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OF MICE AND ELK: PILOT STUDIES ON A KILLED, MUCOSALLY-DELIVERED, *BRUCELLA ABORTUS* VACCINE FOR ELK

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OCTOBER 22, 2018

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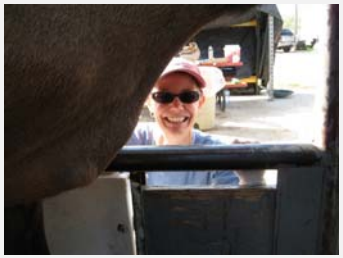

Pauline Nol

Karl Held

Matt McCollum

Morgan Wehtje

Jack Rhyan

"Developing science-based solutions to disease problems at the wildlife/domestic animal interface"

Samantha Bruce

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Dick Bowen's Crew at CSU

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Our theory: multiple mucosal exposures to killed *Brucella abortus* would generate mucosal and systemic protection

Wild boar TB work using oral killed *Mycobacterium bovis* in Spain (Gortazer et al., 2014)

Hank Edwards found it took multiple exposures of *Yersinia* to produce infection

Montmorillonite – carrying agent (Nichols work with CWD; Nichols et al., 2013)

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Purpose: Develop effective, remotely deliverable, inexpensive, safe, brucellosis vaccine for free-ranging elk

4 small projects

- Mouse mucosal vaccination (proof-of-concept)
- Nasal aerosol – elk with montmorillonite/ink (surrogate vaccine)
- Mucosal vaccination – challenge study in elk
- Mucosal delivery trial with montmorillonite/ink (surrogate vaccine)

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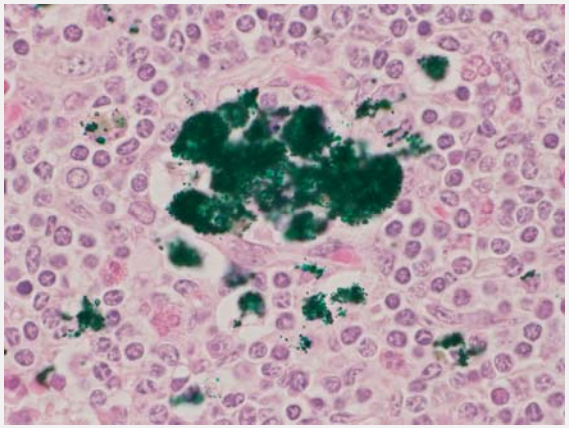
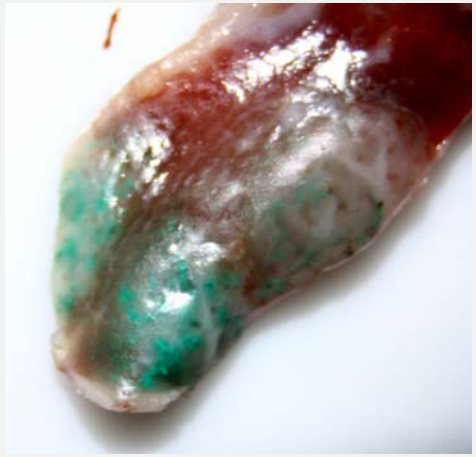
1. Aerosolized left nostril with montmorillonite complexed with tattoo ink

Right parotid lymph node 4 hours post inoculation





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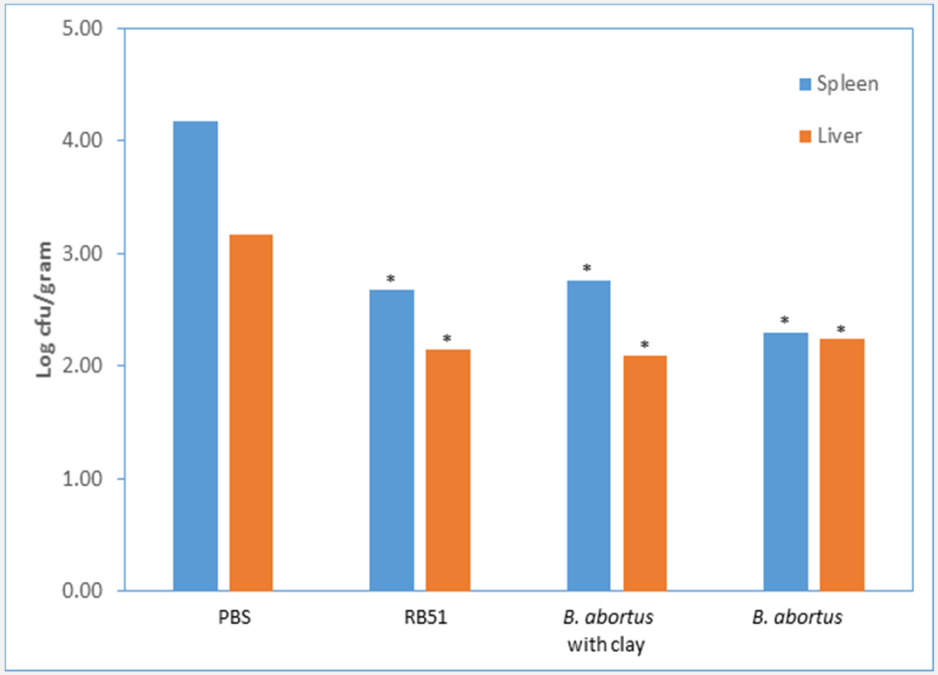
2. Mouse mucosal vaccination with powdered, killed vaccine

Initial studies in mice: Powdered, killed, elk strain *B. abortus* complexed with montmorillonite clay

- Group 1: PBS vaccinated controls (n=15)
- Group 2: RB51 5X10^8 cfu IP (n=15)
- Group 3: Killed *B. abortus* 10^{11} cfu with clay (n=14)
- Group 4: Killed *B. abortus* 10^{11} cfu (n=15)
- Challenge elk strain 10^5 cfu IP

Logrithmic bar graph



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3. Mucosal vaccination pilot study in elk with challenge - Design

11 elk (4 controls, 5 elk vaccinated 4 times, 2 elk vaccinated twice)

Vaccinated by delivering aerosol of powdered vaccine (killed *B. abortus* and montmorillonite) into each nostril and oral pharynx with using 30 ml pipette and rubber bulb (10^{12} cfus/elk)

88% of vaccine mass was montmorillonite

Controls received montmorillonite alone

Controls and vaccinates housed in paddock together

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Mucosal vaccination pilot study – Design (continued)

Elk were moved to BSL3 containment at CSU and challenged 15 weeks later with 10^7 live elk strain *B. abortus* by intraconjunctival inoculation

3 weeks after challenge elk were necropsied and tissues collected and cultured

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Serology Results - Elk Vaccine Pilot

(Buffered Acidified Plate Antigen, Fluorescence Polarization Assay, Complement Fixation)

	1st Vacc (11/29)	4th Vacc (12/15)	Challenge (3/14)	Necropsy (4/6)
Neg Controls n=4	0 positive	1 positive	0 positive	4 positive
2X Vaccinates n=2	0 positive	2 positive	0 positive	2 positive
4X Vaccinates n=5	0 positive	5 positive	1 positive	4 positive

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Pilot elk vaccine trial - Results

Table 1. Colony counts (cfu/~10 mg tissue) from tissues collected from elk

* = pregnant

	Controls				2XVacc		4XVacc				
					Animal ID						
Tissue	B	C	E	K	D*	J	A	F	G*	I	L
Parotid LN	0	0	6	488	14	0	0	0	0	1	0
Mandibular LN	500	13	448		1	222	0	0	152	2	1
Superficial cervical LN	98	16	71	18	8	0	0	0	61	2	18
Mediastinal LN	0	0	6	0	47	0	0	0	6	0	0
Mesenteric LN	3	9	6	3	13	0	0	0	4	1	0
Iliac LN	0	11	89	0	0	2	0	0	0	0	0
Spleen	30	1	264	19	13	0	0	0	4	1	2

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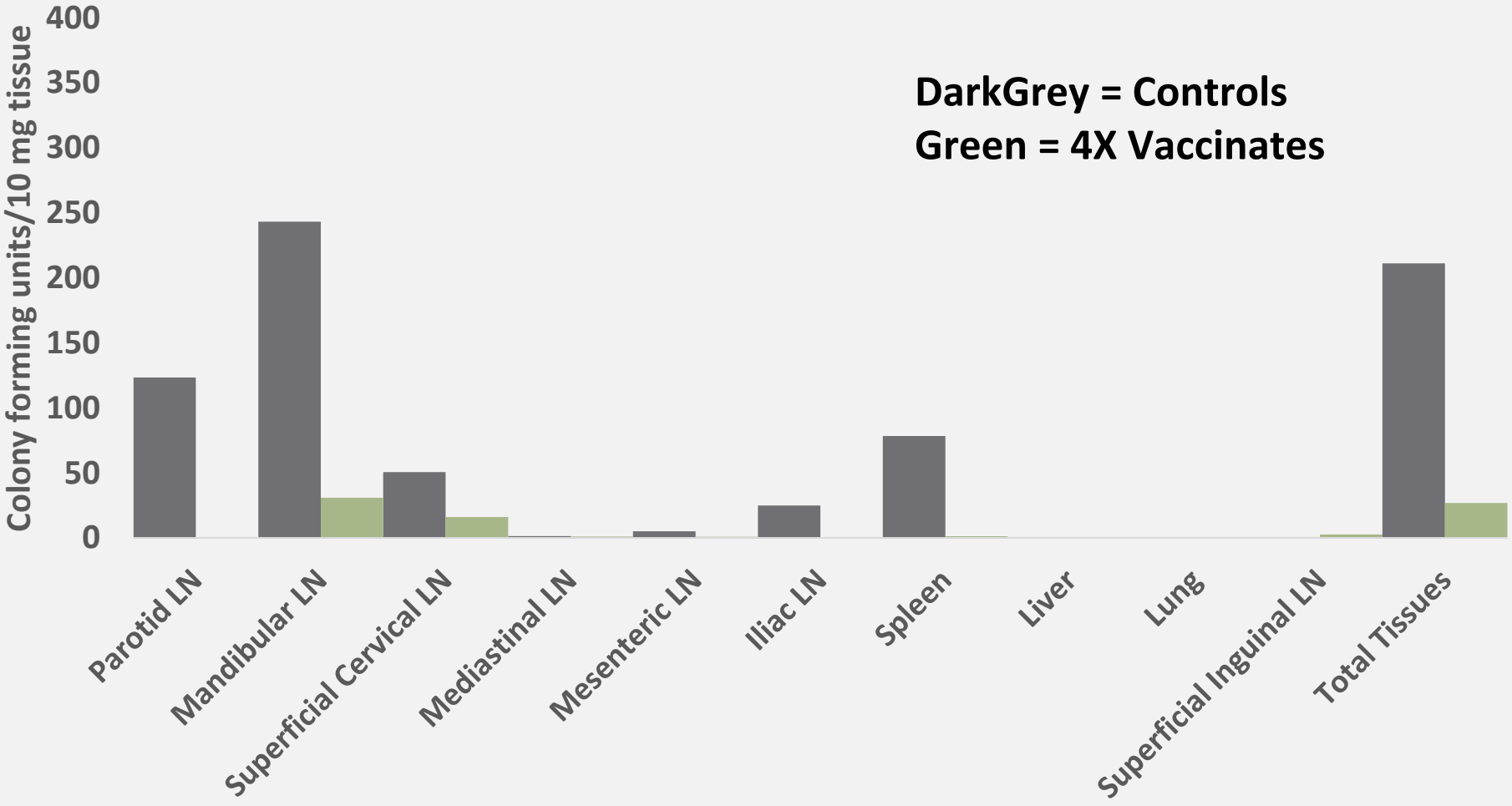
Pilot elk vaccine trial – Results totals

Table 2. Total Colony counts (cfu/~10 mg tissue) from tissues collected from elk

	Controls				2XVacc		4XVacc				
					Animal ID						
	B	C	E	K	D*	J	A	F	G*	I	L
Totals	632	50	890	540	97	224	0	0	227	7	35
Means		528			161				54		

* =pregnant

Culture Results - Controls and 4X Vaccinates



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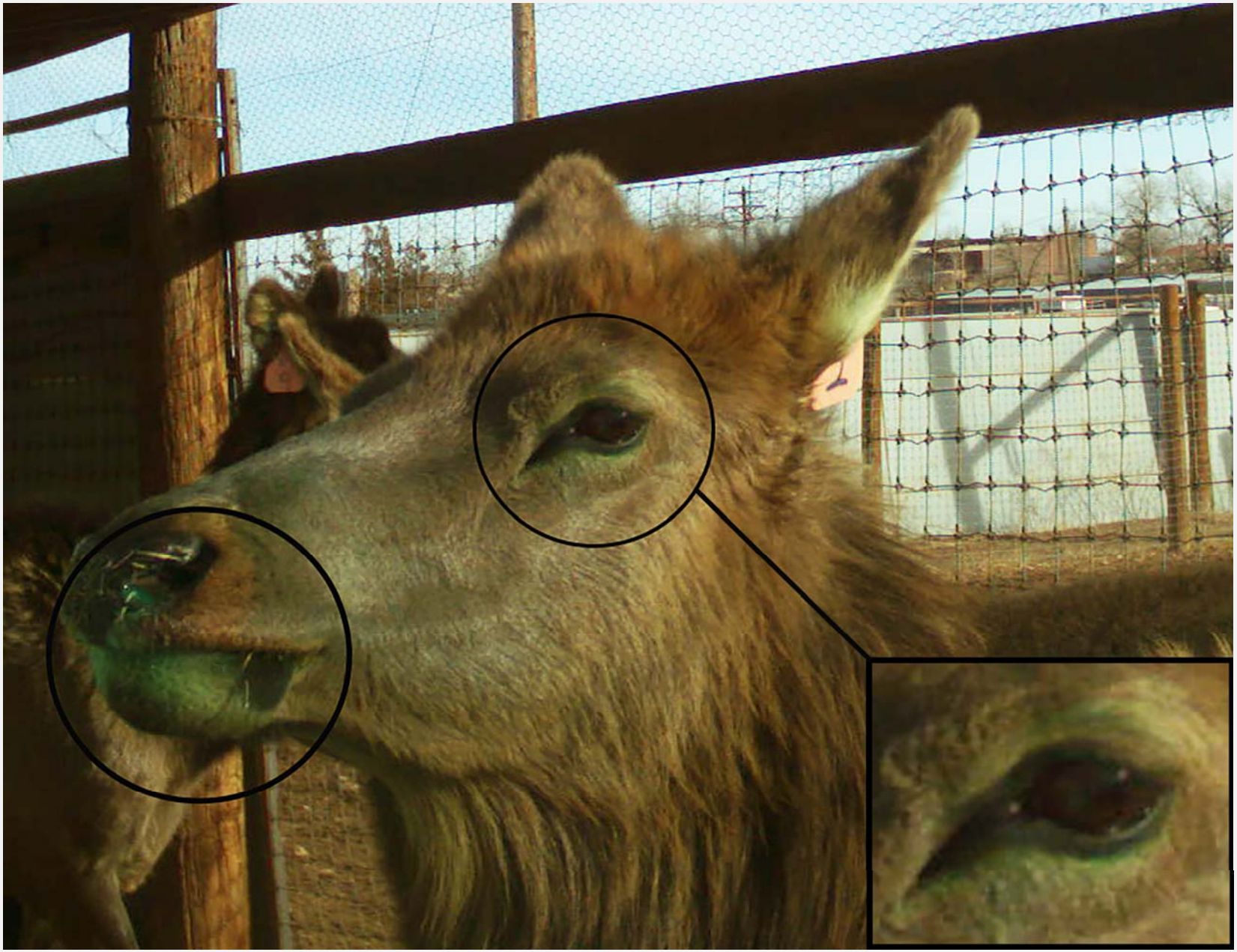
4. Mucosal delivery trial with montmorillonite/ink (surrogate vaccine)

1½ lbs surrogate vaccine/bale

Over 80% of powder was consumed



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Spit collecting – Oral fluids to detect disease

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To the Future!

Colorado State University and USDA/ ARS will continue vaccine work

Questions?

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