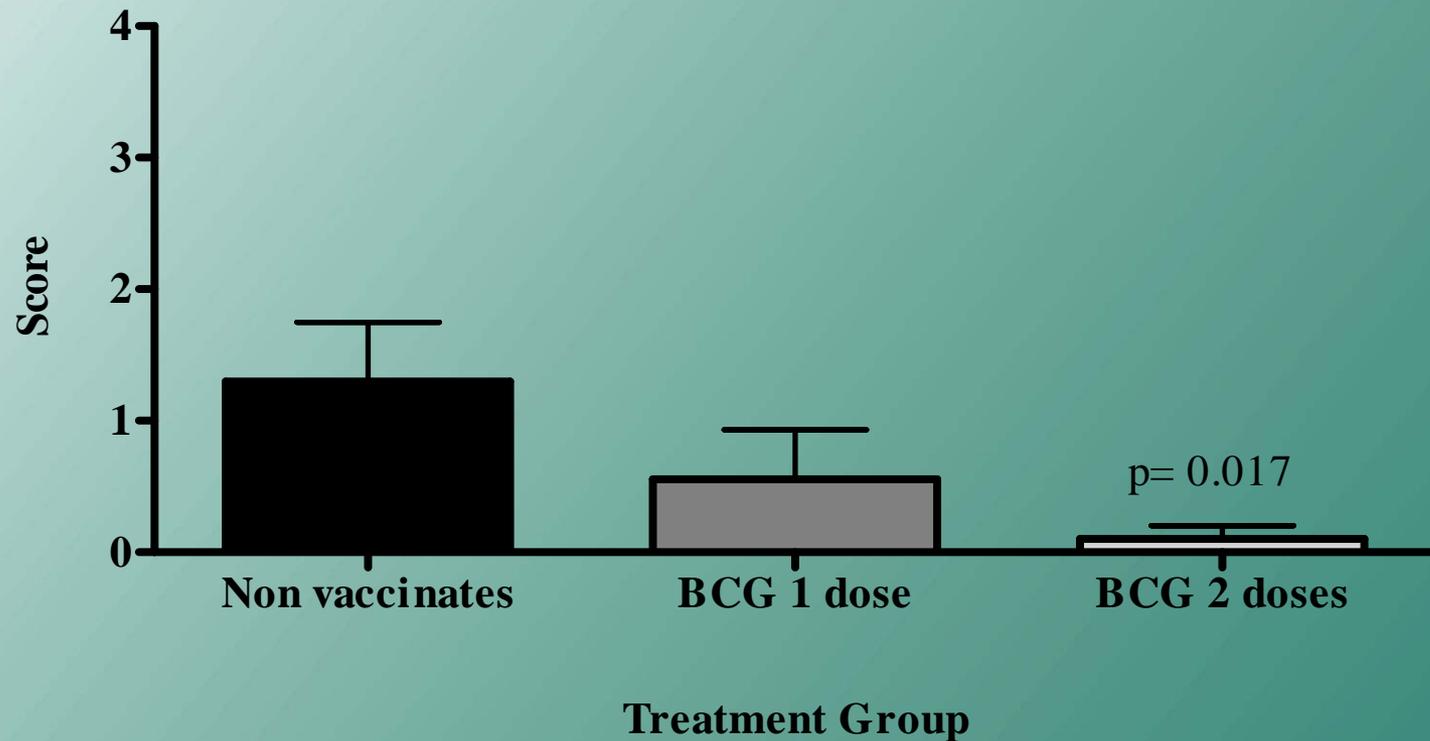
0 = no lesions

1 = small focus (1-2 mm diameter)

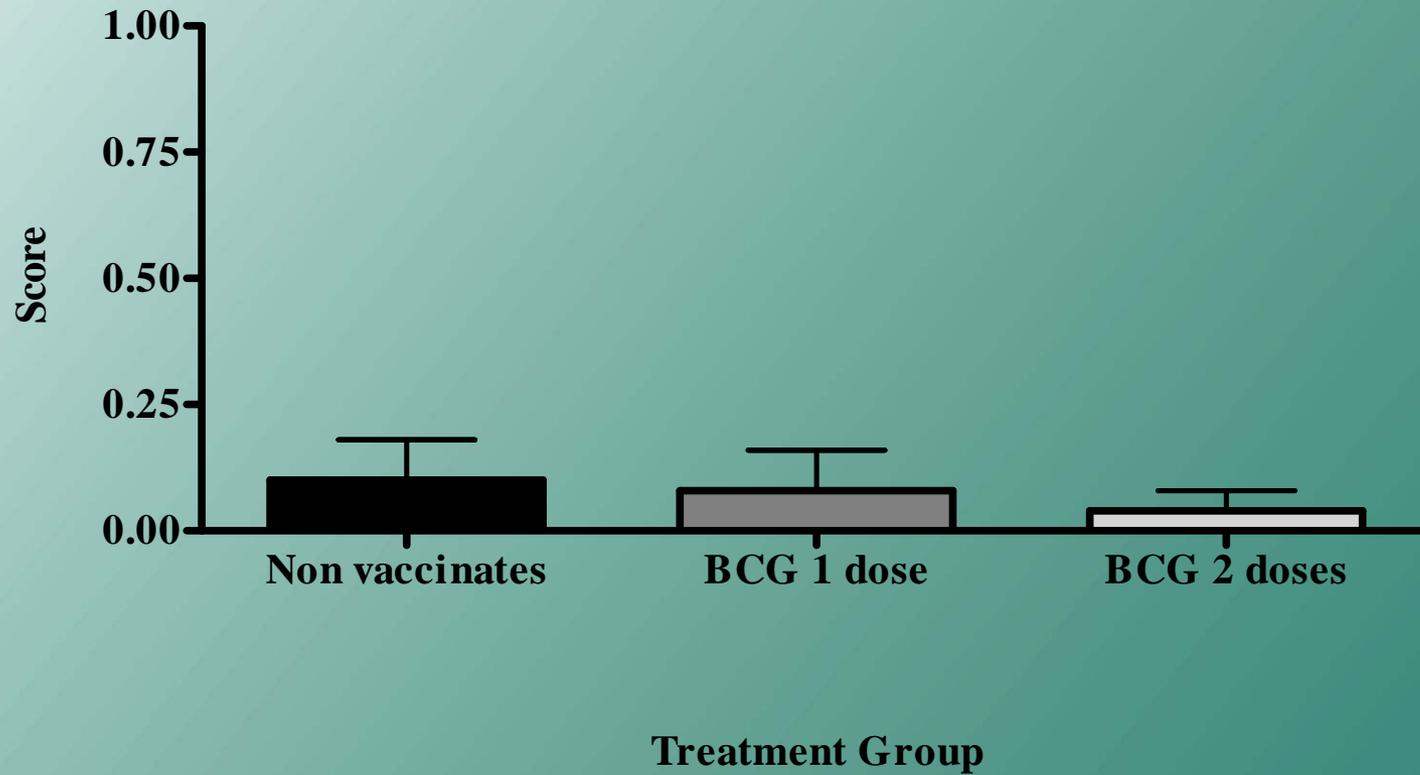
2 = several small foci ≥ 5 mm

3 = extensive necrosis

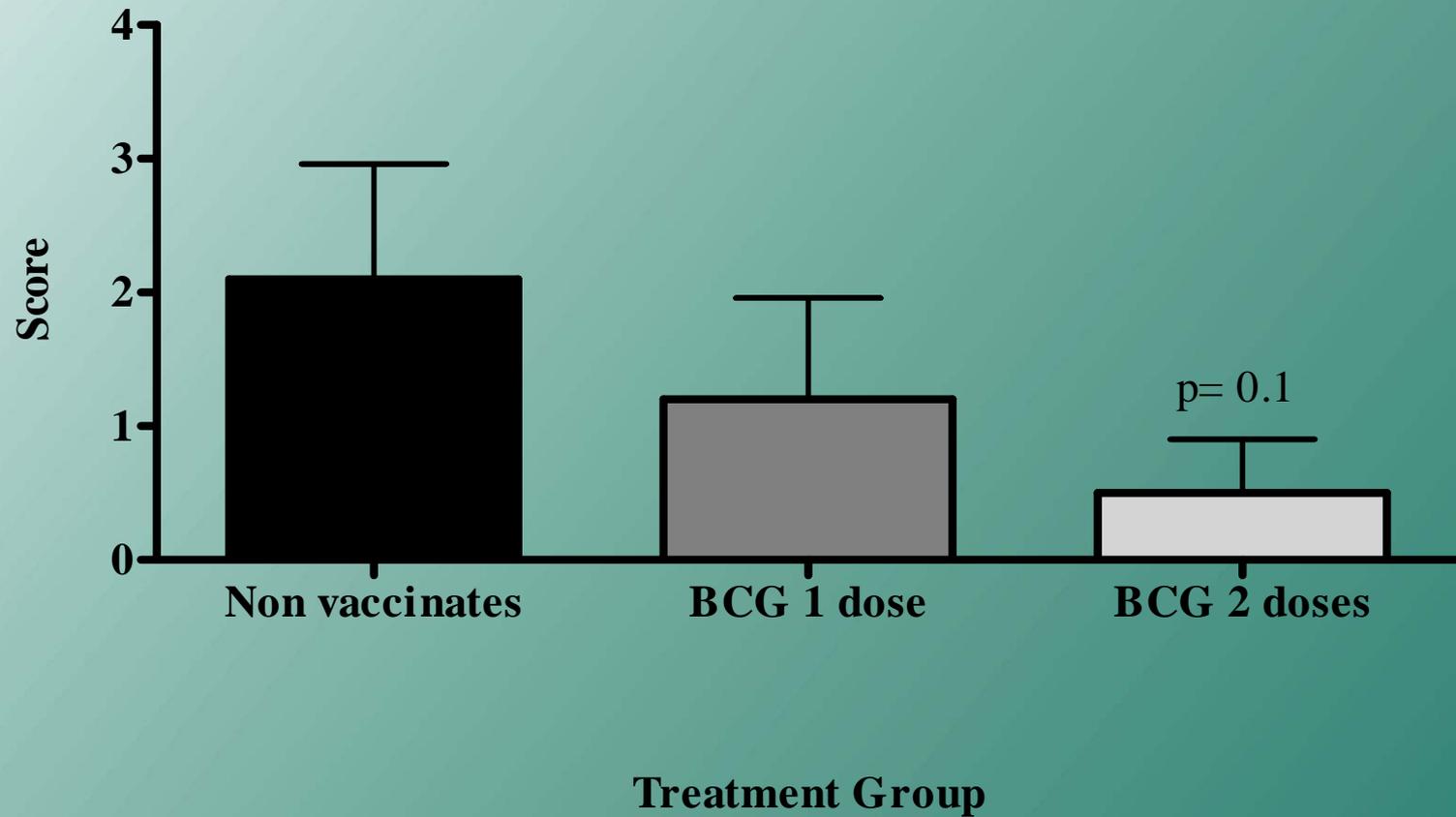
Medial retropharyngeal lymph node

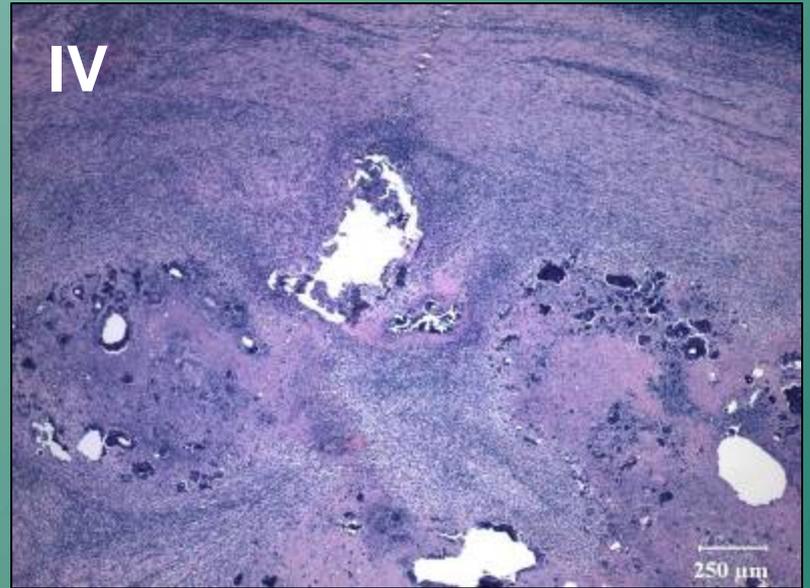
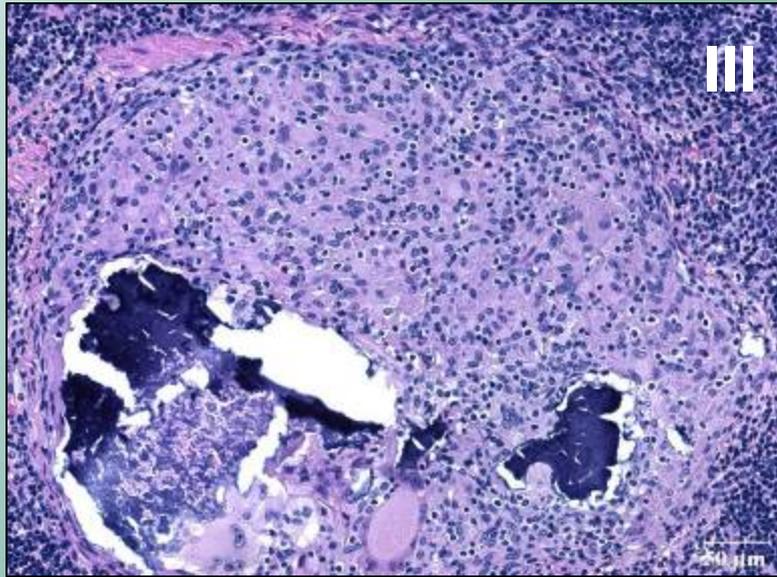
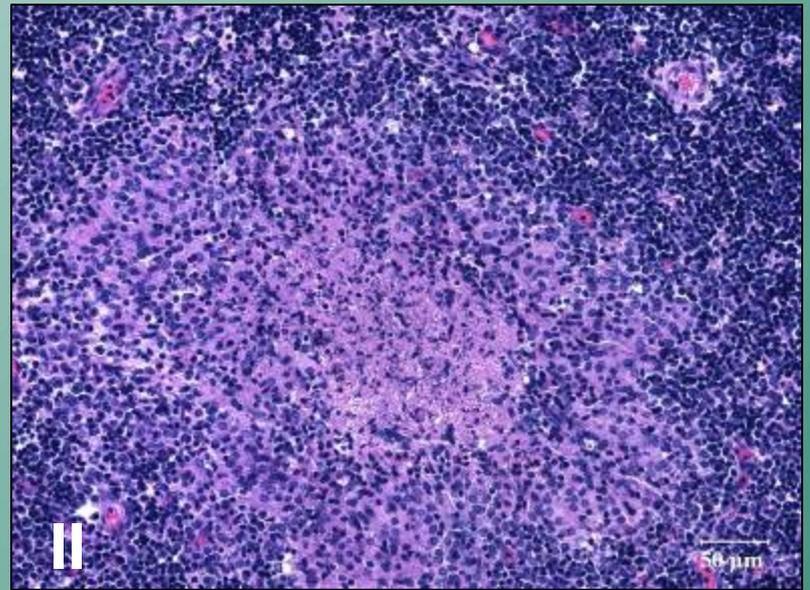
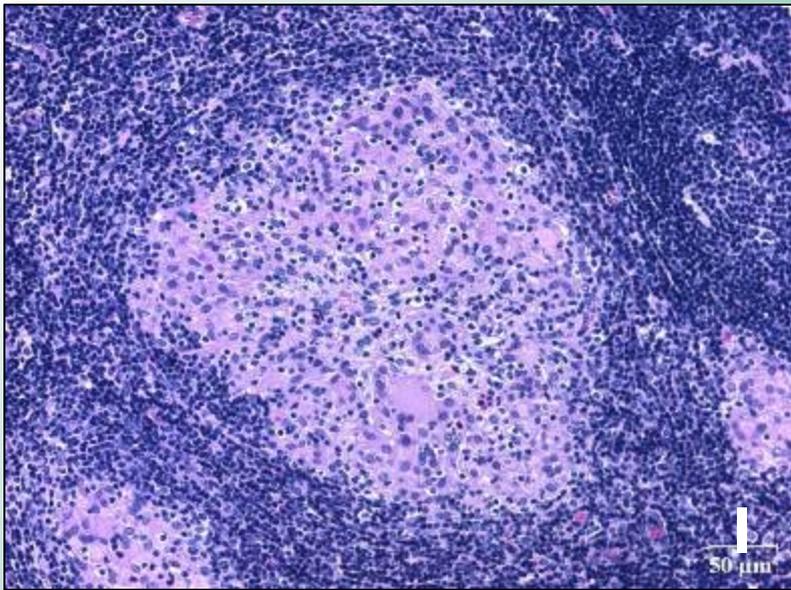


Lung



Total Pathology





Histopathological Staging

Medial retropharyngeal lymph node

	Stage I	Stage II	Stage III	Stage IV	Total
BCG 2 doses	2	3	5	1	11
BCG 1 dose	1	4	3	2	10
Unvaccinated	18	18	11	7	54

Acid fast bacilli

	Stage I	Stage II	Stage III	Stage IV
BCG 2 doses	1	0	5.4	>200 ^b
BCG 1 dose	0	0	11.3	>200
Unvaccinated	0	0.17	0.18	>200

Results - Summary

	Unvaccinated	BCG 1 dose	BCG 2 doses
Gross lesions	5/10	2/10	1/11
Microscopic lesions	6/10	4/10	3/11
Isolation of virulent <i>M. bovis</i>	5/10	3/10	6/11
Isolation of <i>M. bovis</i> BCG	1/10	4/10	3/11
Isolation of <i>M. bovis</i> BCG from tissues with lesions	0/6	0/4	2/3
Isolation of non- tuberculous mycobacteria	1/10	2/10	1/11
Deer from which <i>M.</i> <i>bovis</i> was not isolated and lesions were not seen	2/10	2/10	4/11

Conclusions

- 2 doses of BCG decreased lesion severity at both the gross and microscopic level.
- A single dose of BCG decreased the number and severity of microscopic lesions in the medial retropharyngeal lymph node.

Oral BCG Vaccination in White-tailed Deer

- **Experimental Groups**
- (30 yearling WTD does)
- 1 dose of 10^9 cfu BCG (Danish 1331) orally via lipid-formulated bait (n=8)



NADC caretaker feeding deer lipid-formulated baits

Photo by James Fosse

Oral BCG Vaccination in White-tailed Deer

- **Experimental Groups (cont'd)**
 - 1 dose of 10^9 cfu BCG orally (n=8)
 - 1 dose of 10^6 cfu BCG (SQ) (n=7)
 - 1 dose of placebo orally (n=7)



Pharyngeal administration
of BCG vaccine

Photo by James Fosse

Results

Tissue		Oral liquid	Oral bait	SC	Control
Mediastinal LN	Gross	0/5	0/5	1/6	6/6
	Microscopic	0/5	0/5	3/5	6/6
	Culture	0/5	0/5	0/6	4/6
R Cranial Lung	Gross	1/5	0/5	1/6	6/6
	Microscopic	0/5	0/5	1/6	6/6
	Culture	0/5	0/5	0/6	3/6

Gross Lesions



Group	Right cranial lung			Mediastinal LN		
	None	Mild	Severe	None	Mild	Severe
Oral liquid	5	0	0	5	0	0
Oral bait	5	0	0	5	0	0
SC	5	0	1	2	1	2
Control	0	0	6	0	0	6

Microscopic Lesions



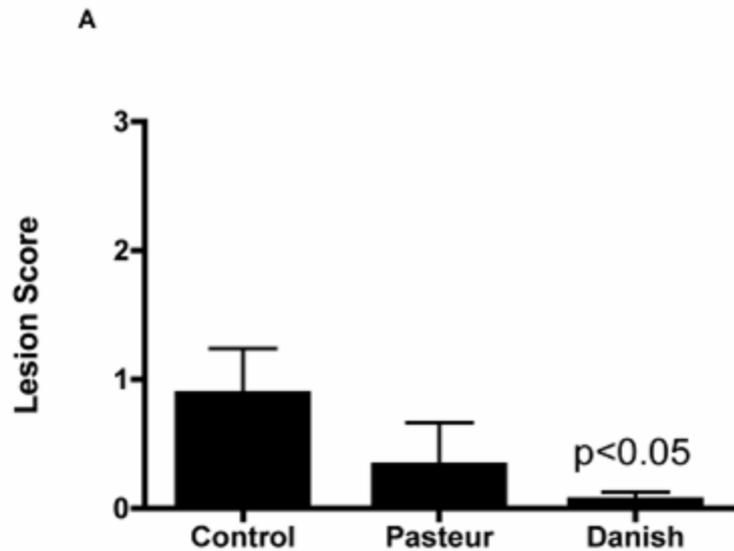
Group	Right cranial lung			Mediastinal LN		
	None	Mild	Severe	None	Mild	Severe
Oral liquid	5	0	0	5	0	0
Oral bait	4	1	0	5	0	0
SC	5	0	1	5	1	0
Control	0	0	6	0	0	6

Conclusions

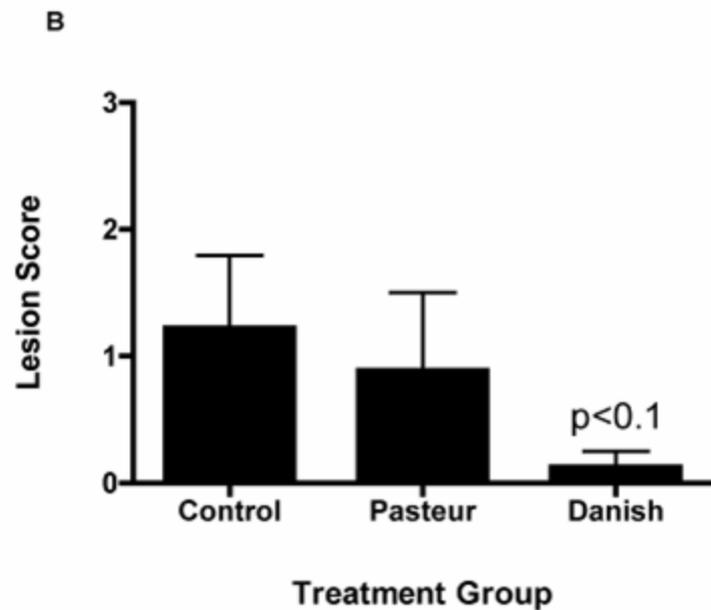
- Vaccination with BCG Danish results in decreased lesion severity
- Oral vaccination may be feasible in white-tailed deer

Vaccination of white-tailed deer with *M. bovis* BCG Pasteur or BCG Danish





Medial retropharyngeal LN



All lymph nodes combined

Medial retropharyngeal LN

	Stage I	Stage II	Stage III	Stage IV	Total
BCG Danish	0	0	0	0	0
BCG Pasteur	2	10	3	8	23
Non-vaccinated	10	5	5	8	28

Lung

	Stage I	Stage II	Stage III	Stage IV	Total
BCG Danish	0	1	2	0	3
BCG Pasteur	0	0	0	0	0
Non-vaccinated	6	9	8	6	29

	Unvaccinated	BCG Danish	BCG Pasteur
Gross lesions	4/9	1/8	2/9
Microscopic lesions	4/9	5/8	3/9
Isolation of virulent <i>M. bovis</i>	6/9	3/8	2/9
Isolation of <i>M. bovis</i> BCG Danish	0/9	4/8	0/9
Isolation of <i>M. bovis</i> BCG Pasteur	0/9	3/8	4/9
Isolation of both BCG Danish and Pasteur from same animal	0/9	1/8	0/9
Isolation of BCG from tissues with lesions	0/9	3/8	1/8
Isolation of non-tuberculous mycobacteria	2/9	1/8	2/9
Deer from which <i>M. bovis</i> was not isolated and lesions were not seen	3/9	3/8	5/9

Vaccine persistence – 250 days

	Unvaccinated	BCG Danish	BCG Pasteur
Gross lesions	0/3	0/3	0/3
Microscopic lesions	0/3	2/3 ^a	1/3 ^b
Isolation of <i>M. bovis</i> BCG Danish	0/3	3/3 ^c	0/3
Isolation of <i>M. bovis</i> BCG Pasteur	0/3	0/3	2/3 ^d
Isolation of BCG from tissues with lesions	0/3	1/3	0/3

^a superficial cervical LN from 1 deer; tracheobronchial, hepatic and prescapular LN from a second deer.

^b hepatic LN from 1 deer.

^c tracheobronchial and mediastinal LN from 1 deer; hepatic, superficial cervical and mediastinal LN from a second deer; superficial cervical LN from a third deer.

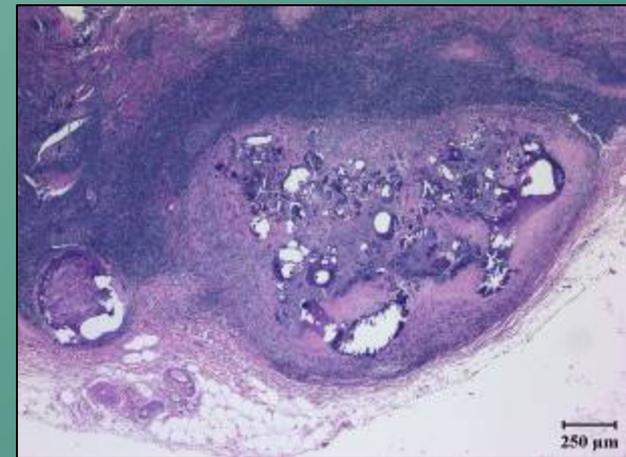
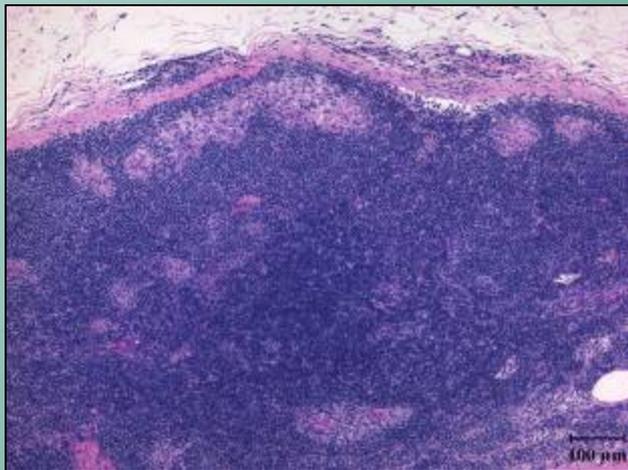
^d prescapular LN from 1 deer; lung from a second deer.



Histopathologic staging of granulomas

Superficial cervical LN (non-challenged)

	Stage I	Stage II	Stage III	Stage IV	Total
BCG Danish	0	3	16	0	19
BCG Pasteur	0	0	0	0	0
Non-vaccinated	0	0	0	0	0

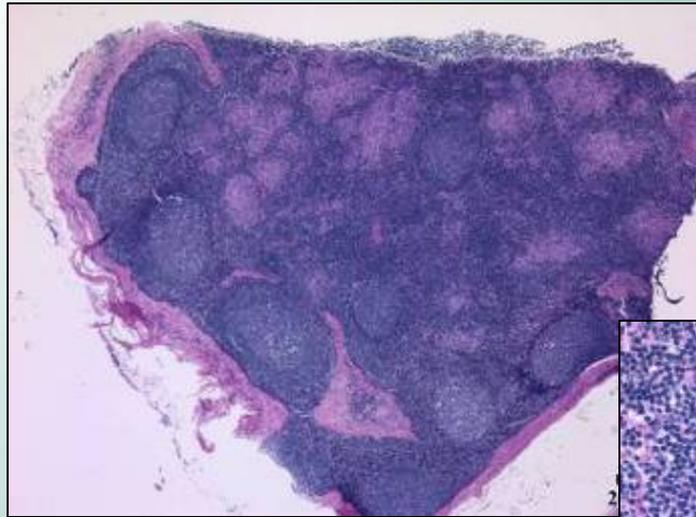


Conclusions

- BCG Danish and Pasteur provide some protection.
- BCG Danish induces microscopic lesion formation in lymph nodes draining the site of vaccination.
- BCG Danish and Pasteur persist for up to 250 days in host tissues.
- BCG Pasteur can be shed and transmitted to other animals.

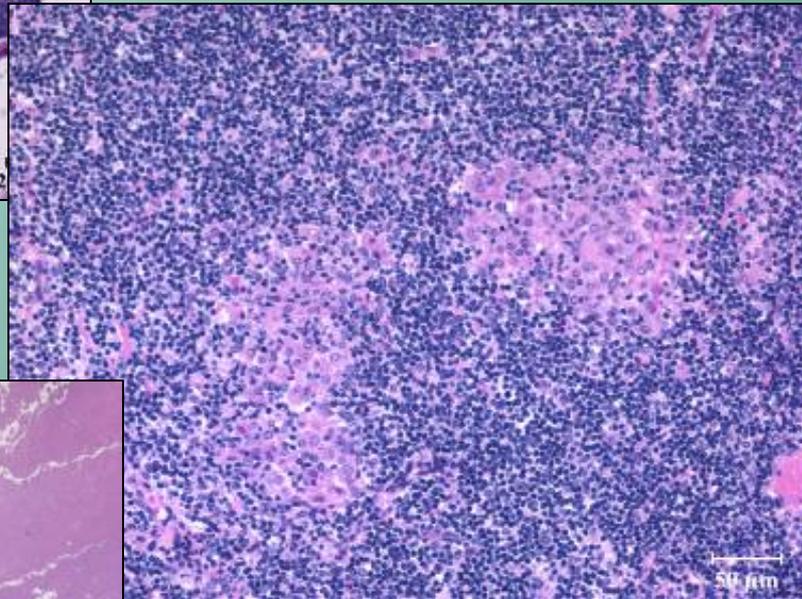
Persistence of BCG Danish

- *Vaccination:*
 - single SC BCG Danish vaccination (n= 21),
 - single Oral BCG Danish vaccination (n=21),
- *Dosages:*
 - SC vaccination: 10^6 CFU of *M. bovis* BCG Danish.
 - Oral: 10^9 CFU of BCG Danish in liquid suspension
 - (deposited in posterior oral cavity).
- *Necropsy:*
 - 14 days, 1, 3, 5, 7, 9, 11 months post-vaccination
- Collect a panel of tissues for culture and histopathology to document persistence



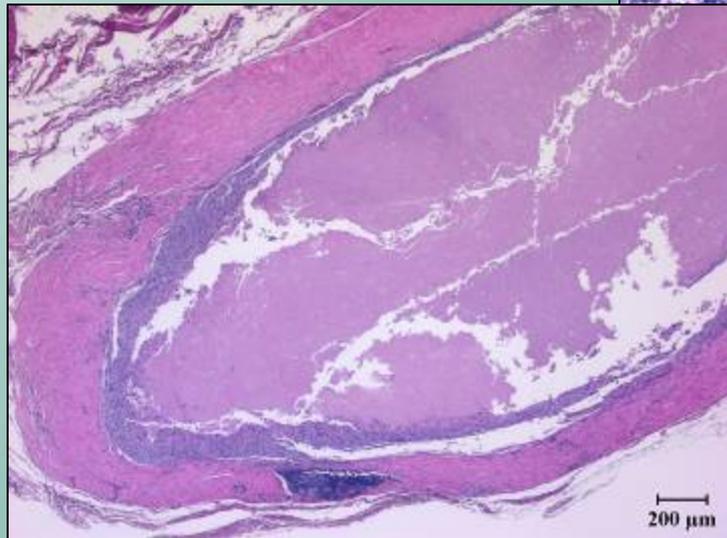
Oral - 1 month

Medial retropharyngeal LN



SC - 3 months

Mediastinal LN



SC - 1 month

Skin injection site

Persistence in Tissue

3 Months after Vaccination

- Oral vaccination
 - Tonsil (1/3)
 - Med retro LN (2/3)
 - Duodenum (1/3)
 - Distal jejunal LN (1/3)
 - Ileocecal LN (1/3)
 - Cecum (1/3)
- SC vaccination
 - Hepatic LN (1/3)
 - Mesenteric LN (1/3)
 - R Prescapular LN (2/3)

Persistence in Tissue

5 Months after Vaccination

- Oral vaccination
No culture positive tissues
- SC vaccination
Hepatic LN (1/3)

Persistence in Tissue

Culture of Muscle

- At no time was BCG isolated from muscle from any vaccinated deer.
 - Shoulder (supraspinatus, triceps muscles)
 - Rear quarter (semimembranosis, semitendinosis, biceps femoris muscles)
 - Tenderloin (sublumbar muscles)
 - Backstrap (epaxial muscles)



Scientists

Dr. Mitchell V. Palmer

Dr. W. Ray Waters

Dr. Tyler Thacker

Technical Support

Rachel Huegel

Pete Lasley

Bart Olthoff

Jessica Pollock

Shelly Zimmerman

Mike Howard

