

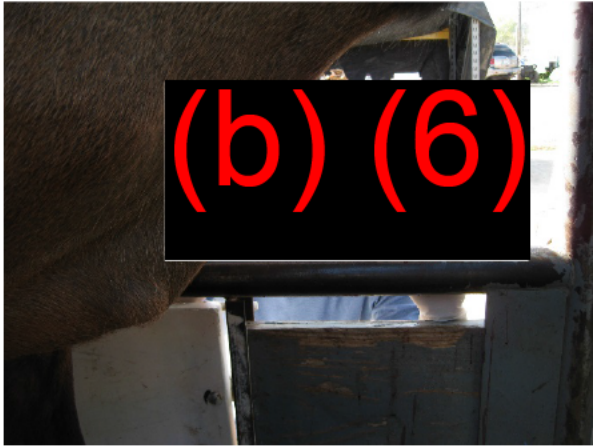
# Wildlife Livestock Disease Investigations Team

## NVSL Review

17 August 2016



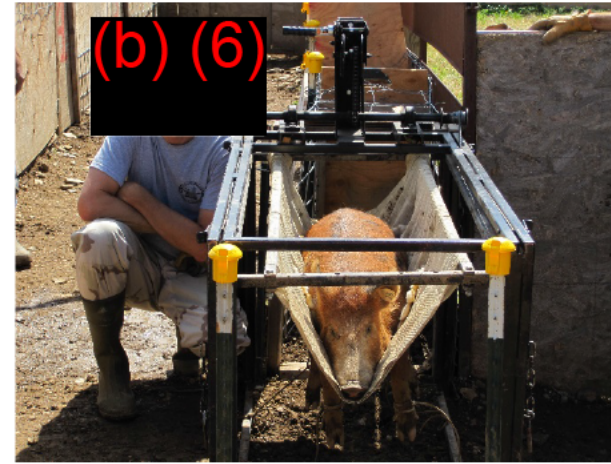
# Wildlife/livestock Disease Investigations Team



Pauline Nol



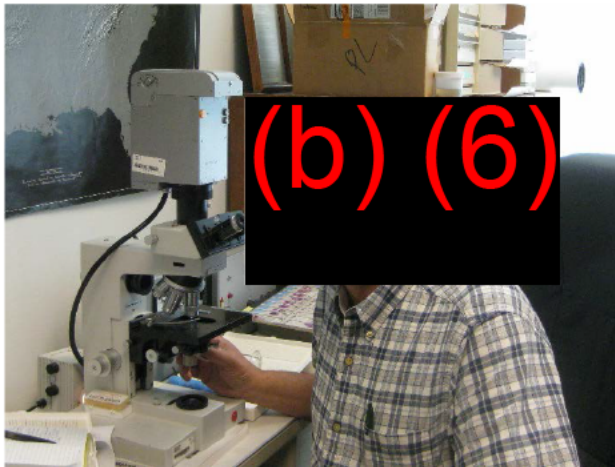
Karl Held



Matt McCollum



Morgan Wehtje



Jack Rhyan



Samantha Bruce

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

# Tools

- Disease detection: Volatile Organic Compound (VOC) analysis
- Disease elimination:
  - ❖ Immunocontraceptive vaccine (GonaCon™)
  - ❖ Brucella and TB vaccines
    - DryDart™ for delivery of parenteral vaccines
    - Spray-dried and other vaccines for mucosal delivery
      - Mouse (Brucella)
      - Feral swine (TB)
    - Using natural exposure as a challenge for *Brucella* vaccine studies
- Establishing conservation herds of brucellosis-negative Yellowstone bison
- A theoretical disease eradication plan



# A Potential New Tool for Remote Disease Detection: Volatile Organic Compounds (VOCs)

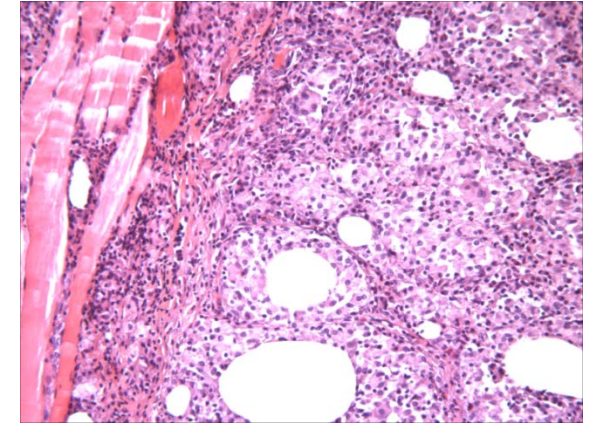
- Detection of unique VOCs or a unique pattern of VOCs from breath or feces from infected animals





# Immunocontraception

- GnRH (GonaCon™)
- Currently 2 ongoing studies in bison
- Preliminary results – efficacious in producing infertility; effective in decreasing transmission of brucellosis



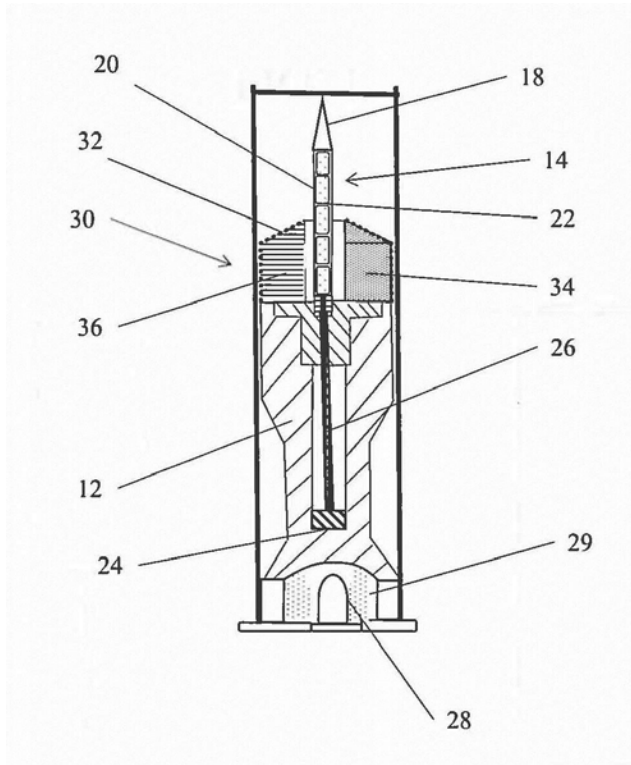
000466





# Darting: DryDart™ Development

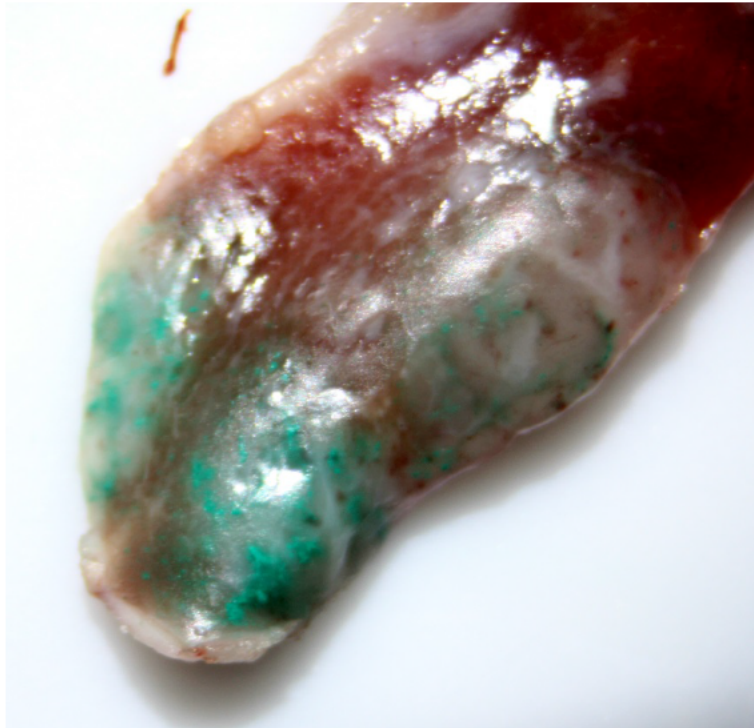
- Developing dart system to deliver lyophilized, powdered, pelleted, or encapsulated vaccines, at range, with accuracy.
- 2X the payload of biobullets; mark injection site.
- Fired from dart gun or shotgun; biodegradable.



000467

# Vaccines

- Develop spray-dried, killed, *B. abortus* vaccine for use on elk feedlines
- Test and implement oral TB vaccine for feral swine.



(b) (6)

000468



# Challenging

- Using natural exposure as a challenge, a potential model for vaccine studies



# Establishing Conservation Herds



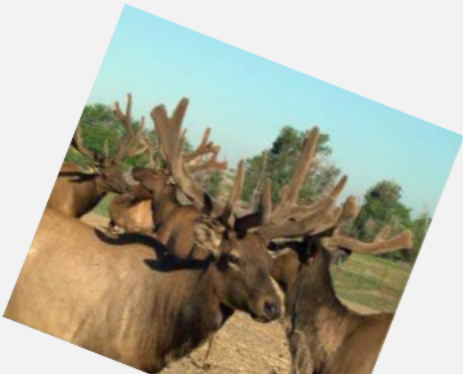
# Wildlife/Livestock Disease Investigations Team (WiLDIT) Brucellosis Research Update

Pauline Nol

U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
Veterinary Services  
Science, Technology, and Analysis Services

September 27, 2016

000471



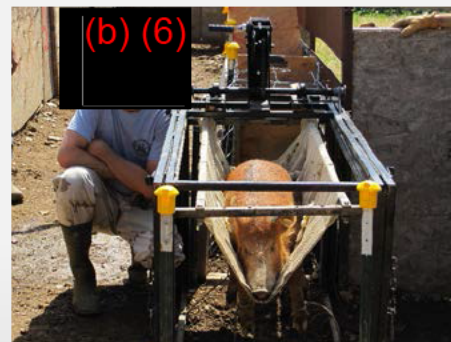
# WILDIT



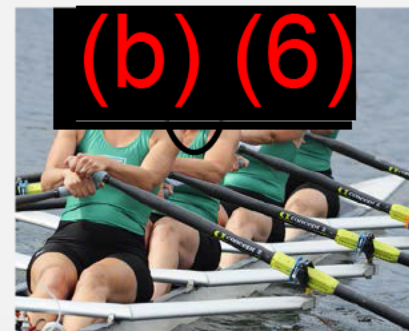
Pauline Nol



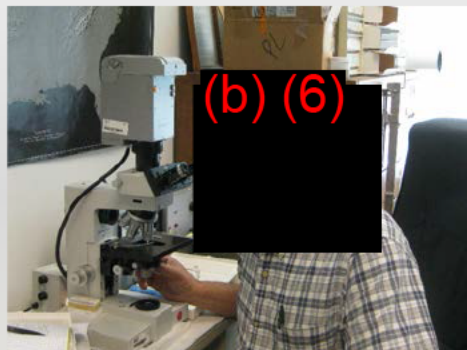
Karl Held



Matt McCollum



Morgan Wehtje



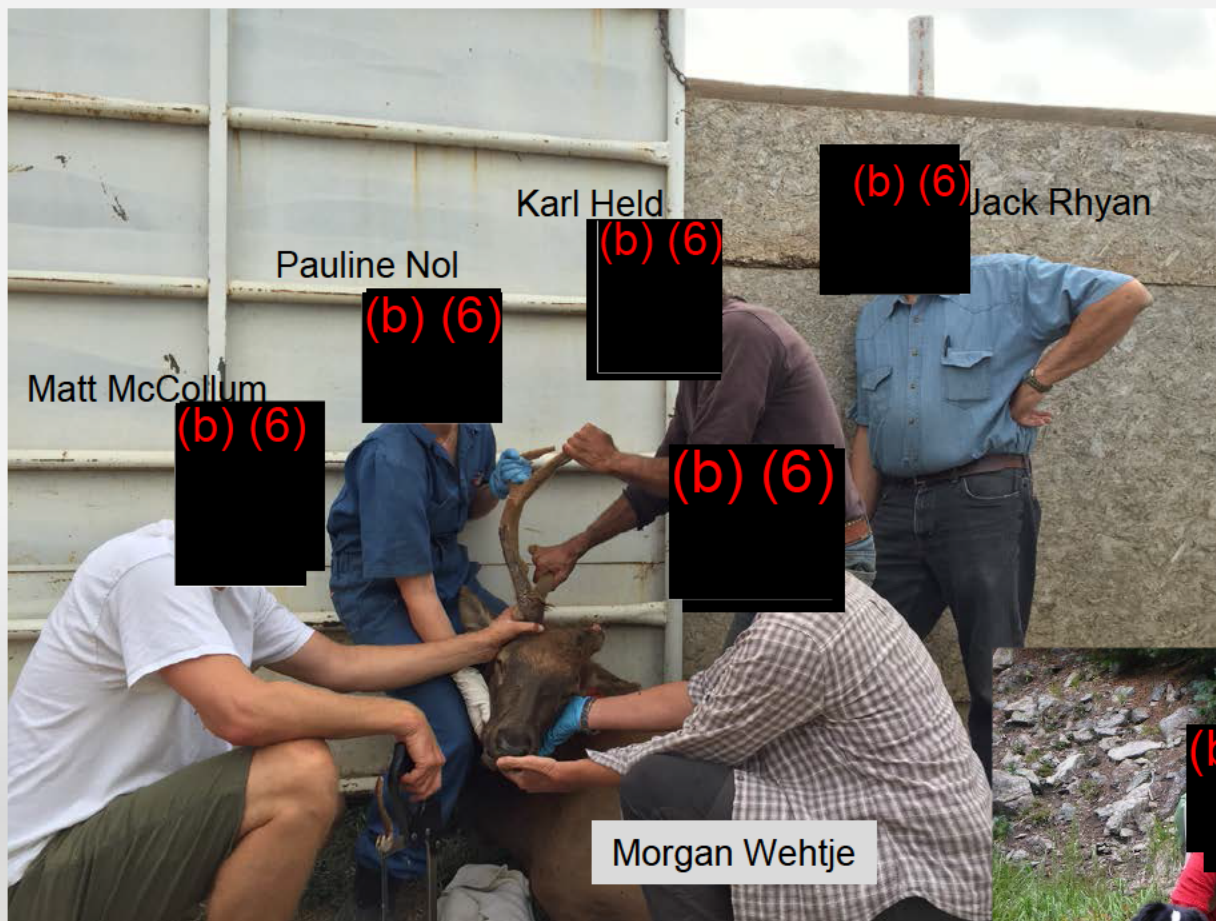
Jack Rhyan

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



Samantha Bruce





# WiLDIT



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

000473

# Management tools for Brucellosis

- Immunocontraception
  - GonaCon<sup>TM</sup>
- Vaccination
  - Dry Dart
  - Mucosal vaccination of killed, powdered vaccine
  - Natural transmission model in elk
- Detection
  - Volatile Organic Compounds

# Immunocontraception

## Background

- In female bison, brucellosis is transmitted if pregnancy occurs
  - In over 300 captures, *B. abortus* was isolated from vagina, milk, blood, feces, & products of parturition
- GonaCon™ (immunocontraceptive vaccine)
  - GnRH linked to sea mollusk protein and therefore looks large and foreign (not recognized as “self”)
  - Combined with adjuvant containing *Mycobacterium avium*



# Immunocontraception

## Current studies

- Study 1: Duration of infertility study in southern Colorado
- Study 2: Management of *B. abortus* in bison through immunocontraception (Corwins Springs, MT)



000476





# Study 1: Duration of infertility in southern Colorado

- Initiated Nov 2011
- Gonacon<sup>TM</sup> treatment group (N=10)
- Non-treatment controls (n=10)

## Results

Number pregnant/number in group

|           | Nov 2011 | Nov 2012 | Nov 2013 | Nov 2014 | Nov 2015 |
|-----------|----------|----------|----------|----------|----------|
| Treatment | 4/10     | 3/9      | 1/10     | 3/9      | 3/10     |
| Control   | 4/10     | 9/9      | 6/9      | 9/9      | 6/9      |

# Study 2: Management of *B. abortus* in bison through immunocontraception

- First cohort (2011)
  - Treatment group (n=15; *B. abortus* +)
    - Sentinels (n=5; *B. abortus* -)
  - Control group (n=14; *B. abortus* +)
    - Sentinels (n=5; *B. abortus* -)
- Second Cohort (2013)
  - Treatment group (n=20; *B. abortus* +)
    - Sentinels (n=6; *B. abortus* -)
  - Control group (n=12; *B. abortus* +)



## Study 2: Management of *B. abortus* in bison through immunocontraception

- First cohort: 15 Controls; 15 GonaCon Vaccs
  - 1<sup>st</sup> year: Controls-77% preg; vaccs- 20% preg
  - 2<sup>nd</sup> year: Controls-77% preg; vaccs- 13% preg
  - 3<sup>rd</sup> year: Controls-90% preg; vaccs- 36% preg
  - 4<sup>th</sup> year: Controls-93% preg; vaccs- 8% preg
- Second cohort: 20 Controls; 20 GonaCon Vaccs
  - 1<sup>st</sup> year: Controls- 90% preg; vaccs- 5% preg
  - 2<sup>nd</sup> year: Controls- 93% preg; vaccs- 26% preg

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results-efficacy of immunocontraception

Number pregnant/number in group

|            | 2013  | 2014  | 2015  | 2016 |
|------------|-------|-------|-------|------|
| Vaccinates | 3/15  | 2/15  | /14   |      |
| Controls   | 11/14 | 10/13 | 10/12 |      |

Number pregnant/number in group

|            | 2015 | 2016 | 2016 |
|------------|------|------|------|
| Vaccinates | 0/20 |      |      |
| Controls   |      |      |      |

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results on the *Brucella* Side

- Control pasture:
  - 14 *Brucella* abortions
  - 3 normal calves born with significant shedding,
  - 4/5 sentinels seroconverted and aborted once or twice;
  - 8 calves seroconverted at 1 year.
  - One control had 1 cult + calf, 2 cult – calves, then cult + abortion.
  - One sentinel had 1 negative calf, seroconverted, 3 culture positive abortions in a row.
  - One seroconversion to negative in control pasture

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results on the *Brucella* Side (cont'd)

- Treatment groups:
  - 1 *Brucella* abortion (2<sup>nd</sup> group); 0 seroconversions
  - 4 conversions to negative



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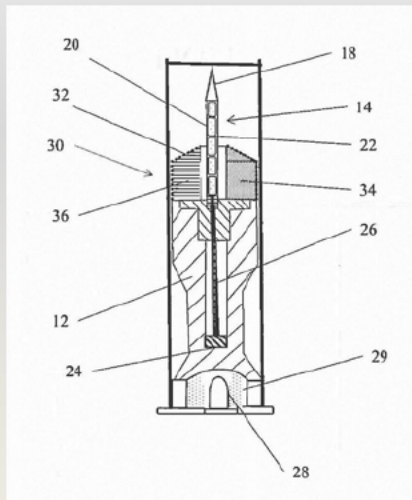
# Vaccination

## Background

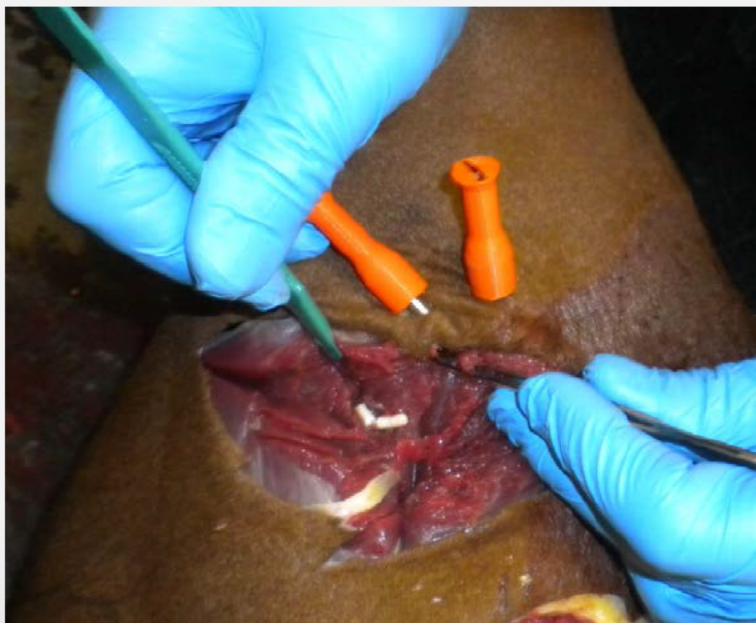
- Need effective/remote delivery of brucellosis vaccines in bison and elk
- Bison: RB51 given in two doses administered ~1 year apart induced increased protection against abortion vs. single dose  
(Olsen et al., 2015. Clinical Vaccine and Immunology 23)
- Elk: Continued research toward effective vaccine and challenge model

# DryDart™

- Dart system to deliver lyophilized, powdered, pelleted, or encapsulated vaccines
- 2X the payload of biobullets
- Marks injection site.
- Fired from dart gun or shotgun
- Biodegradable.







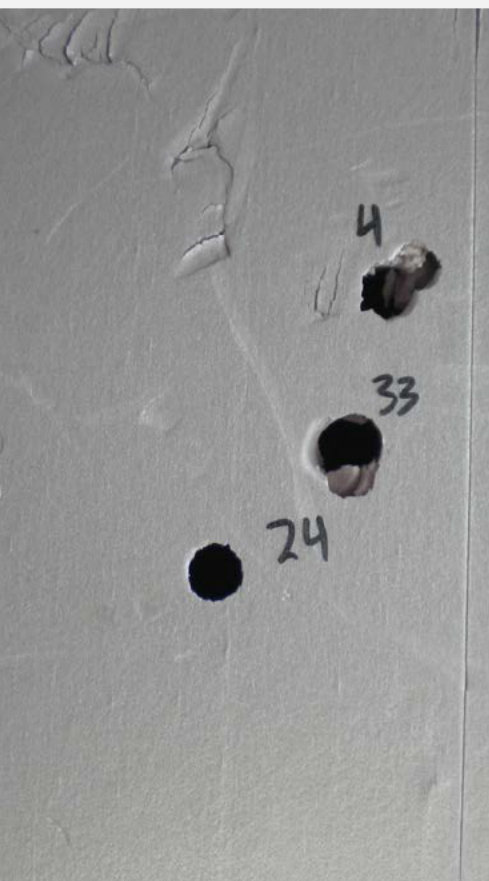
# DryDart™



Pellet delivered by DryDart compared to larger Biobullet placed at site.



000485

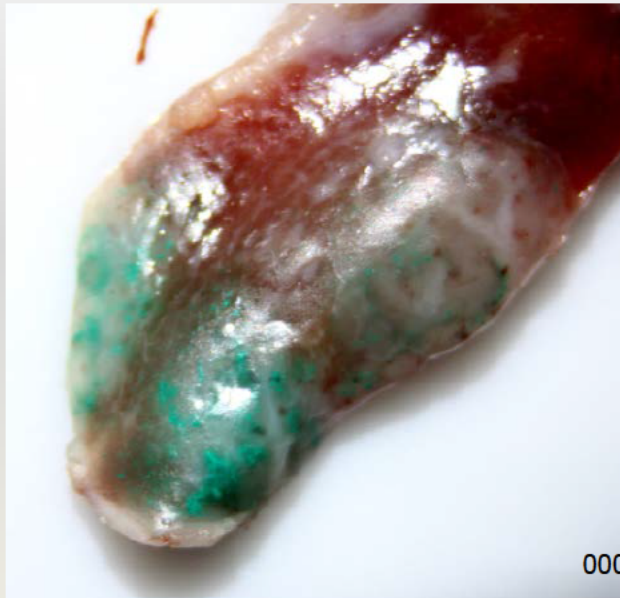


Dart marking injection site and bouncing out after depositing vaccine.



# Mucosal vaccination with powdered, killed vaccine

Goal: Develop killed, *B. abortus* vaccine for use on feedlines.



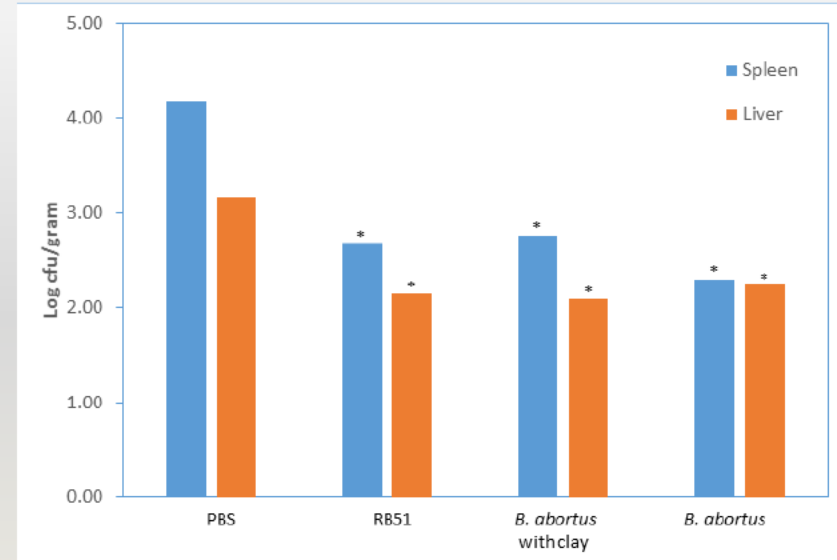
000487

Medial retropharyngeal lymph node with colored clay after intranasal delivery

# Mucosal vaccination with powdered, killed vaccine

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

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



# Developing a model for natural *B. abortus* infection in elk

- Natural exposure as challenge
- Potential model for vaccine studies



000489

# Developing a model for natural *B. abortus* infection in elk

- Study 1: 2014
  - 10 negative elk, 2 undiagnosed elk fetuses
  - In 24 hours, 227 contacts of elk with fetuses
- Study 2: 2016
  - 11 negative elk, 2 elk fetuses, 9 positive pregnant elk



000490



# Developing a model for natural *B. abortus* infection in elk

## Results (so far)

- Study 1
  - No seroconversions
- Study 2
  - No abortions in the 9 pregnant cows
  - Status of calves pending
  - No seroconversions in naïve animals after 90 days



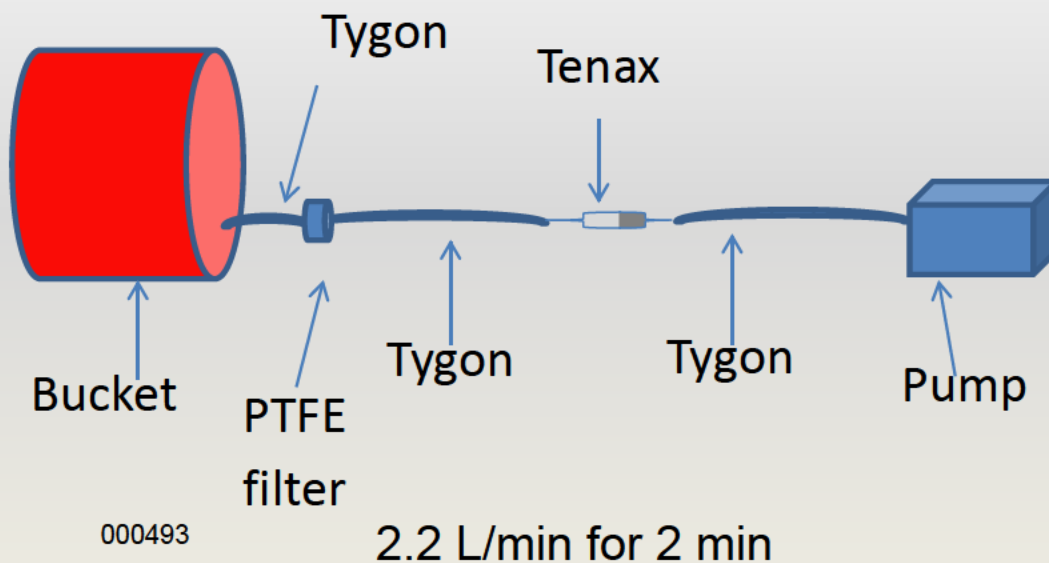
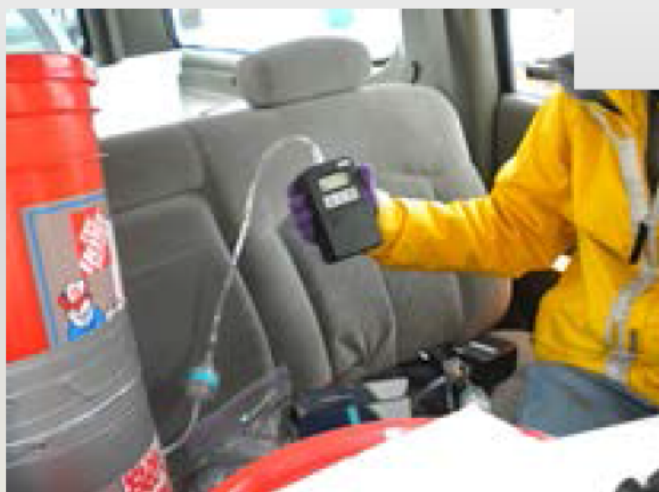


# Detection

Use of Volatile Organic Compounds (VOCs) in breath to distinguish brucellosis-positive animals from negative animals



# Methods: Breath Collection



# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

Bayn et al., 2013 *Analytical Chemistry*. 85 (22): 11146–11152

- 20 *B. abortus* seropositive\* bison
  - 9 housed at Colorado facility (WRF)
  - 11 housed at Montana facility (BQF)†
- 18 seronegative\* controls
  - 8 housed at WRF
  - 10 housed at BQF



\*Based on standard *Brucella* serological tests.

†Samples collected at various time points in different locations in MT.



## Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

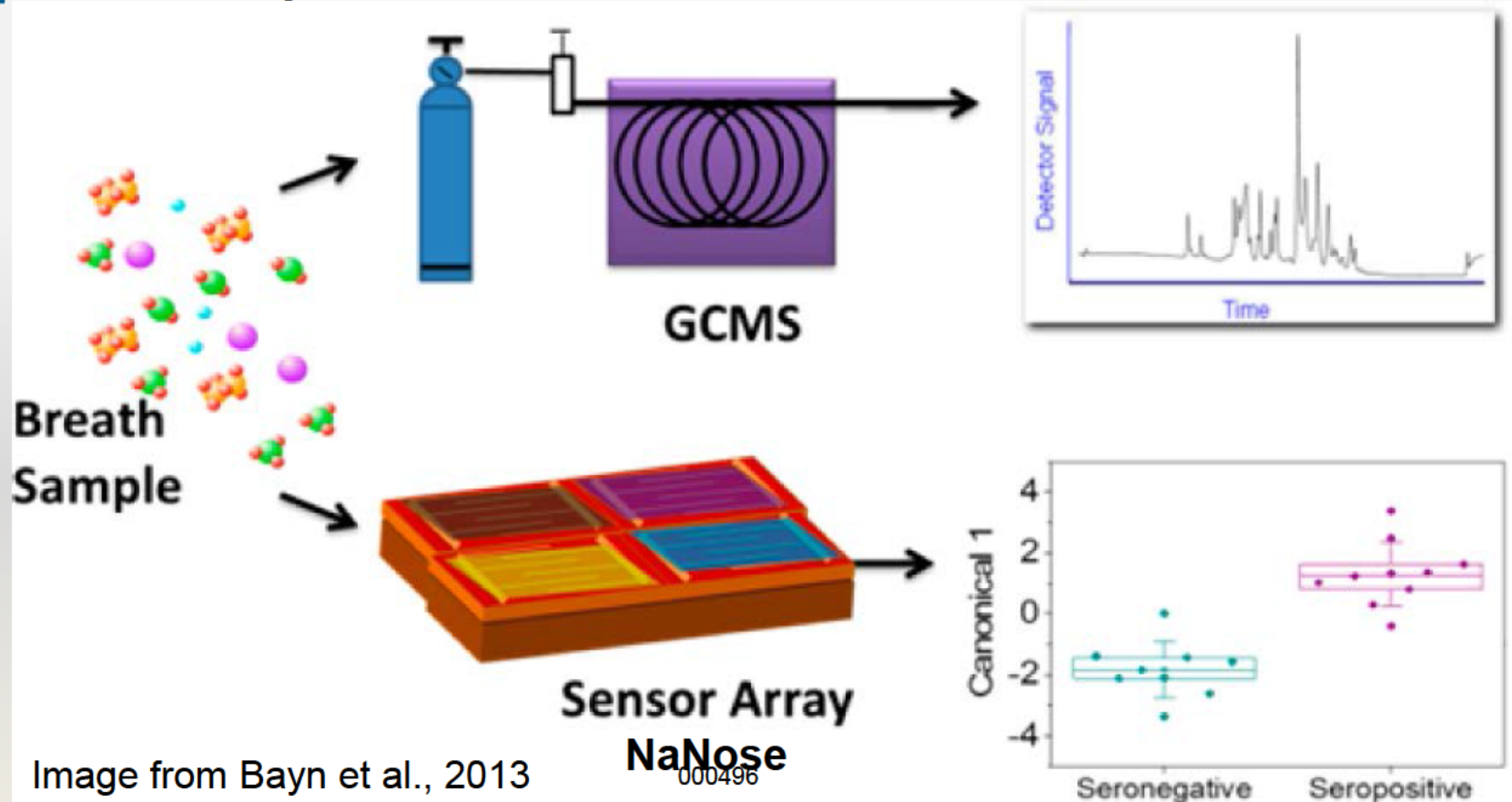
- Breath VOCs collected at Stephen's Creek (n=33)
- 25 seronegative
- 14 seropositive/culture positive
- 10 seropositive/culture negative





Technion-Israel Institute  
of Technology-Haick  
Laboratory

# Methods: Breath Analysis



# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

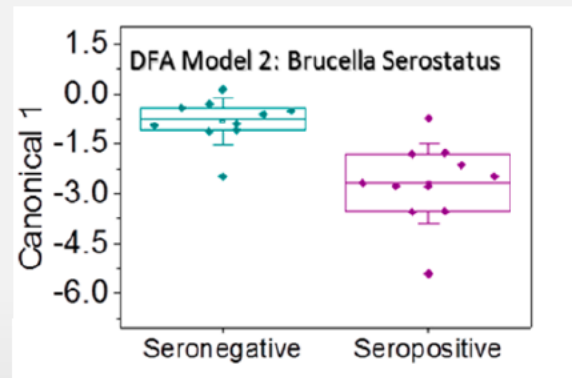
## Results-GC/MS

- 5 VOCs statistically different  
(Wilcoxon/Kruskal-Wallis tests)
  - Heptanal
  - 2-ethyl-1-hexanol
  - Acetophenone
  - Benzaldehyde
  - Octanal

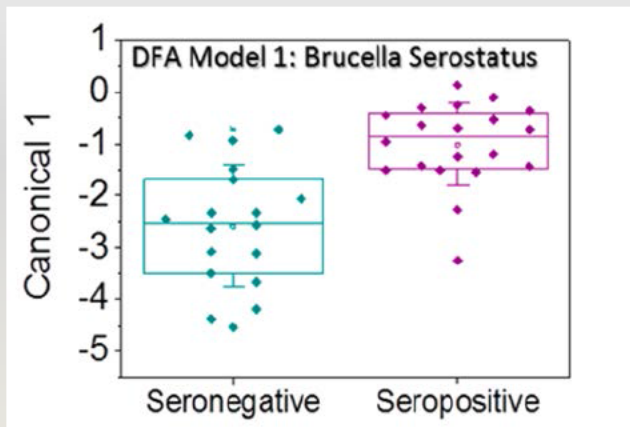


# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

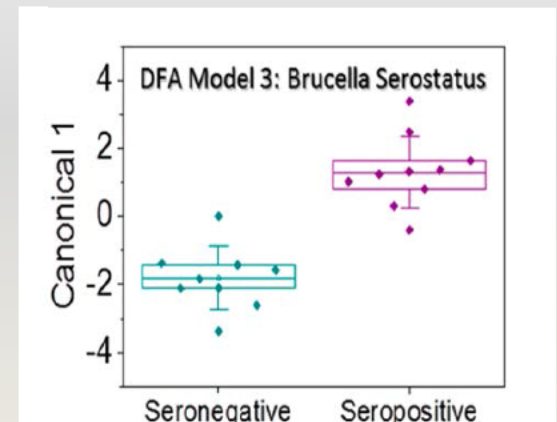
## Results: NaNose



May 2012 BQF only



April/May 2012 WRF and BQF



January 2013 BQF only

# Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

## Results: GCMS

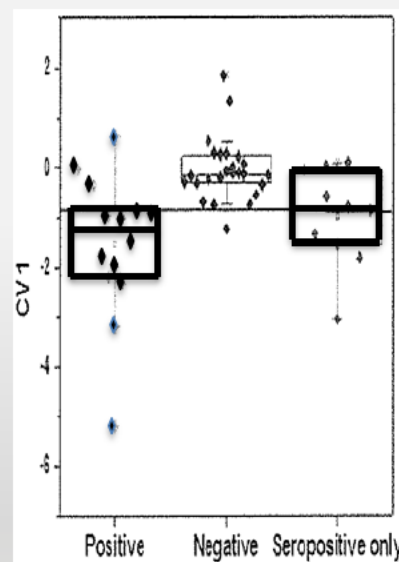
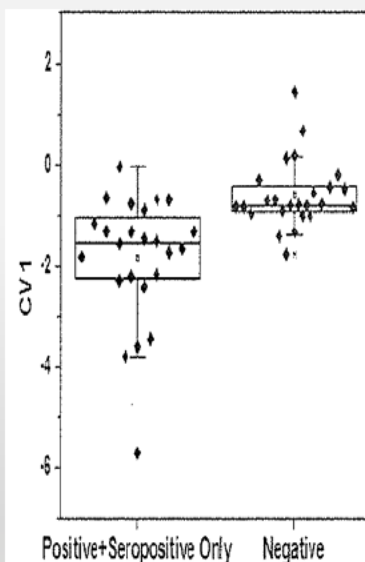
- Culture positive vs negative
- 2 VOCs statistically different
  - Benzene, 1,2,4-trimethyl
  - Decane, 2,6,7-trimethyl





# Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

## Results



|                         | Culture pos/<br>Seropos | Negative |
|-------------------------|-------------------------|----------|
| Culture pos/<br>Seropos | 17                      | 7        |
| Neg                     | 3                       | 22       |

|          | Positive | Negative |
|----------|----------|----------|
| Positive | 10       | 4        |
| Negative | 1        | 24       |

000500

# Summary-VOCs



- Study 1

- Good discrimination between seropositive and seronegative animals can be achieved.
- Environmental factors did not appear to affect the outcome of the models and were consistent over time.

- Study 2

- Good discrimination between culture positive and negative animals, but seropositives are somewhere in the middle.

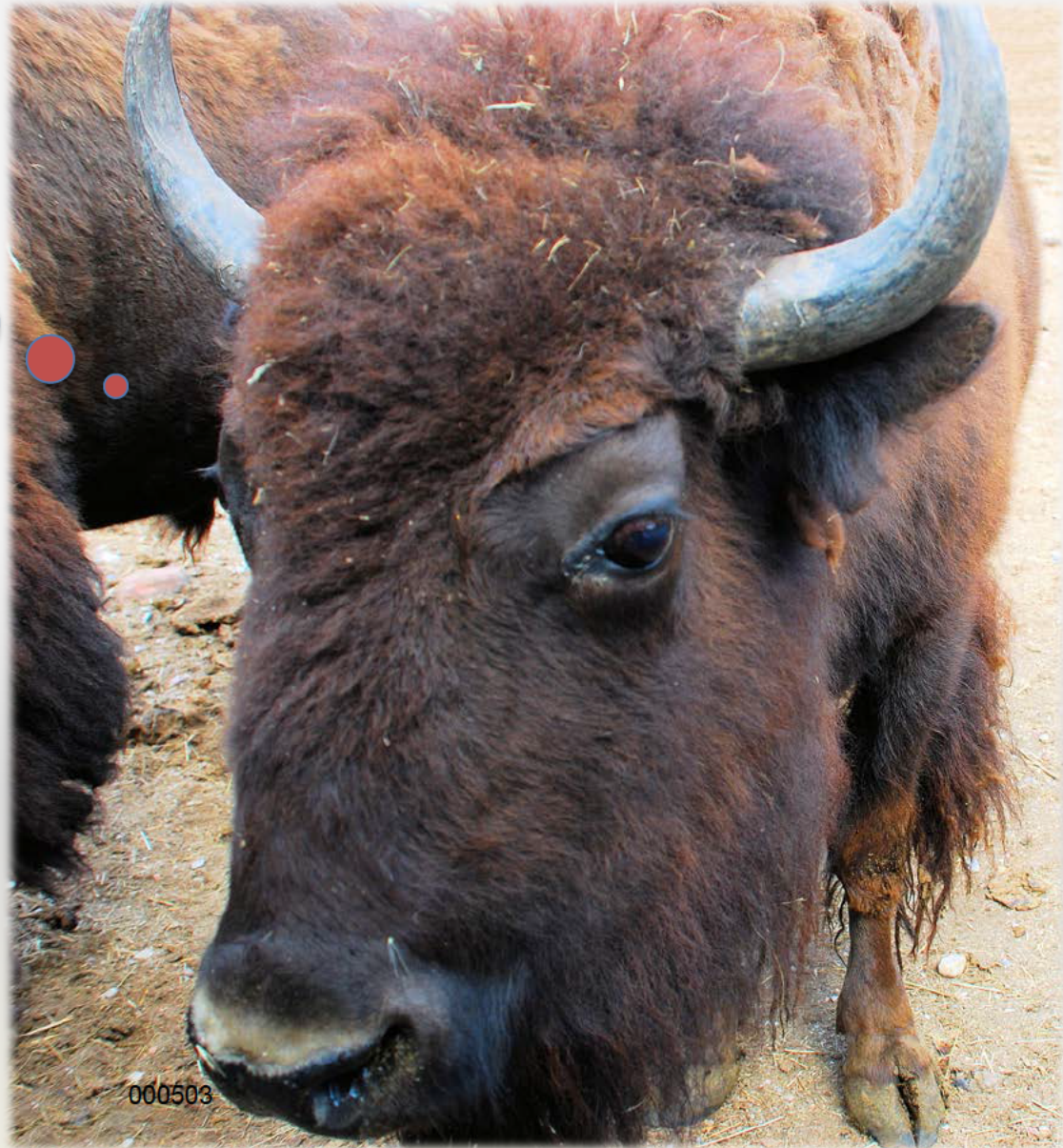
# WiLDIT Future Works

- Dry Dart RB51 study in bison with *B. abortus* challenge
- Remote vaccination of bison calves and yearlings with Dry Dart RB51
- Second mouse study with powdered, killed *B. abortus*
- Powdered, killed *B. abortus* in elk



United States Department of Agriculture

Questions?





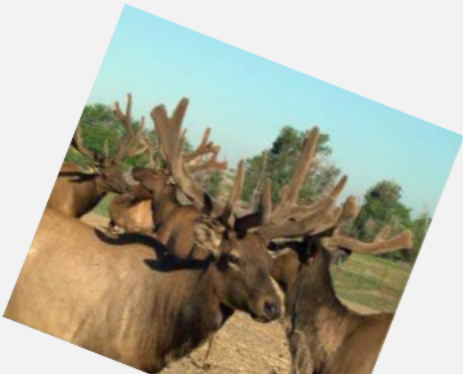
# Wildlife/Livestock Disease Investigations Team (WiLDIT) Brucellosis Research Update

Pauline Nol

U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
Veterinary Services  
Science, Technology, and Analysis Services

September 27, 2016

000504



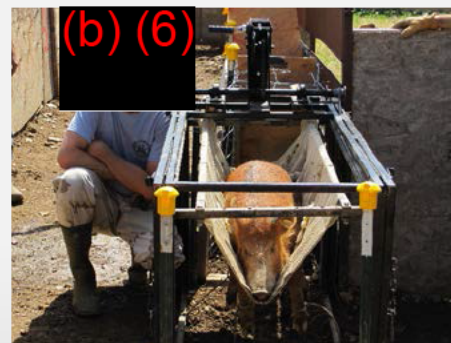
# WiLDIT



Pauline Nol



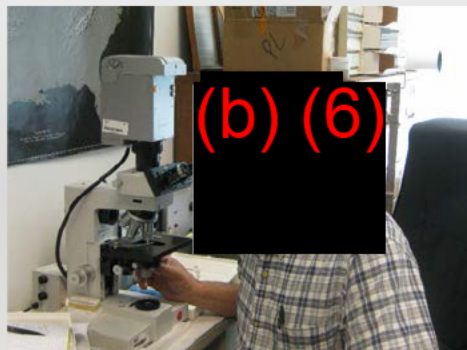
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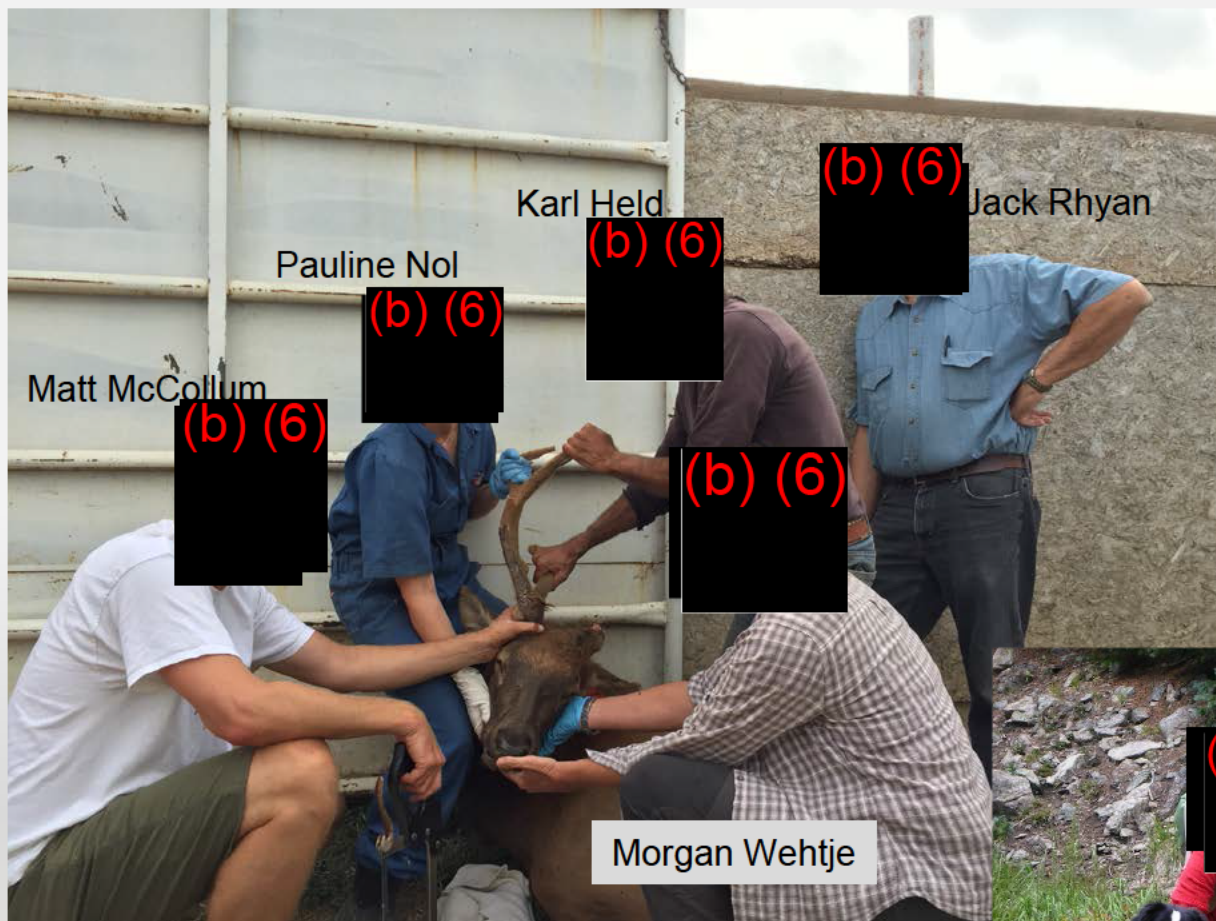
Jack Rhyan

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

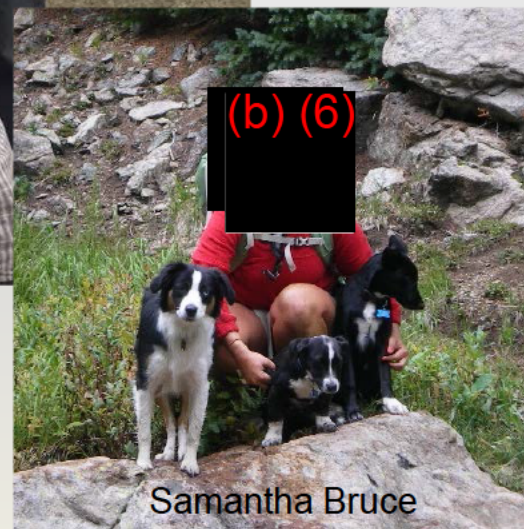


Samantha Bruce





# WiLDIT



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

000506

# Management tools for Brucellosis

- Immunocontraception
  - GonaCon™
- Vaccination
  - Dry Dart
  - Mucosal vaccination of killed, powdered vaccine
  - Natural transmission model in elk
- Detection
  - Volatile Organic Compounds



# Immunocontraception

## Background

- In female bison, brucellosis is transmitted if pregnancy occurs
  - In over 300 captures, *B. abortus* was isolated from vagina, milk, blood, feces, & products of parturition
- GonaCon™ (immunocontraceptive vaccine)
  - GnRH linked to sea mollusk protein and therefore looks large and foreign (not recognized as “self”)
  - Combined with adjuvant containing *Mycobacterium avium*



# Immunocontraception

## Current studies

- Study 1: Duration of infertility study in southern Colorado
- Study 2: Management of *B. abortus* in bison through immunocontraception (Corwins Springs, MT)



000509



# Study 1: Duration of infertility in southern Colorado

- Initiated Nov 2011
- Gonacon<sup>TM</sup> treatment group (N=10)
- Non-treatment controls (n=10)

## Results

Number pregnant/number in group

|           | Nov 2011 | Nov 2012 | Nov 2013 | Nov 2014 | Nov 2015 |
|-----------|----------|----------|----------|----------|----------|
| Treatment | 4/10     | 3/9      | 1/10     | 3/9      | 3/10     |
| Control   | 4/10     | 9/9      | 6/9      | 9/9      | 6/9      |

# Study 2: Management of *B. abortus* in bison through immunocontraception

- First cohort (2011)
  - Treatment group (n=15; *B. abortus* +)
    - Sentinels (n=5; *B. abortus* -)
  - Control group (n=14; *B. abortus* +)
    - Sentinels (n=5; *B. abortus* -)
- Second Cohort (2013)
  - Treatment group (n=20; *B. abortus* +)
    - Sentinels (n=6; *B. abortus* -)
  - Control group (n=12; *B. abortus* +)



## Study 2: Management of *B. abortus* in bison through immunocontraception

- First cohort: 15 Controls; 15 GonaCon Vaccs
  - 1<sup>st</sup> year: Controls-77% preg; vaccs- 20% preg
  - 2<sup>nd</sup> year: Controls-77% preg; vaccs- 13% preg
  - 3<sup>rd</sup> year: Controls-90% preg; vaccs- 36% preg
  - 4<sup>th</sup> year: Controls-93% preg; vaccs- 8% preg
- Second cohort: 20 Controls; 20 GonaCon Vaccs
  - 1<sup>st</sup> year: Controls- 90% preg; vaccs- 5% preg
  - 2<sup>nd</sup> year: Controls- 93% preg; vaccs- 26% preg

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results-efficacy of immunocontraception

Number pregnant/number in group

|            | 2013  | 2014  | 2015  | 2016 |
|------------|-------|-------|-------|------|
| Vaccinates | 3/15  | 2/15  | /14   |      |
| Controls   | 11/14 | 10/13 | 10/12 |      |

Number pregnant/number in group

|            | 2015 | 2016 | 2016 |
|------------|------|------|------|
| Vaccinates | 0/20 |      |      |
| Controls   |      |      |      |

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results on the *Brucella* Side

- Control pasture:
  - 14 *Brucella* abortions
  - 3 normal calves born with significant shedding,
  - 4/5 sentinels seroconverted and aborted once or twice;
  - 8 calves seroconverted at 1 year.
  - One control had 1 cult + calf, 2 cult – calves, then cult + abortion.
  - One sentinel had 1 negative calf, seroconverted, 3 culture positive abortions in a row.
  - One seroconversion to negative in control pasture

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results on the *Brucella* Side (cont'd)

- Treatment groups:
  - 1 *Brucella* abortion (2<sup>nd</sup> group); 0 seroconversions
  - 4 conversions to negative



000515





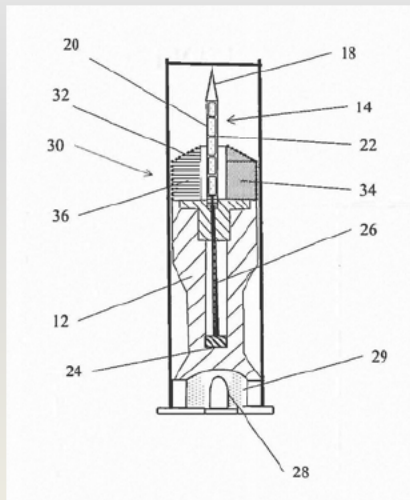
# Vaccination

## Background

- Need effective/remote delivery of brucellosis vaccines in bison and elk
- Bison: RB51 given in two doses administered ~1 year apart induced increased protection against abortion vs. single dose  
(Olsen et al., 2015. Clinical Vaccine and Immunology 23)
- Elk: Continued research toward effective vaccine and challenge model

# DryDart™

- Dart system to deliver lyophilized, powdered, pelleted, or encapsulated vaccines
- 2X the payload of biobullets
- Marks injection site.
- Fired from dart gun or shotgun
- Biodegradable.





# DryDart™



Pellet delivered by DryDart compared to larger Biobullet placed at site.





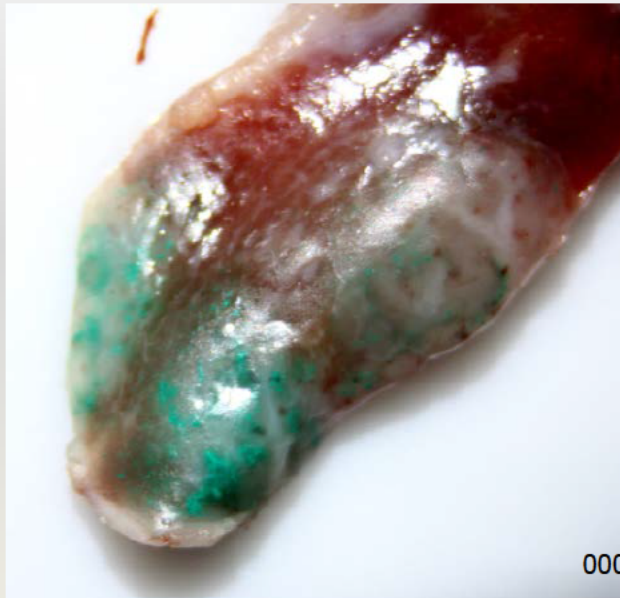


Dart marking injection site and bouncing out after depositing vaccine.



# Mucosal vaccination with powdered, killed vaccine

Goal: Develop killed, *B. abortus* vaccine for use on feedlines.



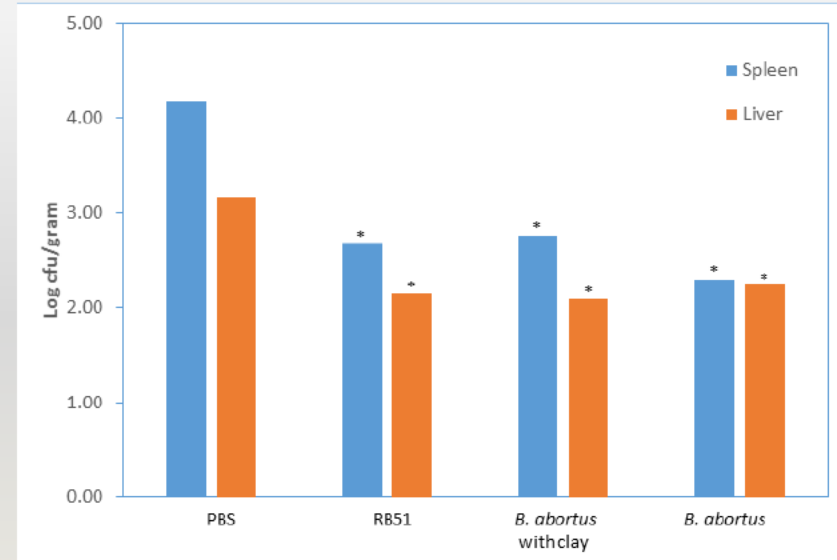
000520

Medial retropharyngeal lymph node with colored clay after intranasal delivery

# Mucosal vaccination with powdered, killed vaccine

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

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



# Developing a model for natural *B. abortus* infection in elk

- Natural exposure as challenge
- Potential model for vaccine studies



000522

# Developing a model for natural *B. abortus* infection in elk

- Study 1: 2014
  - 10 negative elk, 2 undiagnosed elk fetuses
  - In 24 hours, 227 contacts of elk with fetuses
- Study 2: 2016
  - 11 negative elk, 2 elk fetuses, 9 positive pregnant elk



000523





# Developing a model for natural *B. abortus* infection in elk

## Results (so far)

- Study 1
  - No seroconversions
- Study 2
  - No abortions in the 9 pregnant cows
  - Status of calves pending
  - No seroconversions in naïve animals after 90 days

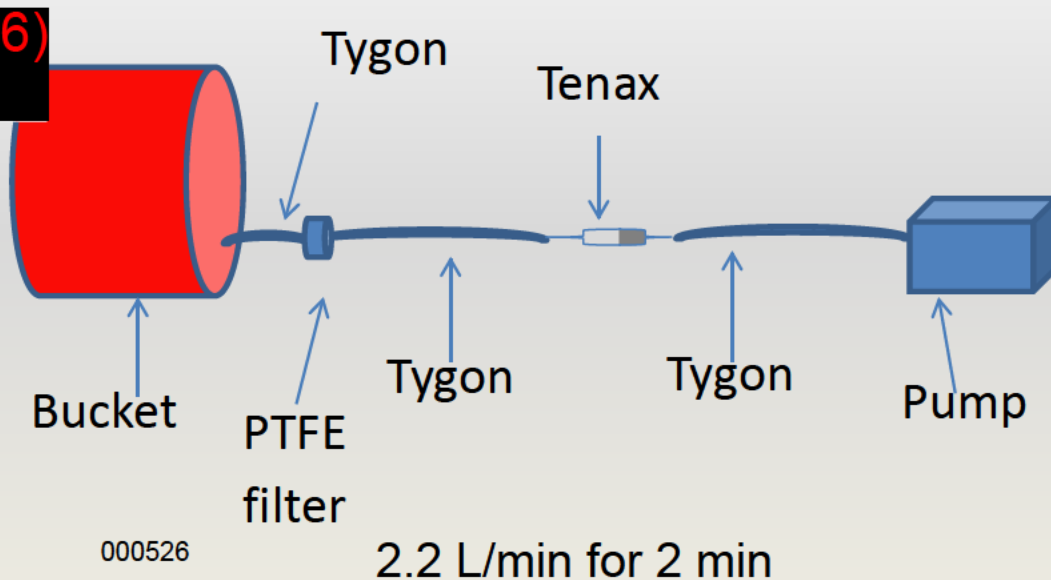
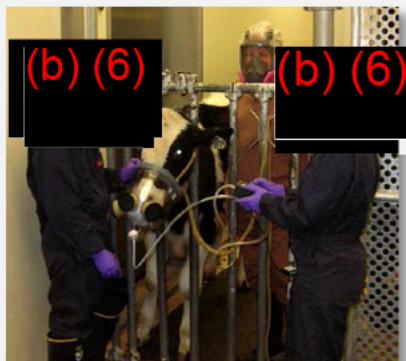


# Detection

Use of Volatile Organic Compounds (VOCs) in breath to distinguish brucellosis-positive animals from negative animals



# Methods: Breath Collection



# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

Bayn et al., 2013 *Analytical Chemistry*. 85 (22): 11146–11152

- 20 *B. abortus* seropositive\* bison
  - 9 housed at Colorado facility (WRF)
  - 11 housed at Montana facility (BQF)†
- 18 seronegative\* controls
  - 8 housed at WRF
  - 10 housed at BQF



\*Based on standard *Brucella* serological tests.

†Samples collected at various time points in different locations in MT.



## Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

- Breath VOCs collected at Stephen's Creek (n=33)
- 25 seronegative
- 14 seropositive/culture positive
- 10 seropositive/culture negative





Technion-Israel Institute  
of Technology-Haick  
Laboratory

# Methods: Breath Analysis

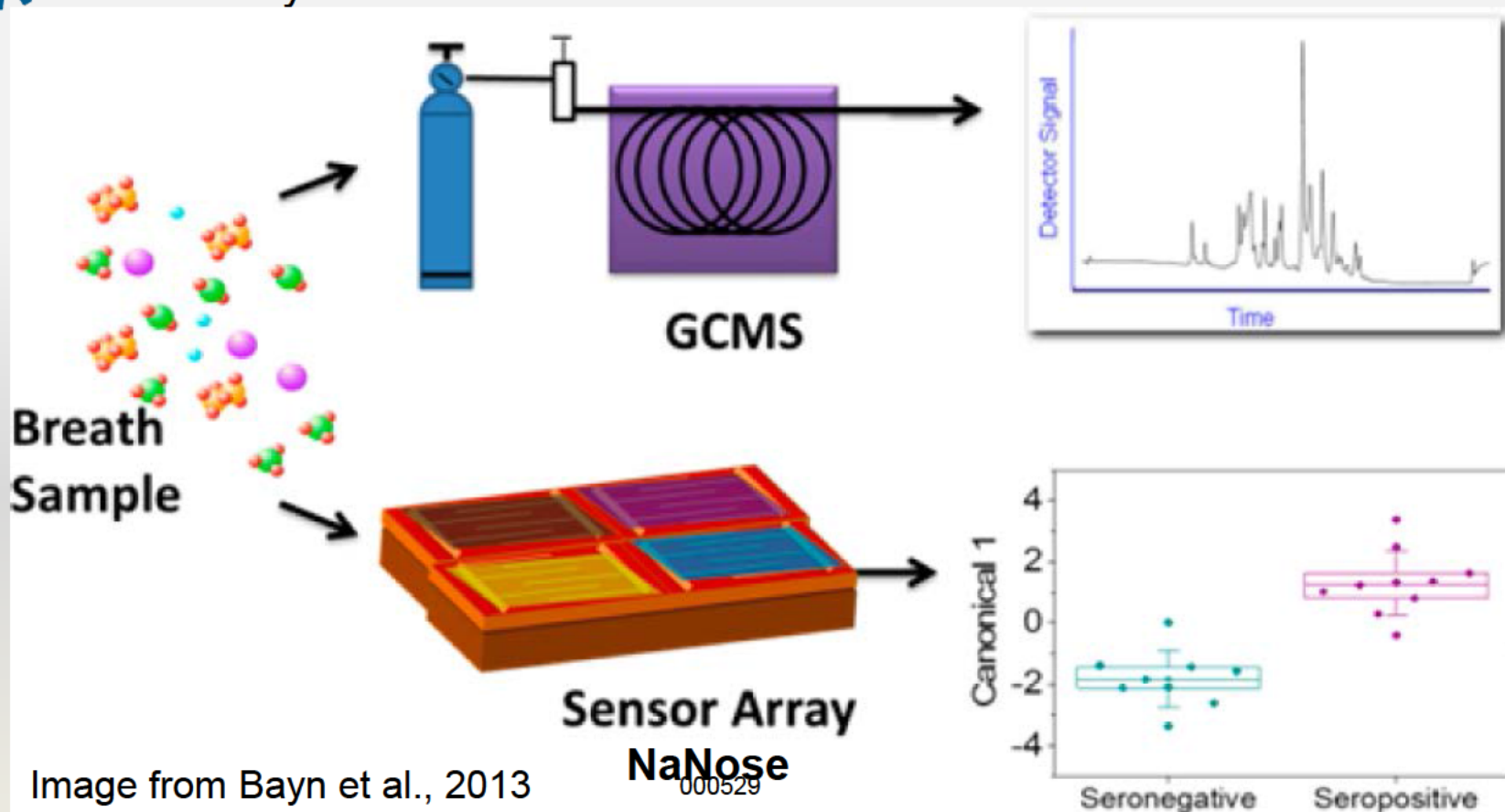


Image from Bayn et al., 2013

NaNose

000529

# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

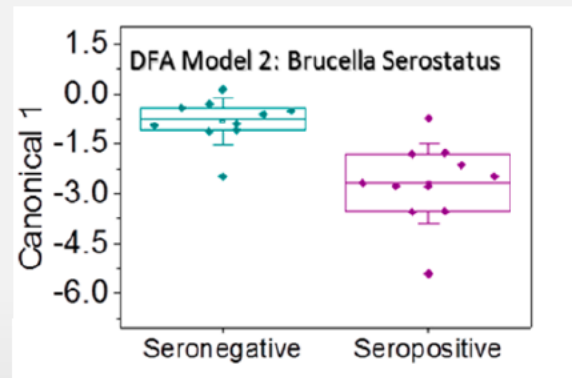
## Results-GC/MS

- 5 VOCs statistically different (Wilcoxon/Kruskal-Wallis tests)
  - Heptanal
  - 2-ethyl-1-hexanol
  - Acetophenone
  - Benzaldehyde
  - Octanal

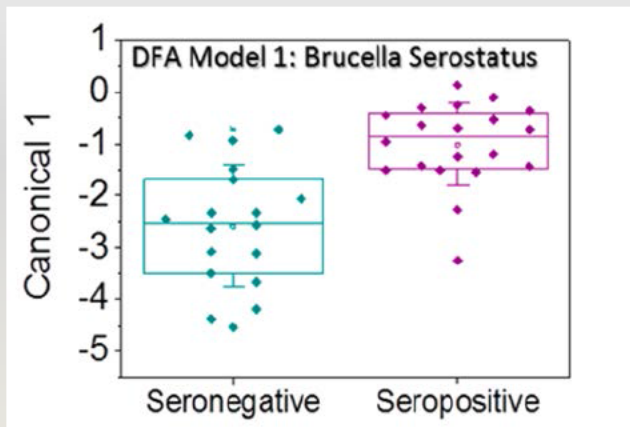


# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

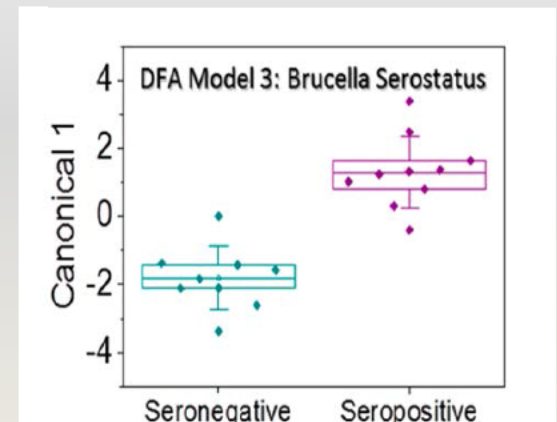
## Results: NaNose



May 2012 BQF only



April/May 2012 WRF and BQF



January 2013 BQF only



# Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

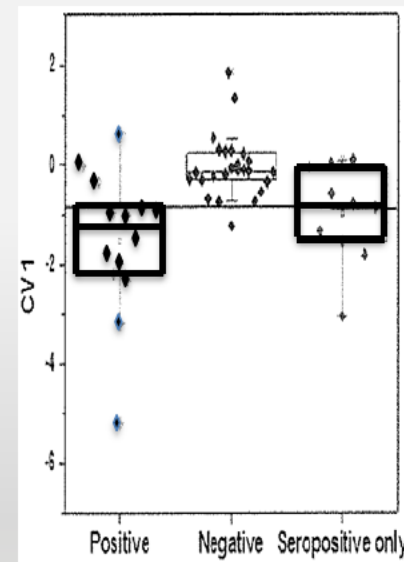
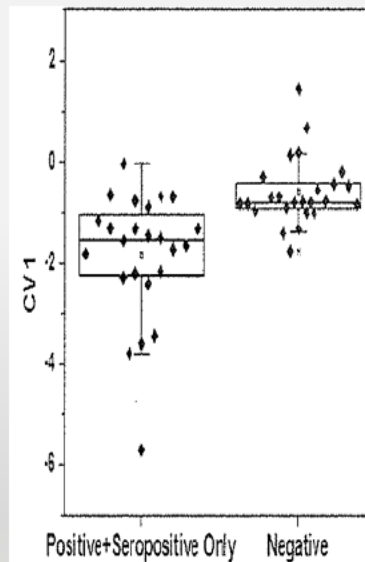
## Results: GCMS

- Culture positive vs negative
- 2 VOCs statistically different
  - Benzene, 1,2,4-trimethyl
  - Decane, 2,6,7-trimethyl



# Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

## Results



|                         | Culture pos/<br>Seropos | Negative |          | Positive | Negative |
|-------------------------|-------------------------|----------|----------|----------|----------|
| Culture pos/<br>Seropos | 17                      | 7        | Positive | 10       | 4        |
| Neg                     | 3                       | 22       | Negative | 1        | 24       |

000533

# Summary-VOCs



- Study 1

- Good discrimination between seropositive and seronegative animals can be achieved.
- Environmental factors did not appear to affect the outcome of the models and were consistent over time.

- Study 2

- Good discrimination between culture positive and negative animals, but seropositives are somewhere in the middle.

# WiLDIT Future Works

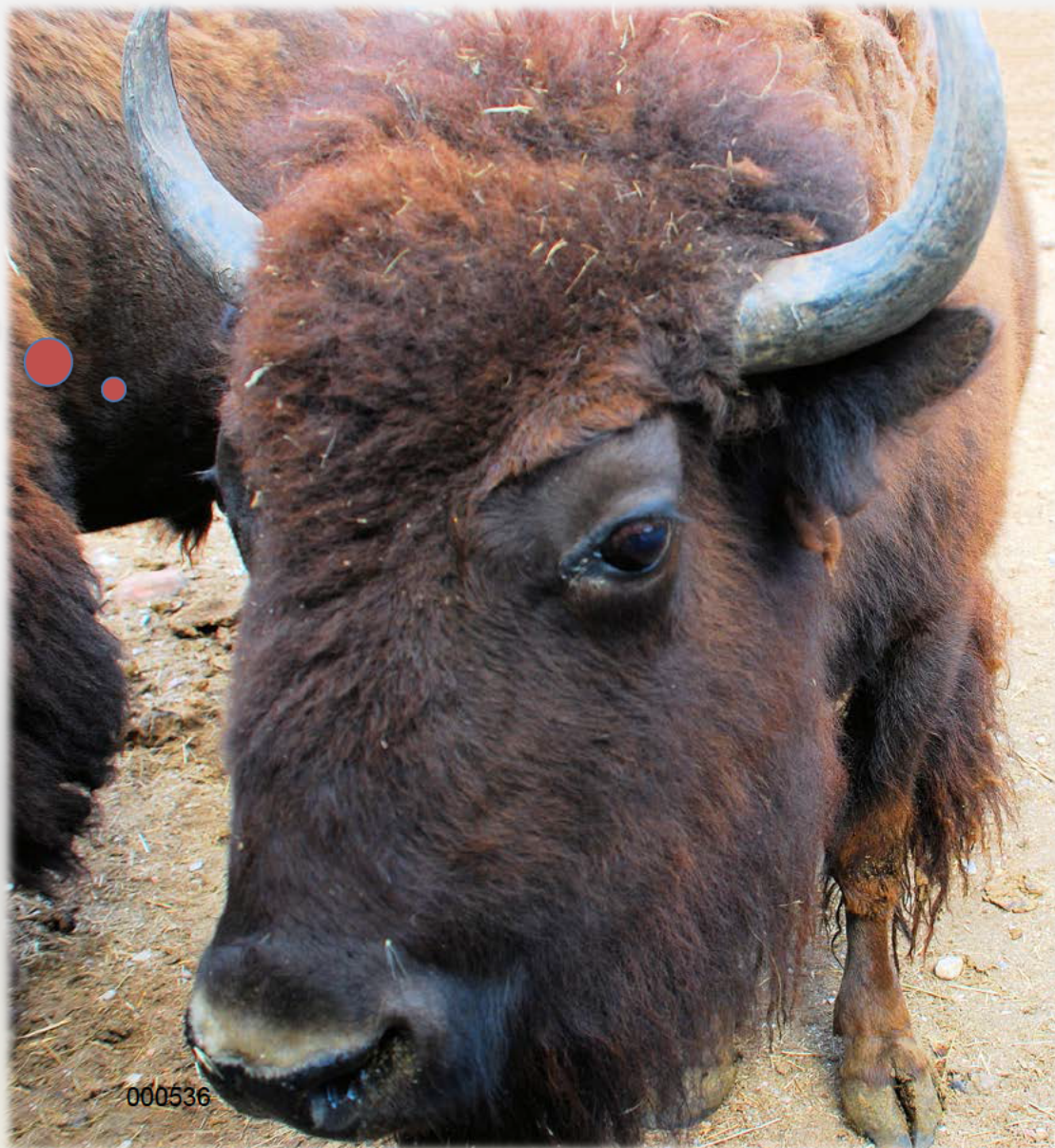
- Dry Dart RB51 study in bison with *B. abortus* challenge
- Remote vaccination of bison calves and yearlings with Dry Dart RB51
- Second mouse study with powdered, killed *B. abortus*
- Powdered, killed *B. abortus* in elk





United States Department of Agriculture

Questions?



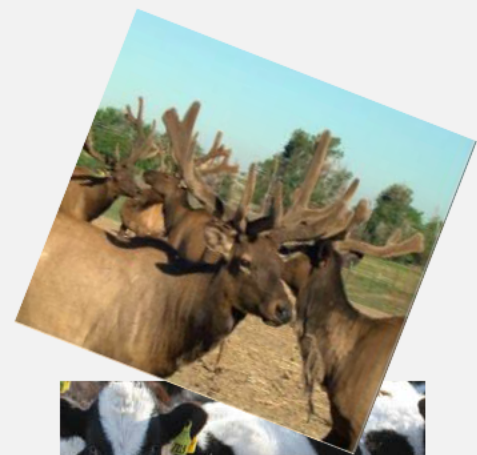
# Wildlife/Livestock Disease Investigations Team (WiLDIT) Brucellosis Research Update

Pauline Nol

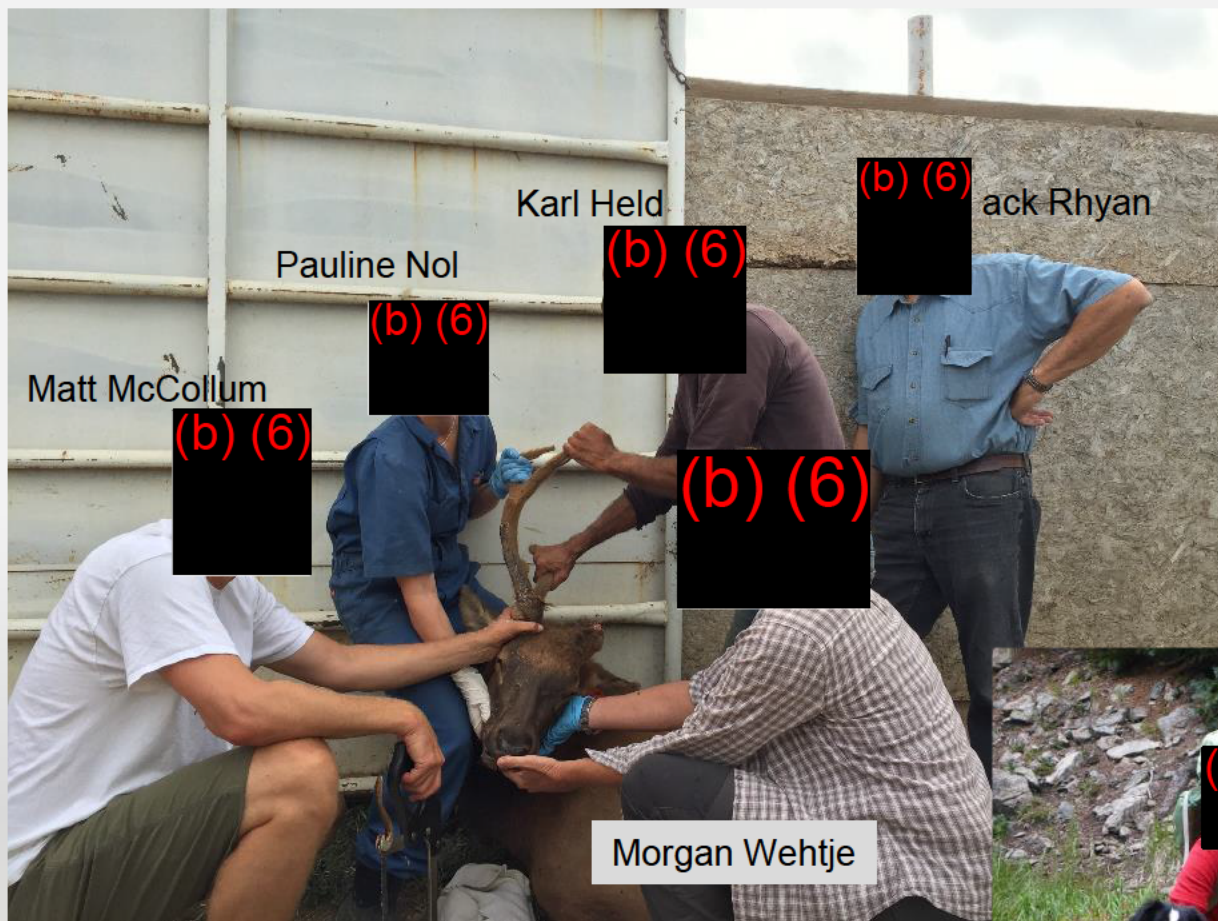
U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
Veterinary Services  
Science, Technology, and Analysis Services

September 27, 2016

000537







# WiLDIT



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

000538

# Management tools for Brucellosis

- Immunocontraception
  - GonaCon™
- Vaccination
  - Dry Dart
  - Mucosal vaccination of killed, powdered vaccine
  - Natural transmission model in elk
- Detection
  - Volatile Organic Compounds
- Conservation



# Immunocontraception

## Background

- In female bison, brucellosis is transmitted if pregnancy occurs
  - In over 300 captures, *B. abortus* was isolated from vagina, milk, blood, feces, & products of parturition
- GonaCon™ (immunocontraceptive vaccine)
  - GnRH linked to sea mollusk protein and therefore looks large and foreign (not recognized as “self”)
  - Combined with adjuvant containing *Mycobacterium avium*



# Immunocontraception

## Current studies

- Study 1: Duration of infertility study in southern Colorado
- Study 2: Management of *B. abortus* in bison through immunocontraception (Corwin Springs, MT)



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# Study 1: Duration of infertility in southern Colorado

- Initiated Nov 2011
- GonaCon™ treatment group (N=10)
- Non-treatment controls (n=10)

## Results

Number pregnant/number in group (percent pregnant)

|           | Nov 2011  | Nov 2012  | Nov 2013  | Nov 2014  | Nov 2015  |
|-----------|-----------|-----------|-----------|-----------|-----------|
| Treatment | 4/10 (40) | 3/9 (33)  | 1/10 (10) | 3/9 (33)  | 3/10 (30) |
| Control   | 4/10 (40) | 9/9 (100) | 6/9 (67)  | 9/9 (100) | 6/9 (67)  |

# Study 2: Management of *B. abortus* in bison through immunocontraception

- First cohort (2012)
  - Treatment group (n=15; *B. abortus* +)
    - Sentinels (n=5; *B. abortus* -)
  - Control group (n=14; *B. abortus* +)
    - Sentinels (n=5; *B. abortus* -)
- Second Cohort (2014)
  - Treatment group (n=20; *B. abortus* +)
    - Sentinels (n=6; *B. abortus* -)
  - Control group (n=14; *B. abortus* +)



# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results-efficacy of immunocontraception

First Cohort:

| Group      | 2013        | 2014       | 2015       | 2016       |
|------------|-------------|------------|------------|------------|
| Treatments | 3/15 (20) * | 2/15 (13)  | 5/14 (36)  | 3/14 (21)  |
| Controls   | 11/14 (79)  | 10/13 (77) | 10/12 (83) | 10/12 (83) |

Second Cohort:

| Group      | 2015     | 2016       |
|------------|----------|------------|
| Treatments | 1/20 (5) | 5/19 (26)  |
| Controls   | n/a      | 13/21 (62) |

\*Number pregnant/number in group (percent pregnant)

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# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results on the *Brucella* Side

- Control pastures:
  - 12 *Brucella* abortions + 1 pos weak calf + 5 pos live calves = 18 “shedding events” from 11 cows
  - All 5 sentinels seroconverted ➡ 6 abortions
  - 8 calves seroconverted at 1 year.
  - One cow had 1 culture pos calf, 2 culture neg calves, then culture pos abortion
  - One cow (sentinel) had 1 negative calf then 3 culture positive abortions
  - One seroconversion to negative

# Study 2: Management of *B. abortus* in bison through immunocontraception

## Results on the *Brucella* Side (cont'd)

- Treatment groups:
  - 1 *Brucella* abortion (2<sup>nd</sup> group); 0 seroconversions
  - 4 seroconversions to negative



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# Vaccination

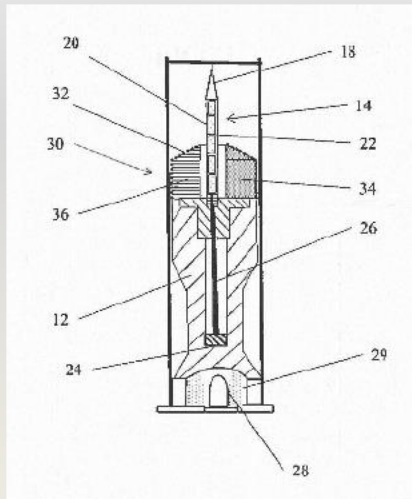
## Background

- Need effective/remote delivery of brucellosis vaccines in bison and elk
- Bison: RB51 given in two doses administered ~1 year apart induced increased protection against abortion vs. single dose  
(Olsen et al., 2015. Clinical Vaccine and Immunology 23)
- Elk: Continued research toward effective vaccine and challenge model



# DryDart™

- Dart system to deliver lyophilized, powdered, pelleted, or encapsulated vaccines
- 2X the payload of biobullets
- Marks injection site.
- Fired from dart gun or shotgun
- Biodegradable.



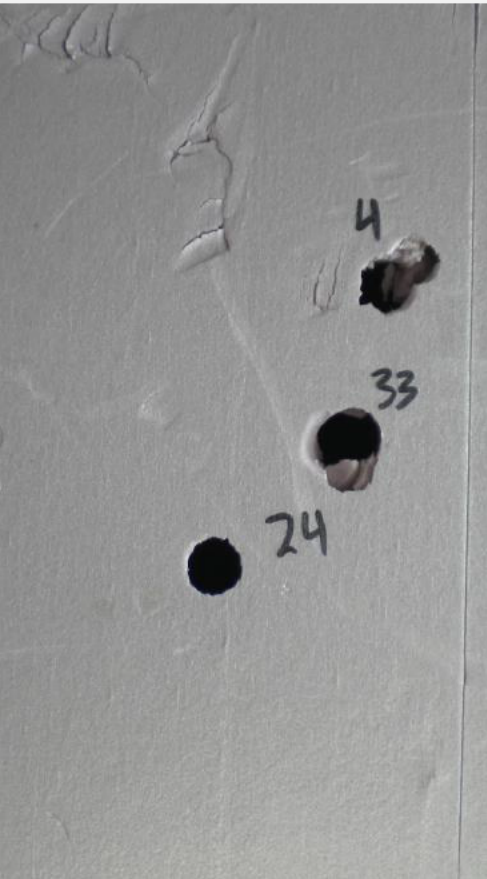


# DryDart™



Pellet delivered by DryDart compared to larger Biobullet placed at site.



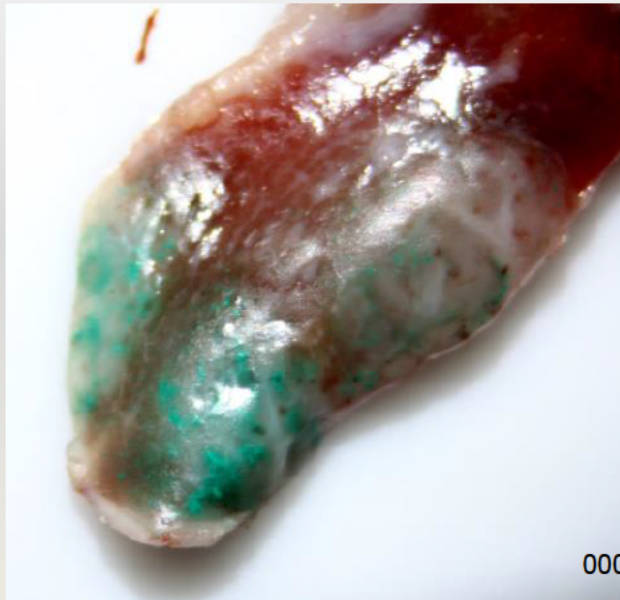


Dart marking injection site and bouncing out  
after depositing vaccine.



# Mucosal vaccination with powdered, killed vaccine

Goal: Develop killed, *B. abortus* vaccine for use on feedlines.



Right parotid lymph node with colored clay after intranasal delivery into left nasal sinus

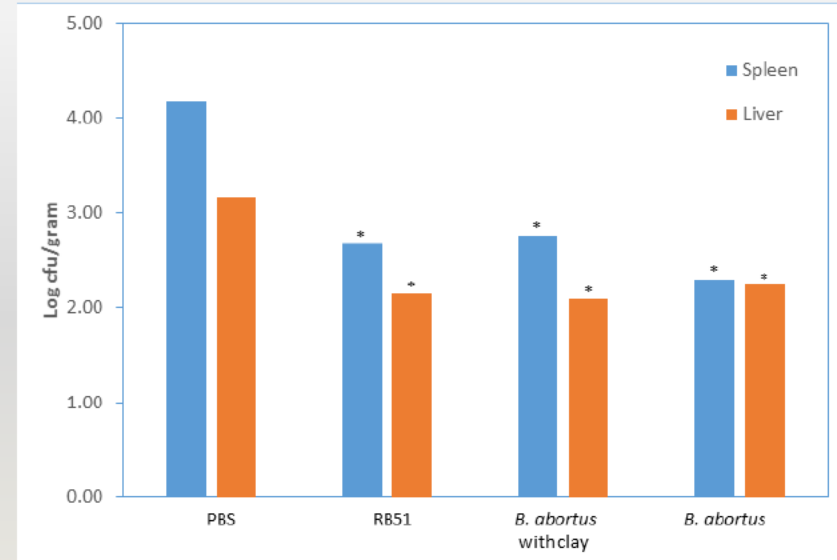
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# Mucosal vaccination with powdered, killed vaccine

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

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



# Developing a model for natural *B. abortus* infection in elk

- Natural exposure as challenge
- Potential model for vaccine studies



000553

# Developing a model for natural *B. abortus* infection in elk

- Study 1: 2014
  - 10 negative elk, 2 undiagnosed elk fetuses
  - In 24 hours, 227 contacts of elk with fetuses
- Study 2: 2016
  - 12 negative elk, 2 elk fetuses, 9 positive pregnant elk
  - No abortions
  - 3 min inspection time with fetus (2 min actual contact)
  - No contact time with calves



000554

# Developing a model for natural *B. abortus* infection in elk

## Results (so far)

- Study 1
  - No seroconversions
- Study 2
  - Culture status of calves that died pending
  - No seroconversions in naïve animals after 90 days



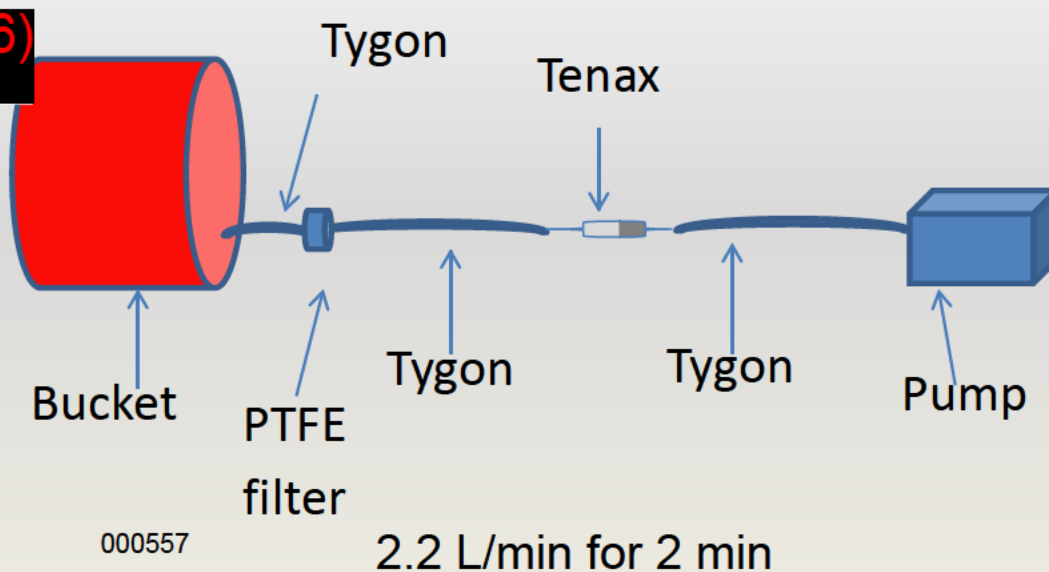


# Detection

Use of volatile organic compounds (VOCs) in breath to distinguish brucellosis-positive animals from negative animals



# Methods: Breath Collection



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Bayn et al., 2013 *Analytical Chemistry*. 85 (22): 11146–11152

- 20 *B. abortus* seropositive\* bison
  - 9 housed at Colorado facility (WRF)
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\*Based on standard *Brucella* serological tests.

†Samples collected at various time points in different locations in MT.

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- 25 seronegative
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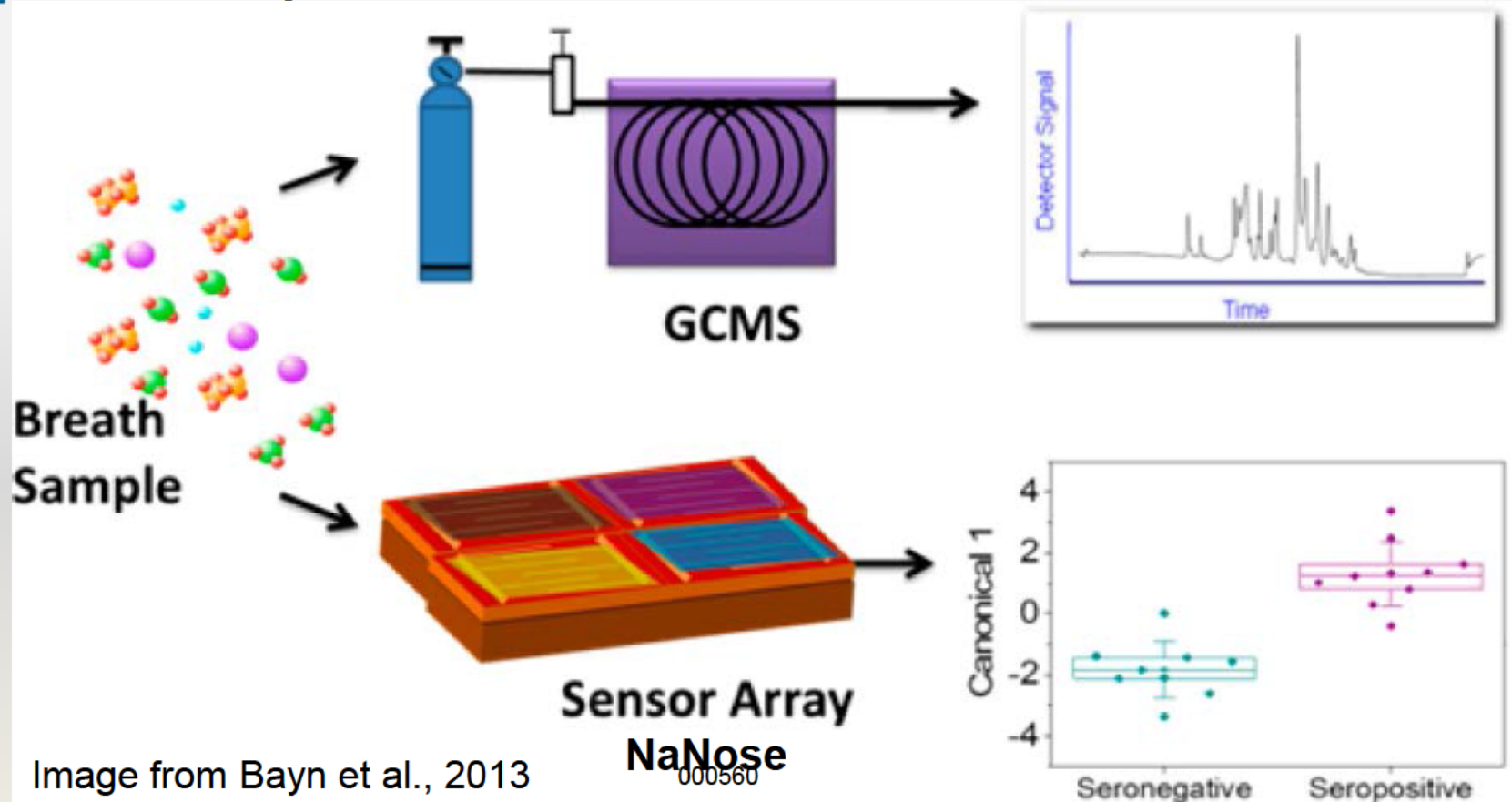






Technion-Israel Institute  
of Technology-Haick  
Laboratory

# Methods: Breath Analysis



# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

## Results-GC/MS

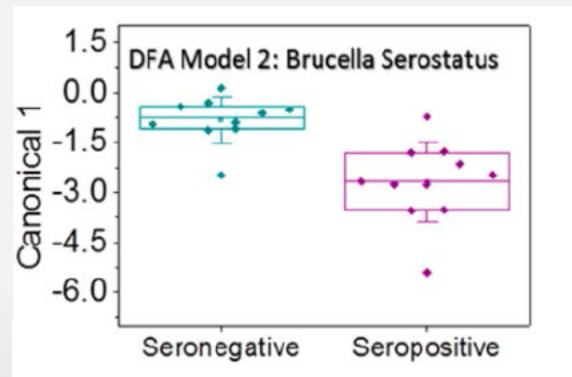
- 5 VOCs statistically different  
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  - Acetophenone
  - Benzaldehyde
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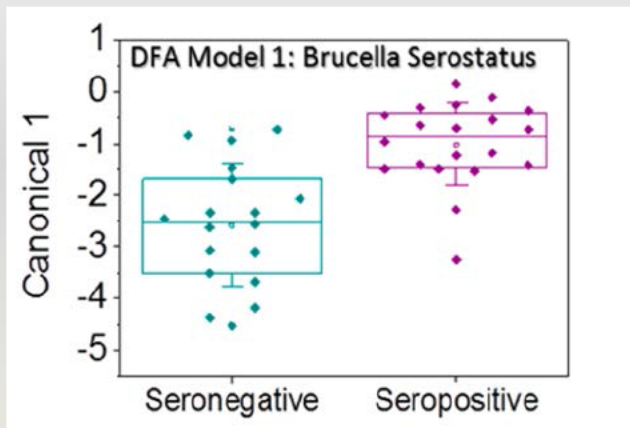
(b) (6)

# Study 1: Detection of volatile organic compounds in *B. abortus*-seropositive bison

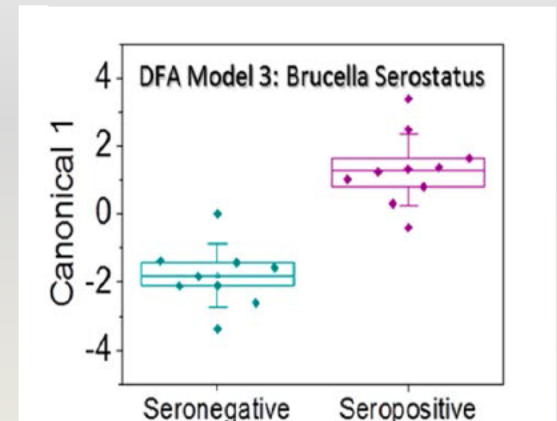
## Results: NaNose



May 2012 BQF only



April/May 2012 WRF and BQF



January 2013 BQF only

# Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

## Results: GCMS

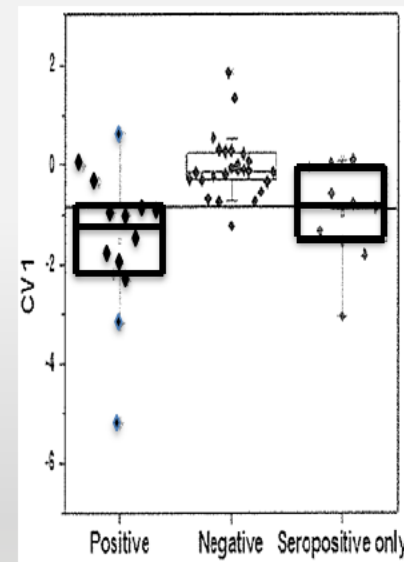
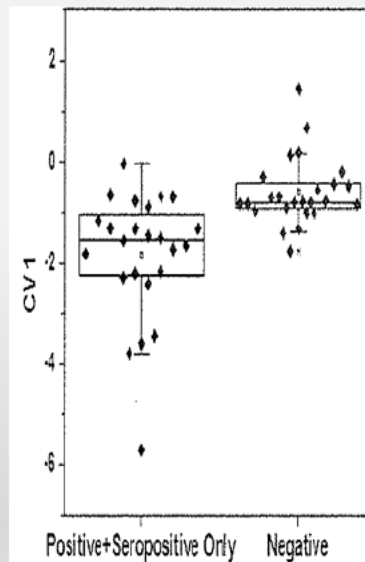
- Culture positive vs negative
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  - Benzene, 1,2,4-trimethyl
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# Study 2: Detection of volatile organic compounds in *B. abortus* culture- positive bison

## Results NaNose



|                         | Culture pos/<br>Seropos | Negative |          | Positive | Negative |
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| Culture pos/<br>Seropos | 17                      | 7        | Positive | 10       | 4        |
| Neg                     | 3                       | 22       | Negative | 1        | 24       |

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# Summary-VOCs



- Study 1

- Good discrimination between seropositive and seronegative animals can be achieved.
- Environmental factors did not appear to affect the outcome of the models and were consistent over time.

- Study 2

- Good discrimination between culture positive and negative animals, but seropositives are somewhere in the middle.

# Conservation

- Establishment of brucellosis-free bison herds of Yellowstone genetics outside of the GYA





United States Department of Agriculture



Northern Colorado (Soapstone Prairie)



USFS Midewin National Tallgrass Prairie,  
Illinois

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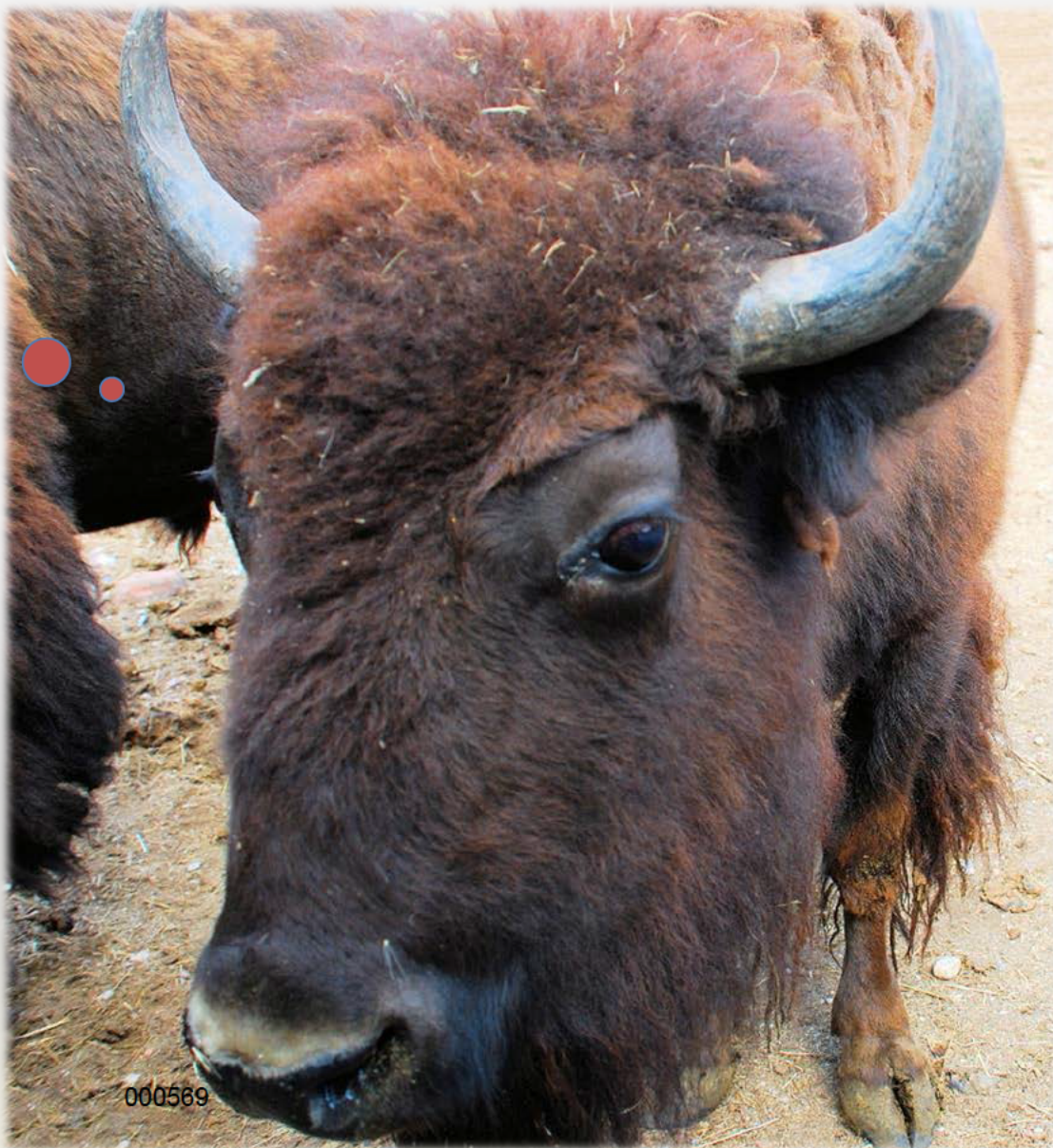
# WiLDIT Future Works

- Dry Dart RB51 study in bison with *B. abortus* challenge
- Remote vaccination of bison calves and yearlings with Dry Dart RB51
- Second mouse study with powdered, killed *B. abortus*
- Powdered, killed *B. abortus* in elk
- Continued work on elk natural transmission model
- Continued work on VOCs
- Continued work on conservation herd establishment



United States Department of Agriculture

Questions?





# Thanks to the Folks that Helped Make it Happen!

## Immunocontraception Studies

Becky Frey  
Ryan Clarke  
Brent Thompson  
Dennis Tilton  
Patrick  
Kate Schoenecker  
Doug Eckery  
Darcy Mora  
APHIS WS  
National Park Service  
Nature Conservancy  
Zapata Ranch

## Killed Vaccine Study

Richard Bowen  
Nikki Marlenee  
CSU Dept. Biomedical Sciences

## Elk Studies

Brandon Skurlock  
Cheyenne Stewart  
Mary Wood  
Hank Edwards  
Mark Nelson  
WYGFC  
COPW  
CO Dept Ag.  
CSU  
APHIS VS

## VOC Studies

Becky Frey  
Ryan Clarke  
Hossam Haick  
Alona Bayn  
Orna Adams  
Nisreen Shehada  
Christine Ellis  
Brent Thompson  
John Treanor  
Technion, Israel  
APHIS WS

## Bison Conservation

Jennifer Barfield  
CSU  
City of Fort Collins  
Larimer Cty. CO  
National Park Service

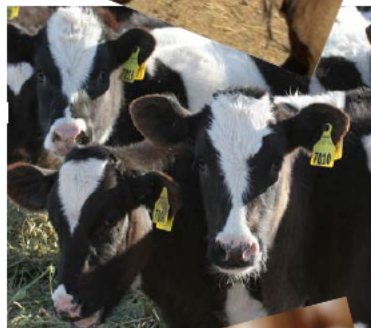


# Veterinary Services

Tools to detect and eliminate  
brucellosis from wild populations:  
current research

Jack Rhyan  
Wildlife/Livestock Disease  
Investigations Team

U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
Veterinary Services  
October 25, 2015





# Wildlife/livestock Disease Investigations Team

(b) (6)



Pauline Nol



Karl Held



Matt McCollum

Samantha Bruce

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

# Tools

- Disease detection: Volatile Organic Compound (VOC) analysis
- Disease elimination:
  - Immunocontraceptive vaccine (GonaCon™)
  - Brucella vaccines
    - DryDart™ for delivery of parenteral vaccines
    - Spray-dried vaccines for oral delivery
    - Using natural exposure as a challenge, a potential model for vaccine studies

A theoretical disease eradication plan

# A Potential New Tool for Remote Disease Detection: Volatile Organic Compounds (VOCs)

- Detection of unique VOCs or a unique pattern of VOCs from breath or feces from infected animals



# Detection of volatile organic compounds in *Brucella abortus*-positive bison

Bayn et al., 2013 *Analytical Chemistry*. 85 (22): 11146–11152

- 20 *Brucella abortus* seropositive\* bison (9 housed at Colorado facility and 11 housed at Montana facility†)
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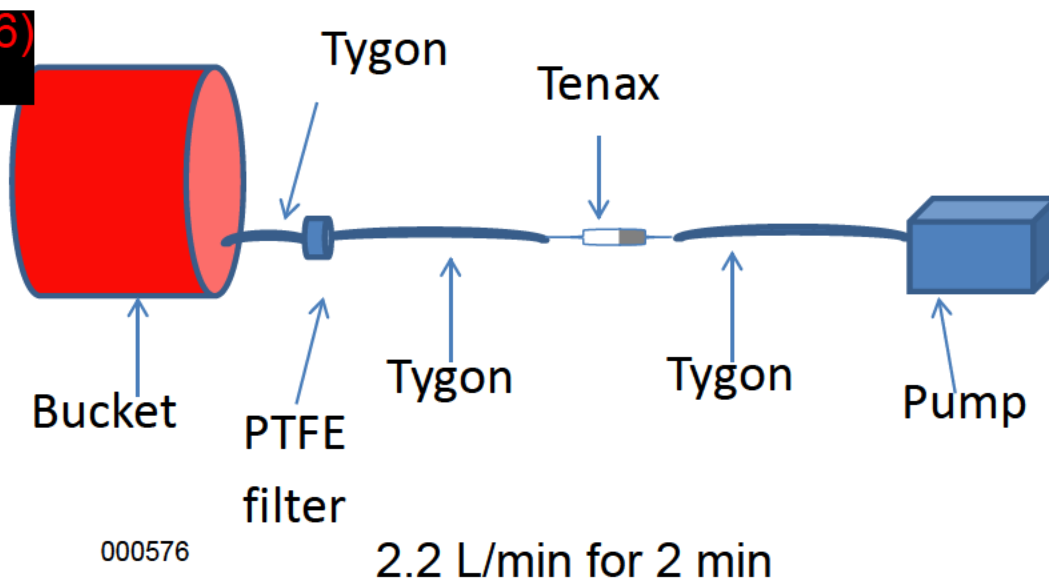
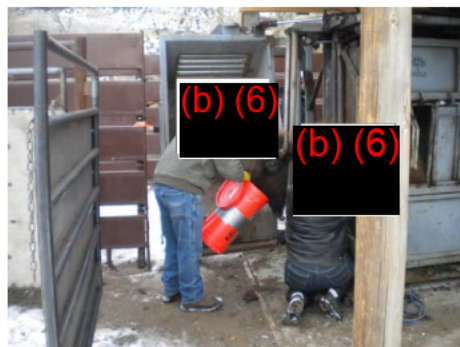
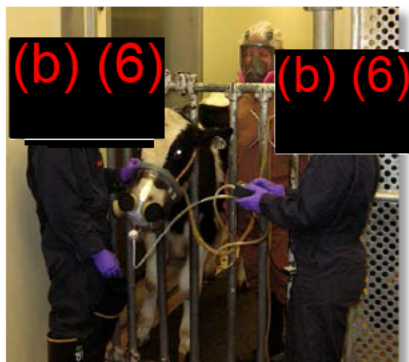


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# Methods: Breath Collection





Technion-Israel Institute  
of Technology-Haick  
Laboratory

# Methods: Breath Analysis

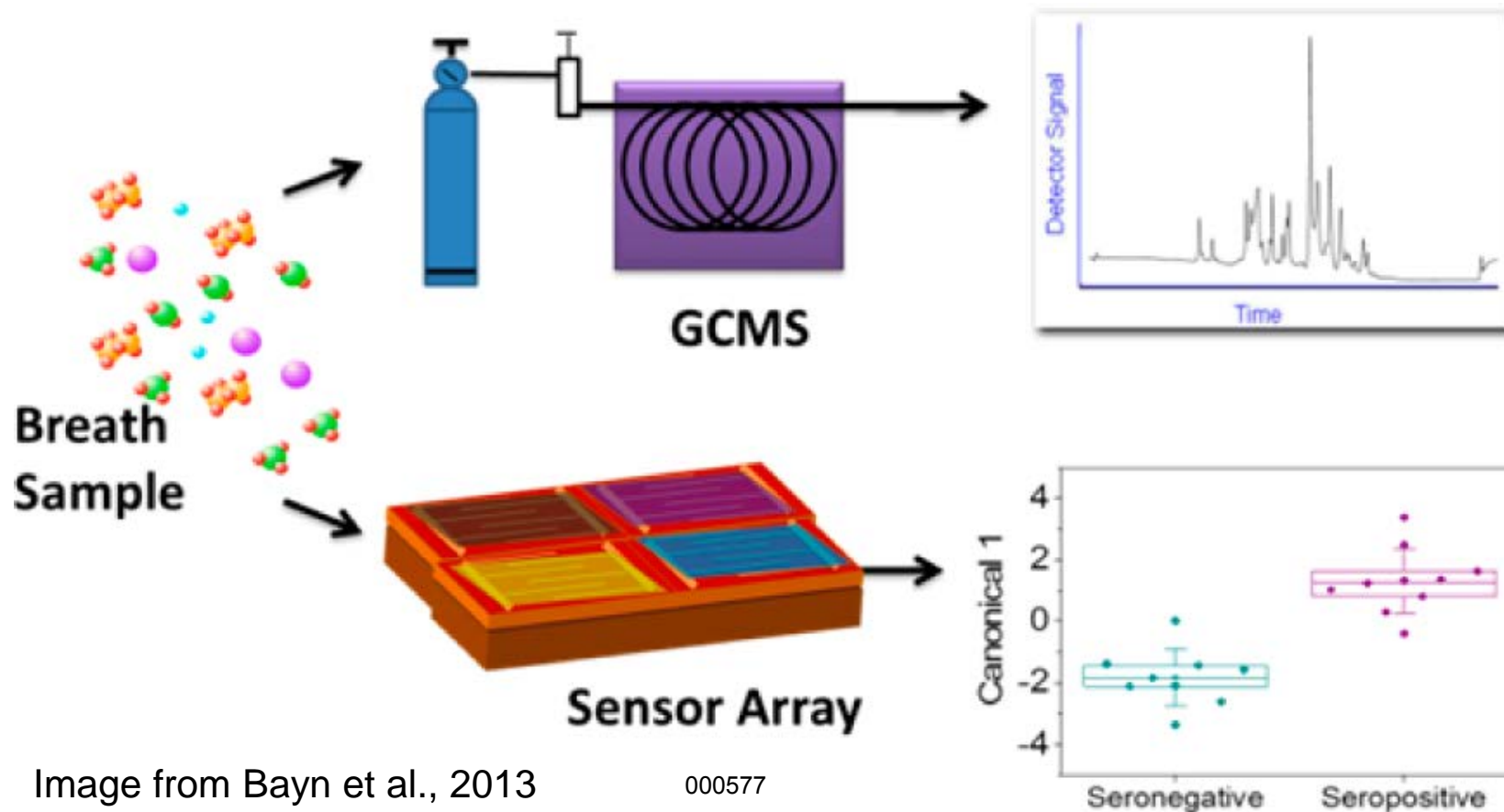


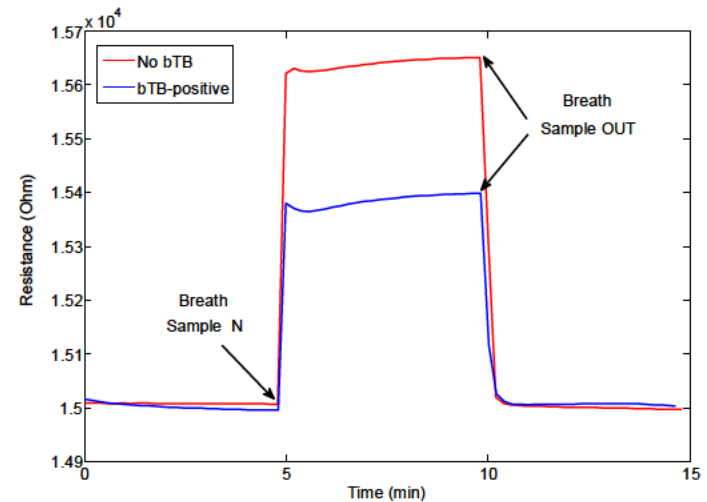
Image from Bayn et al., 2013

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# NaNose

## Nanomaterials-based sensor array

- Comprised an array of 21 cross reactive sensors
- Discriminant Factor Analysis Models



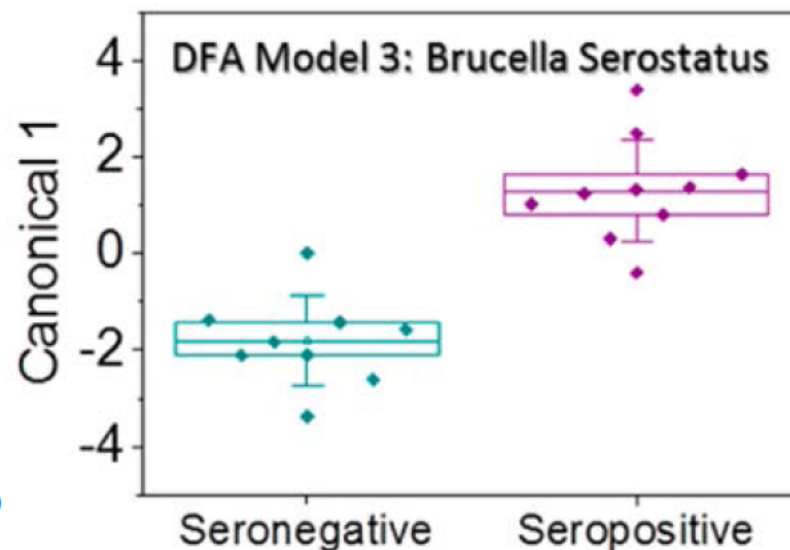
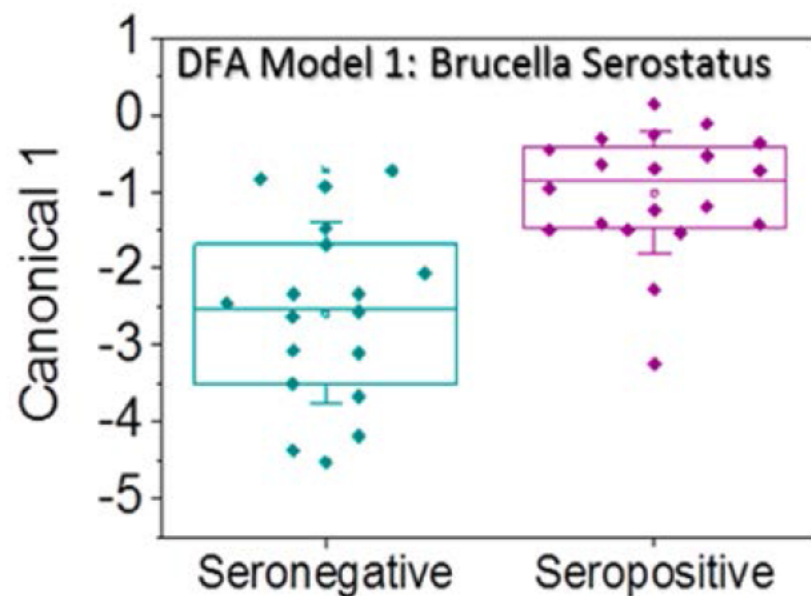
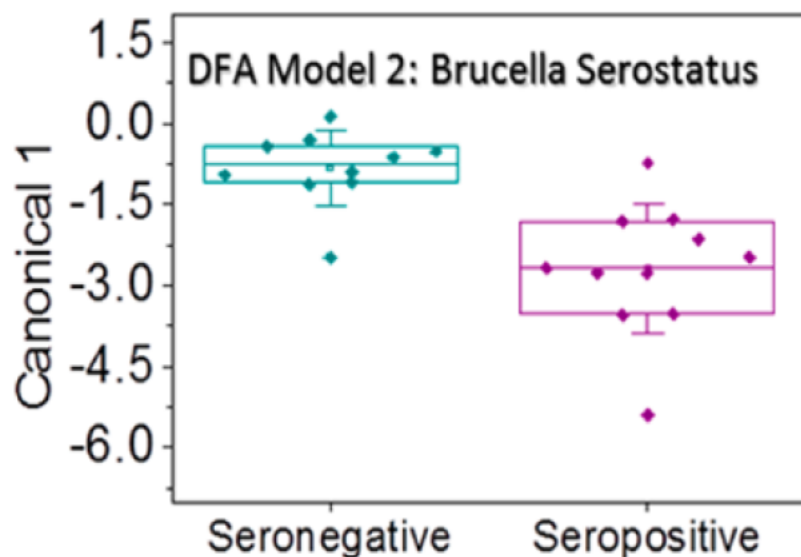
# Results

- GC/MS
  - 5 VOCs statistically different  
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    - Heptanal
    - 2-ethyl-1-hexanol
    - Acetophenone
    - Benzaldehyde
    - Octanal



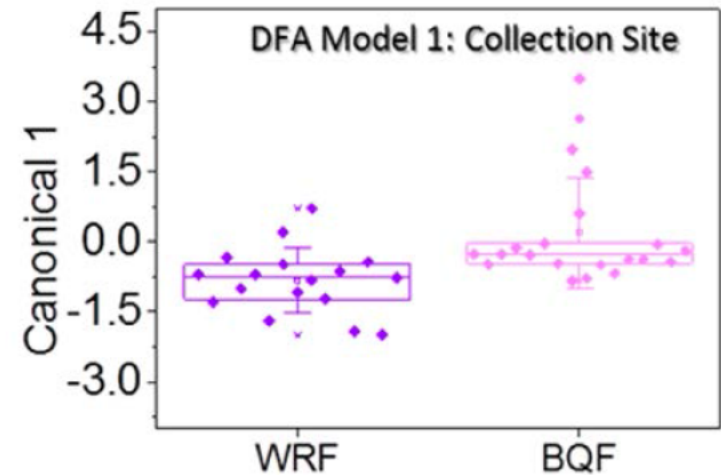
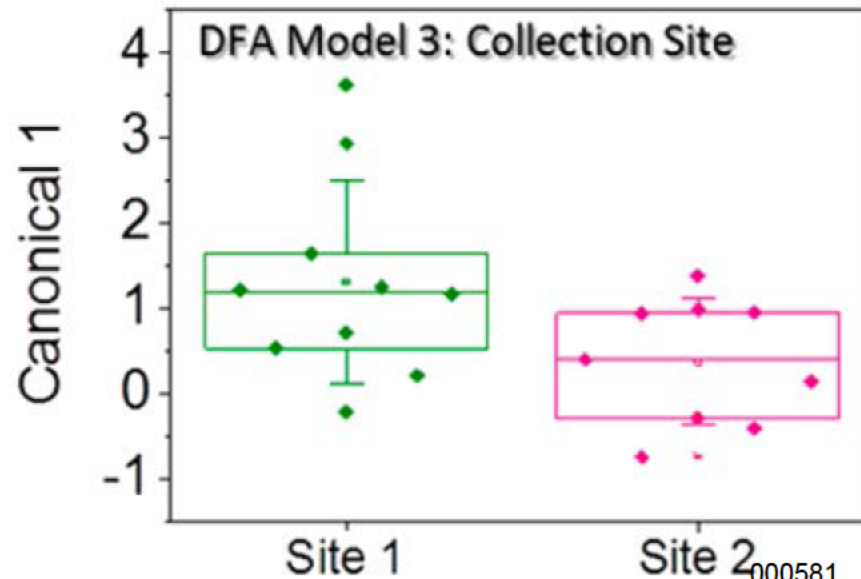


# Results-NaNose Serostatus



# Results-NaNose

## Collection Site



# Results

| DFA<br>Model | Target<br>Group                                    | Control<br>Group                                   | No. of<br>Animals | TN | FP | FN | TP | Se      | Sp      |
|--------------|--|--|-------------------|----|----|----|----|---------|---------|
| 1            | <b>Pos</b> Bison<br>CO and MT<br>April/May<br>2012 | <b>Neg</b> Bison<br>CO and MT<br>April/May<br>2012 | 38                | 18 | 2  | 5  | 13 | 72<br>% | 90<br>% |
| 2            | <b>Pos</b> Bison<br>MT<br>May 2012                 | <b>Neg</b> Bison<br>MT<br>May 2012                 | 21                | 8  | 3  | 1  | 9  | 90<br>% | 73<br>% |
| 3            | <b>Pos</b> Bison<br>MT<br>Sites 1 & 2<br>Jan 2014  | <b>Neg</b> Bison<br>MT<br>Sites 1 & 2<br>Jan 2014  | 20                | 9  | 1  | 1  | 8  | 89<br>% | 90<br>% |

# In Summary

- Good discrimination between seropositive and seronegative animals can be achieved.
- Environmental factors did not appear to affect the outcome of the models and were consistent over time.
- Results are supportive of further work





# Tools to Eliminate Brucellosis from Wildlife

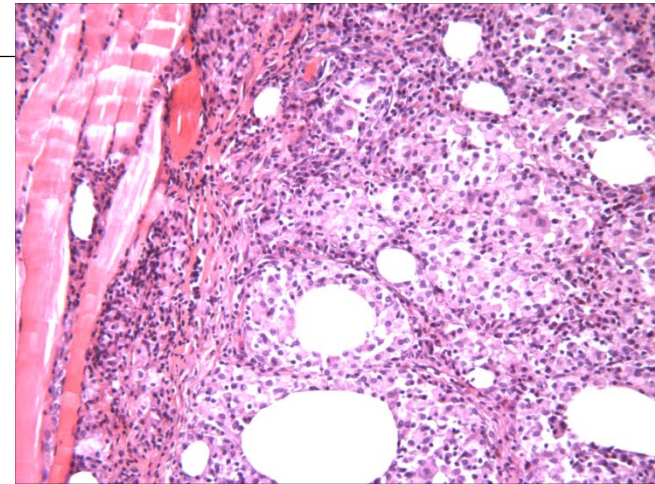


# Immunocontraception - GonaCon™

- GnRH is very **small** and is considered by the body as “**self**”-neither condition will lead to the production of antibodies
- The goal is to make GnRH look **large** and **foreign**
- This is done by linking GnRH to a large foreign protein found in the sea mollusk

# Immunocontraception

- GnRH (GonaCon™)
- Benefits
  - Single injection produces 2-3 yr+ sterility in most species tested
  - Animals are anestrus
  - Approved for use in wild deer and horses.
- Liabilities
  - 5-15% of deer become permanently sterile (5 years)
  - Injection site reactions in some species
  - Protection from a vaccine not 100%



# In Female Bison, Brucellosis is only transmitted if pregnancy occurs

- In over 300 captures, *Brucella abortus* was isolated from vagina, milk, blood, feces, & products of parturition.





# Pilot study: Idaho bison

- 2002 – 2008
- vaccinated 4-year-olds June 2002

|                 | 2002<br>pregs/dams | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 |
|-----------------|--------------------|------|------|------|------|------|------|
| controls        | 4/5                | 3/5  | 4/4  | 1/3  | 2/3  | 3/3  | 2/3  |
| Treat-<br>ments | 4/6                | 0/6  | 0/6  | 0/5  | 0/5  | 0/5  | 0/5  |

# Pilot study – Idaho bison

- Controls: 15 calves/21 reproductive years
- Treatments: 0 calves/32 reproductive years
- Animals in mid or late pregnancy when vaccinated had normal pregnancies

# Dose-response study

- 2003-2008
- Vaccinated virgin 2 year-olds May 2003

|           | 2004<br>pregs/dams | 2005 | 2006 | 2007 | 2008 |
|-----------|--------------------|------|------|------|------|
| Controls  | 0/5                | 5/5  | 4/5  | 2/3  | ND   |
| Low Dose  | 0/5                | 2/5  | 3/5  | 2/4  | ND   |
| Med Dose  | 0/5                | 3/5  | 2/5  | 0/2  | 0/1  |
| High Dose | 0/4                | 0/4  | 1/4  | 1/4  | 0/2  |

# Dose-response study

- Controls: 11 pregnancies/13 reproductive years
- Low dose: 7 pregnancies/14 reproductive years
- Med dose: 5 pregnancies/13 reproductive years
- High dose: 2 pregnancies/14 reproductive years



# Current Bison Contraception Studies

- Duration of infertility study in southern Colorado - 18 bison (Sand dunes)



000592

# GonaCon™ results: Southern Colorado herd

## Duration of Infertility

Number pregnant/number in group

|            | Nov 2011* | Nov 2012 | Nov 2013 | Nov 2014 |
|------------|-----------|----------|----------|----------|
| Treatments | 4/10      | 3/9      | 1/10     | 3/9      |
| Controls   | 4/10      | 9/9      | 6/9      | 9/9      |



# Corwin Springs contraceptive study

First group: 15 Controls; 15 GonaCon Vaccs

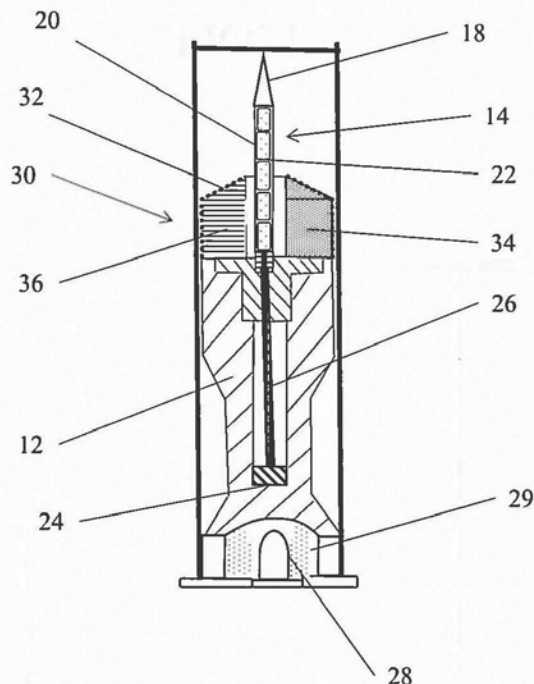
- 1<sup>st</sup> year: Controls-77% preg; vaccs-20% preg
- 2<sup>nd</sup> year: Controls-77% preg; vaccs-13% preg
- 3<sup>rd</sup> year: Controls-90% preg; vaccs-36% preg

Second group: 20 Controls; 20 GonaCon Vaccs

- 1<sup>st</sup> year: Controls-90% preg; Vaccs-5% preg
- Control pasture: 12 *Brucella* abortions; 3 normal calves born with significant shedding, 4/5 sentinels seroconverted and aborted once or twice; 8 calves seroconverted at 1 year.
- Vaccinated pastures: 0 *Brucella* abortions; 0 seroconversions

# DryDart™ Development

- Developing dart system to deliver lyophilized, powdered, pelleted, or encapsulated vaccines, at range, with accuracy.
- 4X the payload of biobullets; mark injection site.
- Fired from dart gun or shotgun; biodegradable.



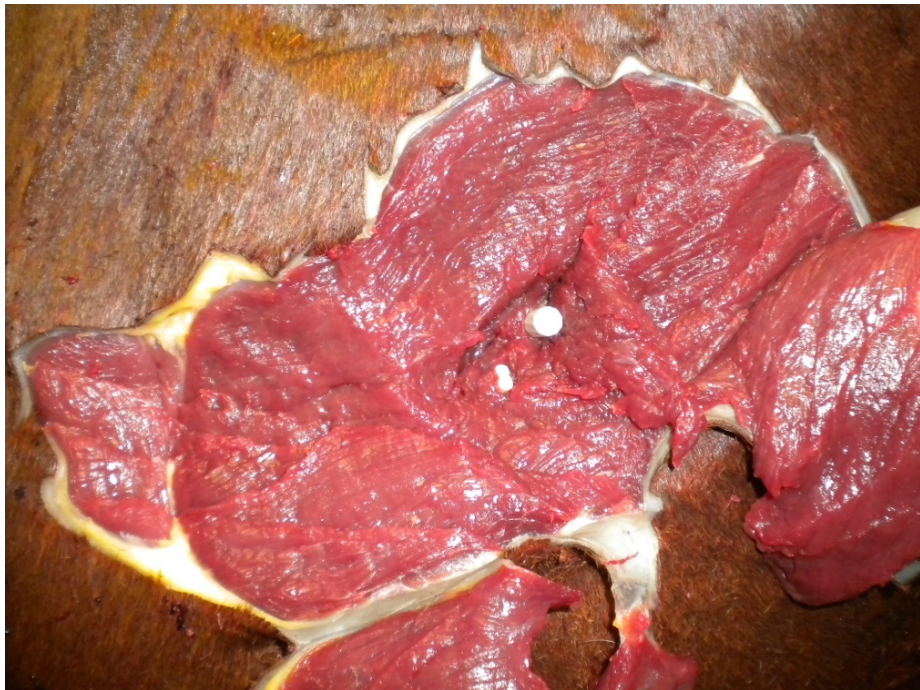


# DryDart™





# DryDart™

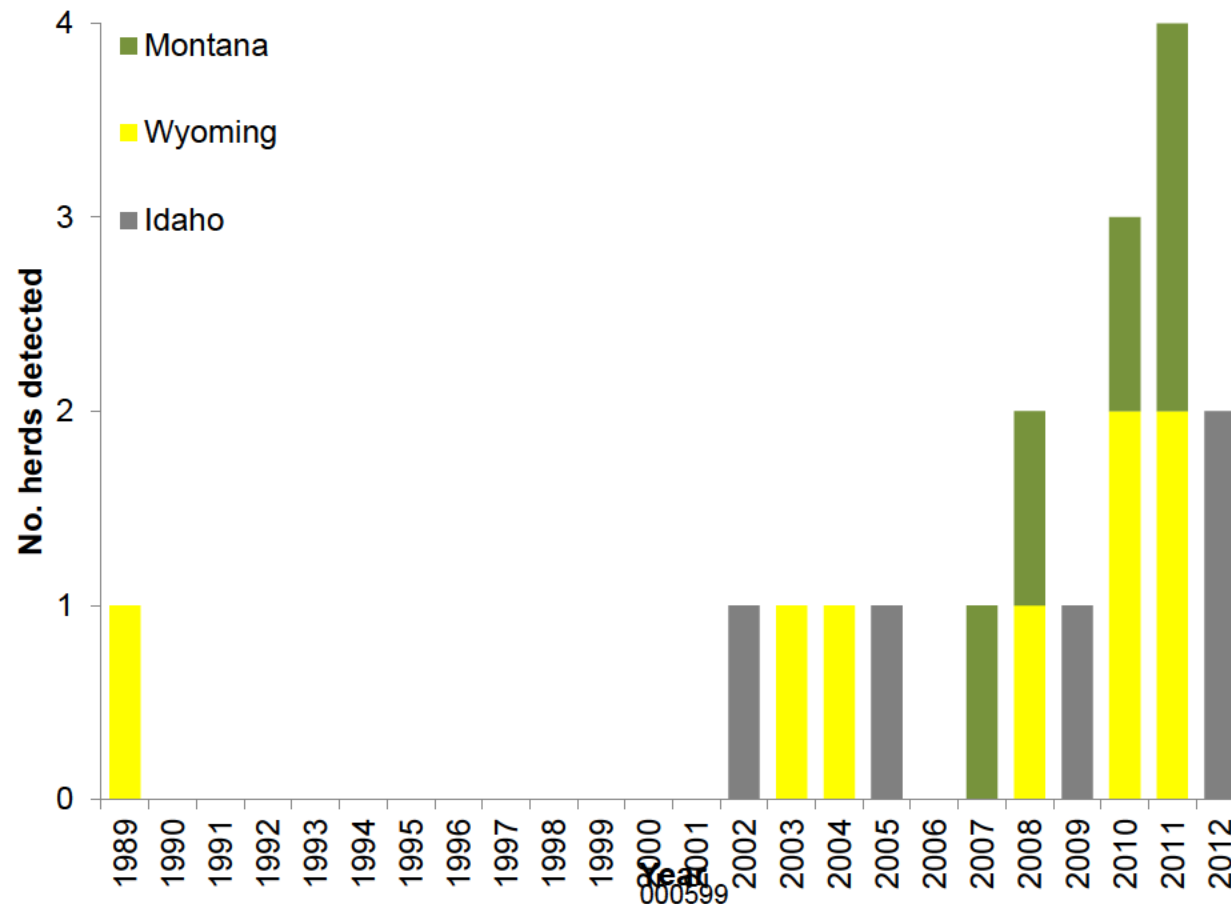


# Elk: Recent Increase of Brucellosis

- 1988-89: Case of brucellosis transmission from wildlife to cattle
- 1990-2001: No cases of transmission to cattle
- 2002-2013: 17 cases of transmission from elk to cattle and ranched bison



Figure 1. Number of *B. abortus*-positive domestic cattle and ranched bison herds (combined) detected each year between 1989 and 2012





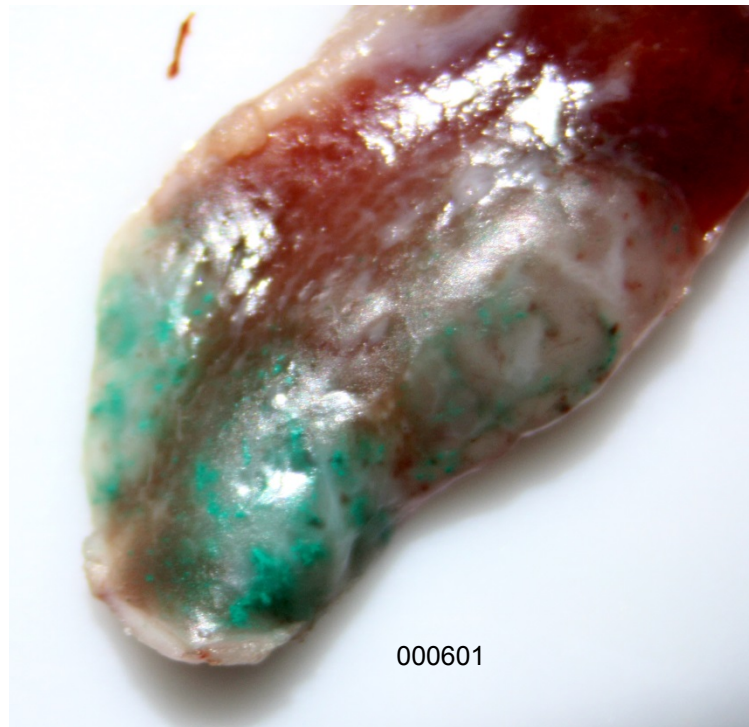
# Causes for Increase in Elk?

- Elk population increases
- Land use changes
- Wolves



# Vaccines: killed, spray-dried, finely powdered vaccine

- Goal: Develop spray-dried, killed, *B. abortus* vaccine for use on feedlines.



000601

# Ongoing Mouse Study – Powdered killed *B. abortus* complexed with montmorillonite clay

- If results are encouraging, proceed with elk study

# Using natural exposure as a challenge, a potential model for vaccine studies

- Goal: Develop vaccine model using natural exposure as challenge.
- 10 elk, 2 undiagnosed elk fetuses
- In 24 hours, 227 contacts of elk with fetuses



000603





# A Potential Plan to make Bovine Brucellosis a Foreign Animal Disease

- 20-30 year time frame
- Start with agencies and groups that agree
- Adaptive plan
- 5 phases:
  - Preparation phase – 1-2 years (meetings, budget planning, Public education, NEPA compliance, research, modeling, cost-benefit analysis)
  - Pilot study & vaccination phase – 5 years (bison vaccination, baseline surveillance, elk pilot studies, NEPA compliance, research, Public education)

## 5 phases (continued)

- Disease reduction phase – 10 years (decreasing transmission in elk and bison, NEPA compliance, Public education, research)
- Mop-up phase 5-10 years (bison: actions to eliminate transmission in last positive bison and elk, Public education, NEPA compliance)
- Surveillance phase – 5-10 years (continue monitoring)

# Nonlethal techniques to stop transmission - bison

- Remote vaccination of calves and yearlings
  - Test & treat
    - Field test (FPA, card)
    - Rx: 1. immunocontraception – 3+ years infertility
    - 2. therapeutic vaccination
    - 3. sustained release rifampin
- Booster in 3 years if needed
- Downside: requires hands-on bison work; requires animal ID

## Nonlethal techniques to stop transmission - elk

- Oral vaccination on feedgrounds and temporary bait stations
- Gradual incremental reduction and elimination of elk feedgrounds
  - Habitat improvement
  - Pilot studies with rigorous research
  - Vaccination and immunocontraception to mitigate against transmission to cattle
  - Elimination of feedgrounds would decrease transmission of other diseases, i.e., CWD



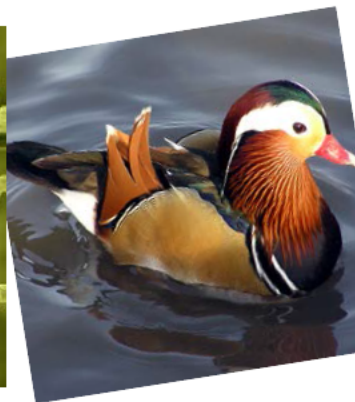


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# Questions?



# VS Wildlife Disease Group

## Wildlife/Livestock Disease Investigations Team/GYA Team (WiLDIT/GYA)

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

Pauline Nol  
Matt McCollum  
Karl Held  
Jack Rhyan  
Becky Frey  
Ryan Clarke





# Administrative History

- 1997 Dr. Arnoldi created position under WRD. Purpose to assist with GYA bruc and engage in “interface diseases” and “game farm” diseases.
- 1999 When Dr. Buish went to NVSL, Dr. Arnoldi put position under her supervision.
- Dr. Torres put position under Dr. Gilsdorf. Dr. Torres also directed group to begin work on FMD in wildlife.
- Currently WiLDIT and GYA Teams part of WR
- Collaborate with ARS, NWRC, CSU and others.



# Mission

- Serve APHIS/VS in area of wildlife/livestock interface and game farm diseases:
  - Serve as part of GYA Core Team to promote and achieve brucellosis elimination from GYA wildlife
  - Provide and disseminate knowledge of interface diseases to VS and others
  - Liaise with State and Federal agencies including wildlife and game farm authorities
  - Develop science-based solutions to disease problems at the wildlife/domestic animal interface

# 4 Activity Areas

- Consultation/liaison
- Developmental work (collaborating with ARS, NWRC, YNP, CSU, others)
- Diagnostics/  
surveillance
- Training



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# Major interface diseases engaged in since 2000

- Brucellosis in GYA wildlife and feral swine
- TB in deer
- CWD in cervids
- FMD threat in North American wildlife

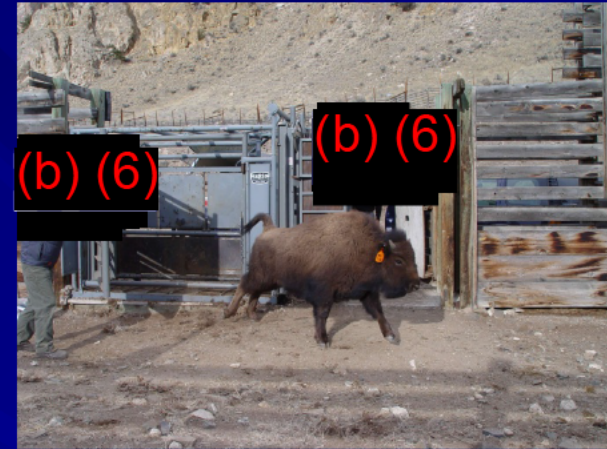


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# Current work brucellosis

- Work with YNP in brucellosis surveillance & vaccination
- Develop nonlethal strategies to eradicate brucellosis from GYA bison and elk
  - Bison: contraception, sustained release rifampin, therapeutic vaccination
  - Elk: oral recombinant RB51, contraception





# Current work-brucellosis

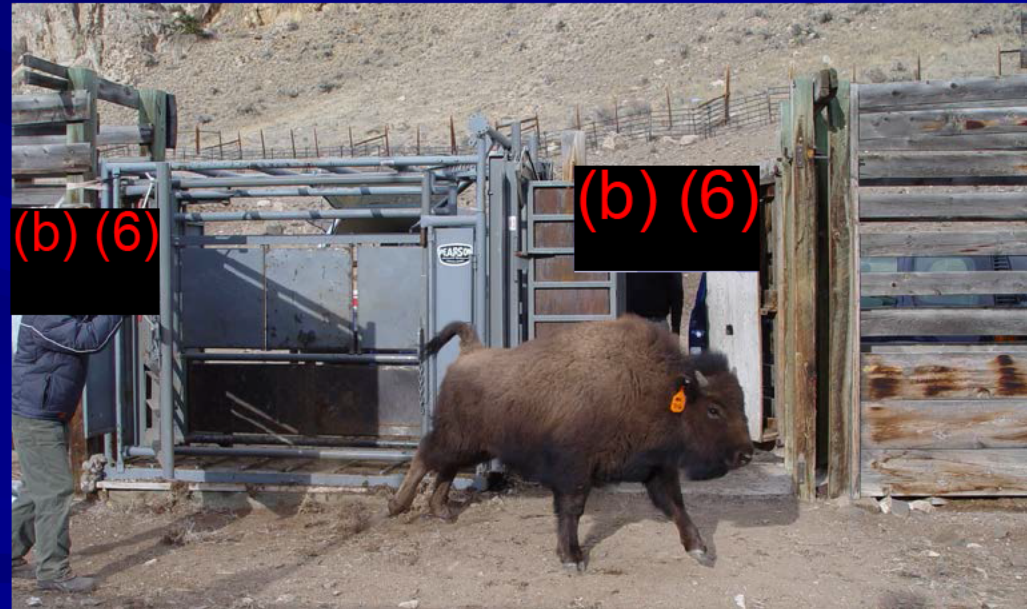
- Elk/bison/cattle: ecology of disease
- Bison: venereal transmission
- Feral swine: oral vaccination for *B. suis*
- Bison: detection of brucellosis by breath analysis for VOCs
- Multispecies: lipid antigens uniquely produced by individual *Brucella* spp.





# Bison Quarantine Feasibility Study (completed)

- Graduated 148 adults and offspring
- No latent infections detected





# First YNP calf born on Tribal land April 22, 2012



000618

# Contraception Studies

- Demonstrate alternatives to test and slaughter
- Principle: if we prevent pregnancy, we prevent shedding and transmission
- Ongoing studies should evaluate
  - (1) duration of infertility following single injection
  - (2) efficacy in prevention of *B. abortus* shedding

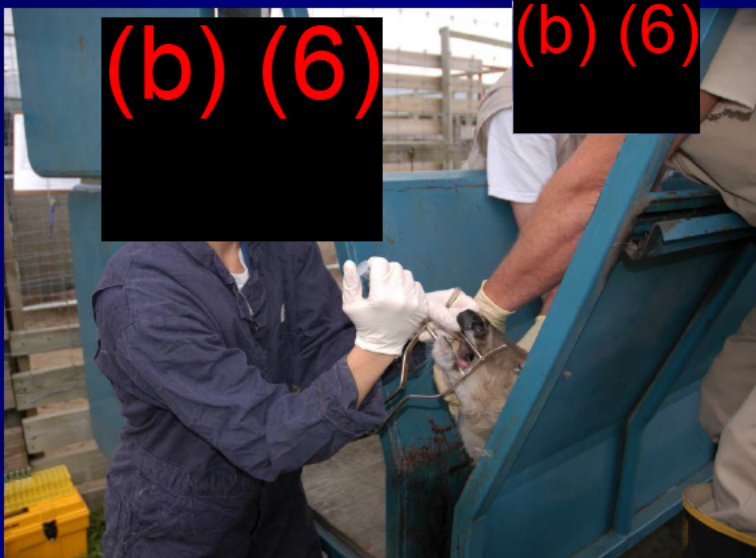


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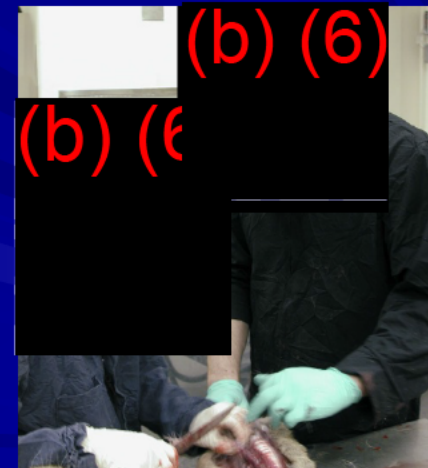
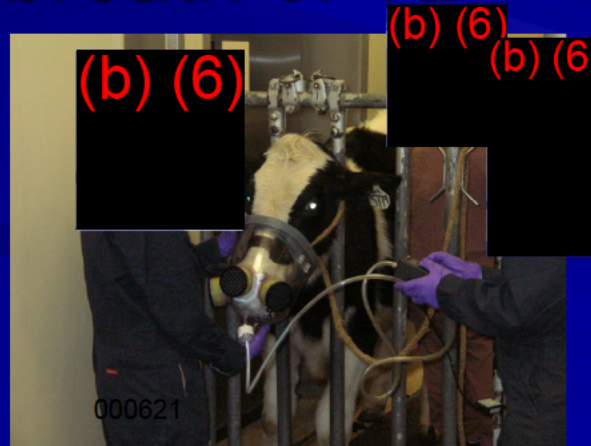
# Current TB Work

- Development of oral TB vaccines for eventual field trial in Michigan deer and Molokai feral pigs
- Detection of VOCs for the screening/diagnosis of TB in wildlife and livestock



# TB Results

- Oral BCG vaccine shown efficacious in white-tailed deer and wild boar (IREC-Spain)
- Current studies evaluating safety and tissue clearance
- Unique VOCs and VOC profiles demonstrated in breath of TB infected cattle



# FMD Susceptibility & Transmission in North American Wildlife

- Experimental infections in bison, elk, pronghorn, & mule deer.

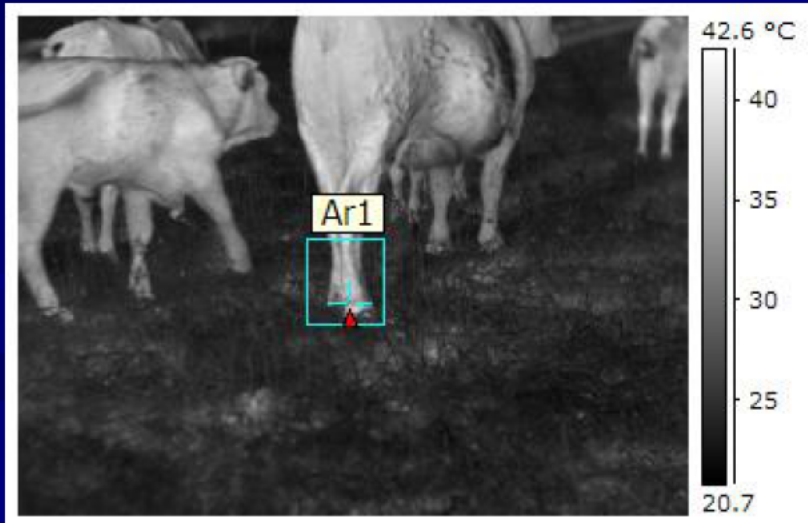




# Current/Future Work – Wildlife Disease Surveillance and Control

## ■ Development and demonstration of techniques for wildlife disease surveillance

- Use of drones for census and disease detection
- Thermal imaging to conduct census and detect febrile animals





# Current/Future Work – Feral Swine

- Techniques for feral swine (develop and prove 4 point program using temporary feeding sites)
  - Implement feeding sites to keep feral swine from leaving area
  - Motion-triggered infrared imaging at feeder site for disease monitoring (fever or lesions)
  - Detect disease-specific VOCs
  - Using pig-specific feeder: kill, contraceptive or vaccinate (*B. suis*, TB, swine flu, CSF, FMD, etc.)



# Hockaday/Swanson Wildlife Research Facility (APHI/APHIS Wildlife Pens)



- On CSU land south of NWRC – shared between APHIS and CSU-APHI
- Built primarily with end-of-year \$\$, grant money, and excess property
- Currently housing bison, feral pigs, and white-tailed deer







United States Department of Agriculture



United States Department of Agriculture

# Developing Monitoring and Management Tools for Diseases at the Wildlife/Livestock Interface

Pauline Nol DVM, MS, PhD

U.S. Department of Agriculture  
Animal and Plant Health Inspection Service  
Veterinary Services  
Science, Technology, and Analysis Services

September 5, 2017

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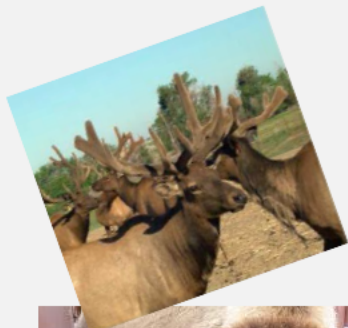
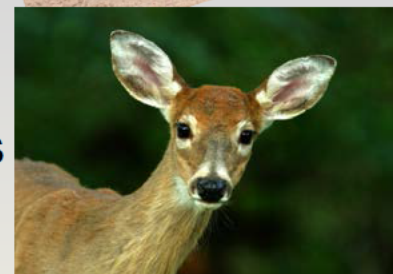


Photo: Brandon Schmidt



www.dowild.com

“The key to developing effective tools for the management of disease involving wildlife is the sufficient understanding of conditions required for the persistence of pathogens”

Delahey et al., *in* Management of Diseases in Wild Mammals, 2009

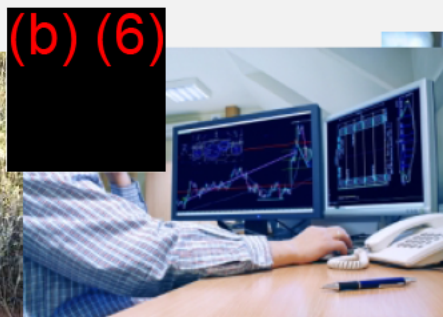




[www.energysafe.ru](http://www.energysafe.ru)



<http://www.thespectrum.com/>

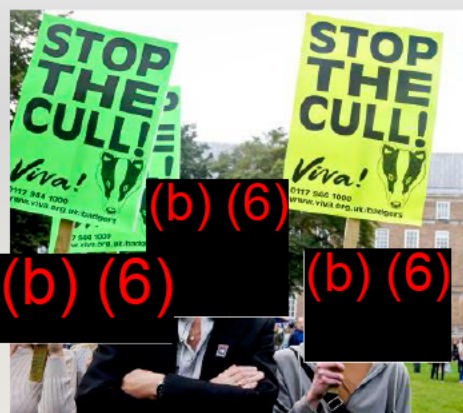


<http://www.jpstechnologies.com/>



<http://career.iresearchnet.com/>

# Management of wildlife diseases requires a multidisciplinary approach!!



[www.energysafe.ru](http://www.energysafe.ru)



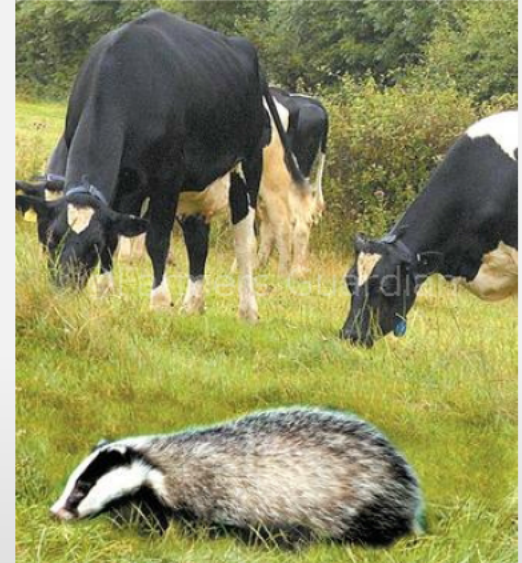
[www.energysafe.ru](http://www.energysafe.ru) 000628



<https://blackgirlnerds.com/>

# What do we need to know?

- Presence/Absence?
- Significance?
- Species involved?
- Reservoir species?
- Distribution?
- Ecology of involved species?
- Ecology of disease/Mode of transmission?
- Risk factors?



# Ideal Tools for Wildlife Disease Monitoring and Management

- Efficient
- Adequate/appropriate population coverage
- Cost effective
- Acceptable to public

## **Innovative!**

# Veterinary Services Developmental Work

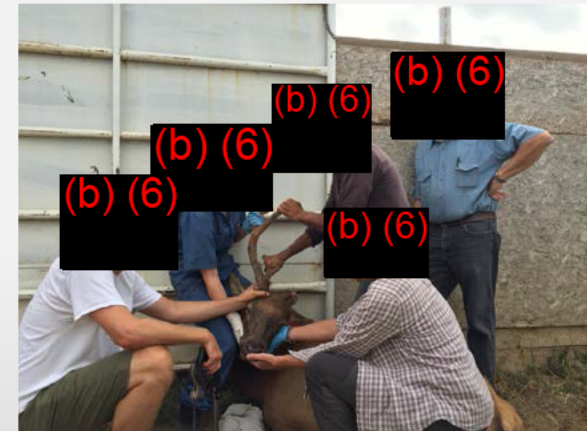
## Detection

Volatile organic compounds

- Oral fluids

## Management

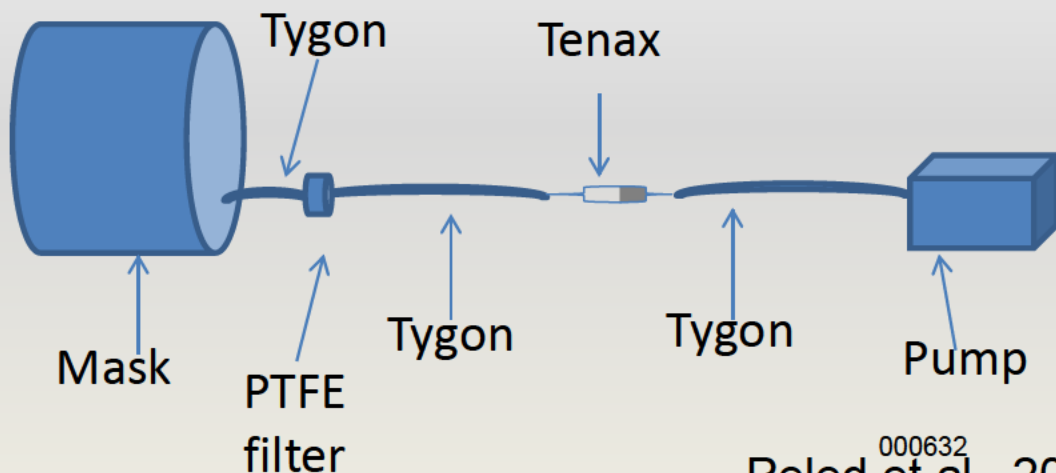
- Vaccine and vaccine delivery
- Immunocontraception
- Quarantine
- Assisted Reproduction

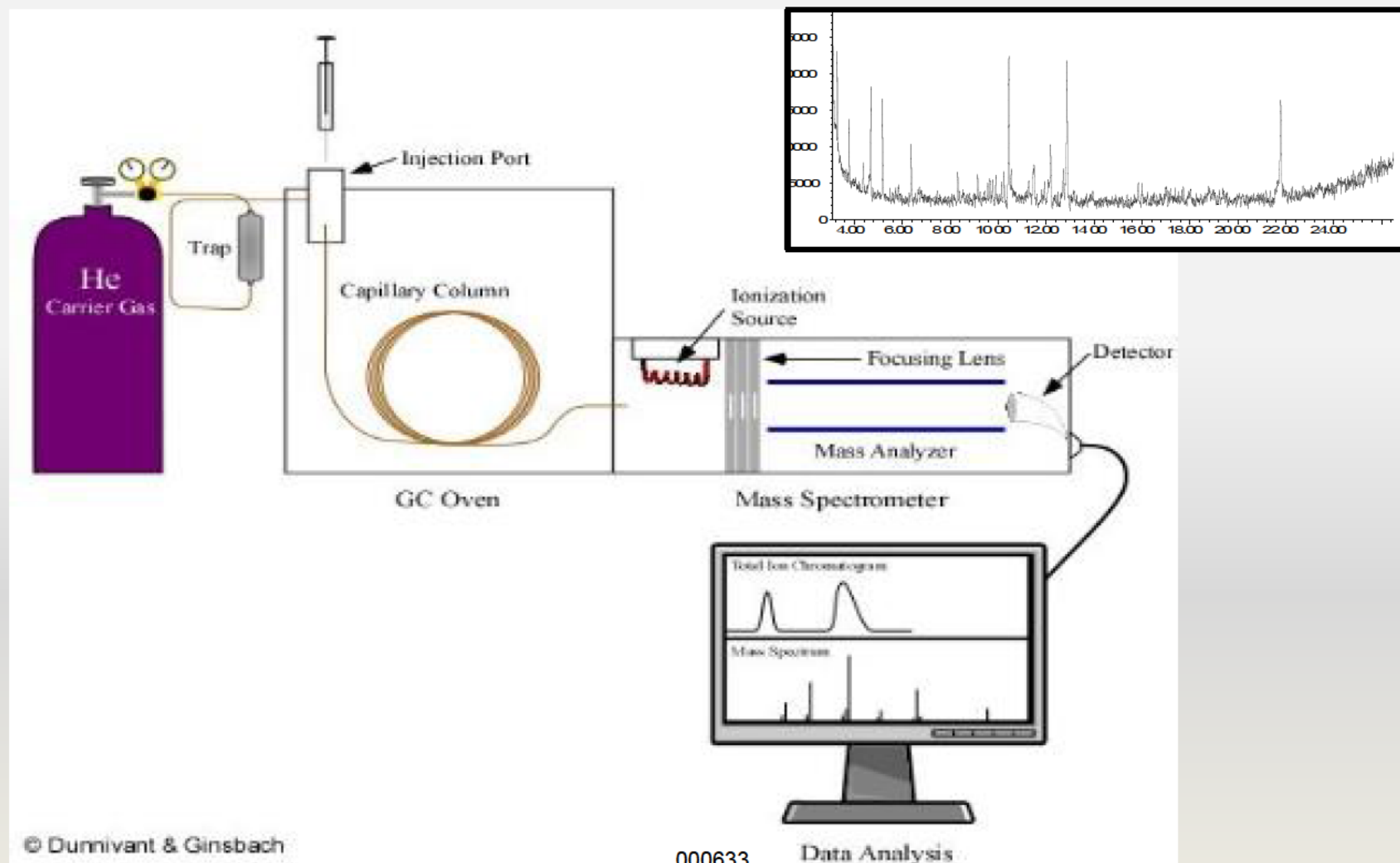


Wildlife Livestock Disease  
Investigations Team



## Volatile organic compounds





## Oral fluids

Field trials with  
Swineapples in wild  
pigs in Florida and  
Georgia, USA



Captive trials  
with elk using  
paint rollers  
and mineral

Non-target species using  
Swineapples

Swineapple





## Vaccination

- Dry Dart
  - Remote delivery of vaccine
- Mucosal vaccination
  - killed, powdered vaccine
  - Oral vaccine incorporated into baits



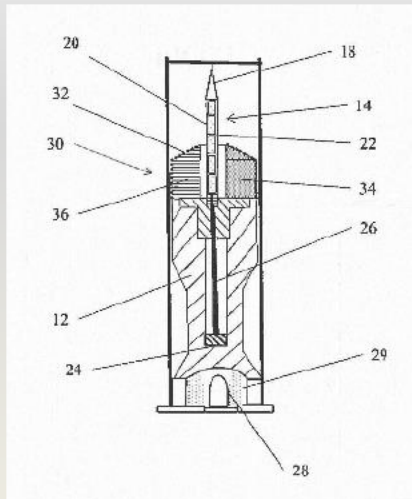
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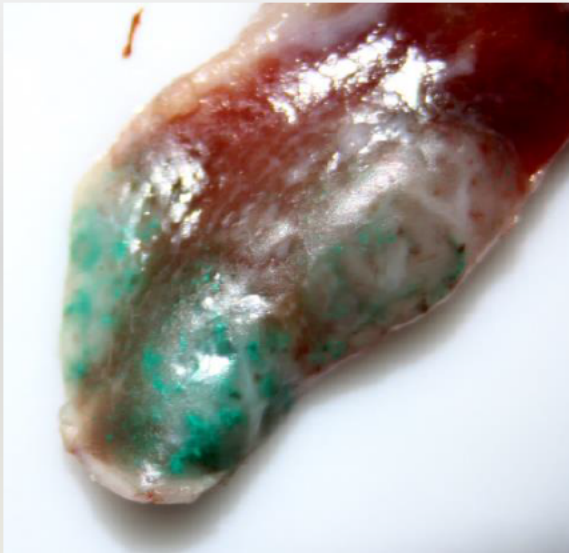
## DryDart™

- Dart system to deliver lyophilized, powdered, pelleted, or encapsulated vaccines
- 2X the payload of biobullets
- Marks injection site.
- Fired from dart gun or shotgun
- Biodegradable.



# Mucosal vaccination

- Powdered, killed vaccine
- Killed *Brucella abortus* vaccine for use on elk feedlines.



Right parotid lymph node with colored clay after intranasal delivery into left nasal sinus

# Mucosal vaccination

- Killed *Mycobacterium bovis* vaccine for use in feral swine
- Oral BCG in white-tailed deer

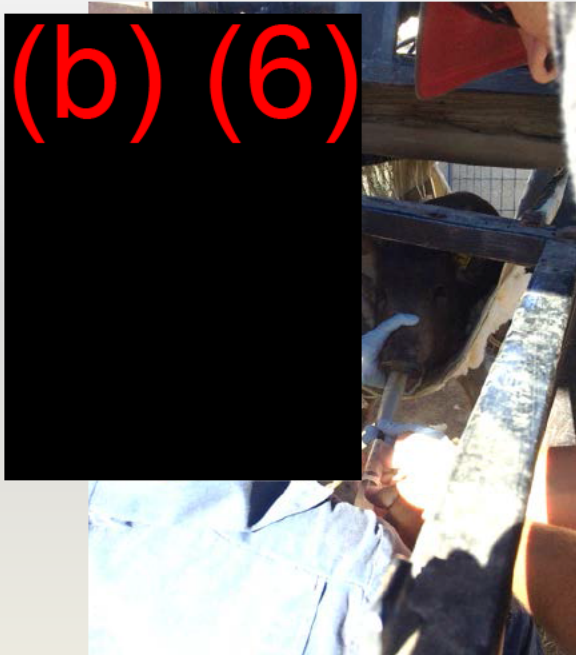


Photo by James Fosse

## Immunocontraception



- Tool to mitigate brucellosis
- GonaCon<sup>TM</sup> (GnRH-based vaccine)
- Prevents pregnancy in bison (~60%)
- Non pregnant bison cows do not transmit *B. abortus*



# Assisted Reproduction for Bison Conservation

- Optimization of embryo transfer and *in vitro* fertilization technologies in bison
- Allows preservation of valuable bison genetics

Dr. Jennifer Barfield, Department of Biomedical Sciences,  
Colorado State University



## Quarantine for Bison Conservation

- Establishment of brucellosis-free bison herds of Yellowstone genetics outside of the GYA



# Thanks to the Many, Many Collaborators!!!

## Immunocontraception

APHIS Veterinary Services  
APHIS Wildlife Services  
USGS  
National Park Service  
Nature Conservancy  
Zapata Ranch

## Oral Fluids

University of Florida  
University of Georgia  
Colorado State University

## Quarantine

APHIS Veterinary Services  
Montana Dept. Agriculture  
Montana Fish Wildlife and Parks  
National Park Service

## Vaccines

USDA Agricultural Research Services  
IREC, University of la Mancha, Spain  
Colorado State University  
APHIS Veterinary Services  
Virginia Tech  
Otago Innovations

## VOCs

Rovira i Virgili University, Spain  
IREC, University of la Mancha, Spain  
Technion University, Israel  
APHIS Wildlife Services  
USDA Agricultural Research Services  
APHIS Veterinary Services

## Assisted Reproduction

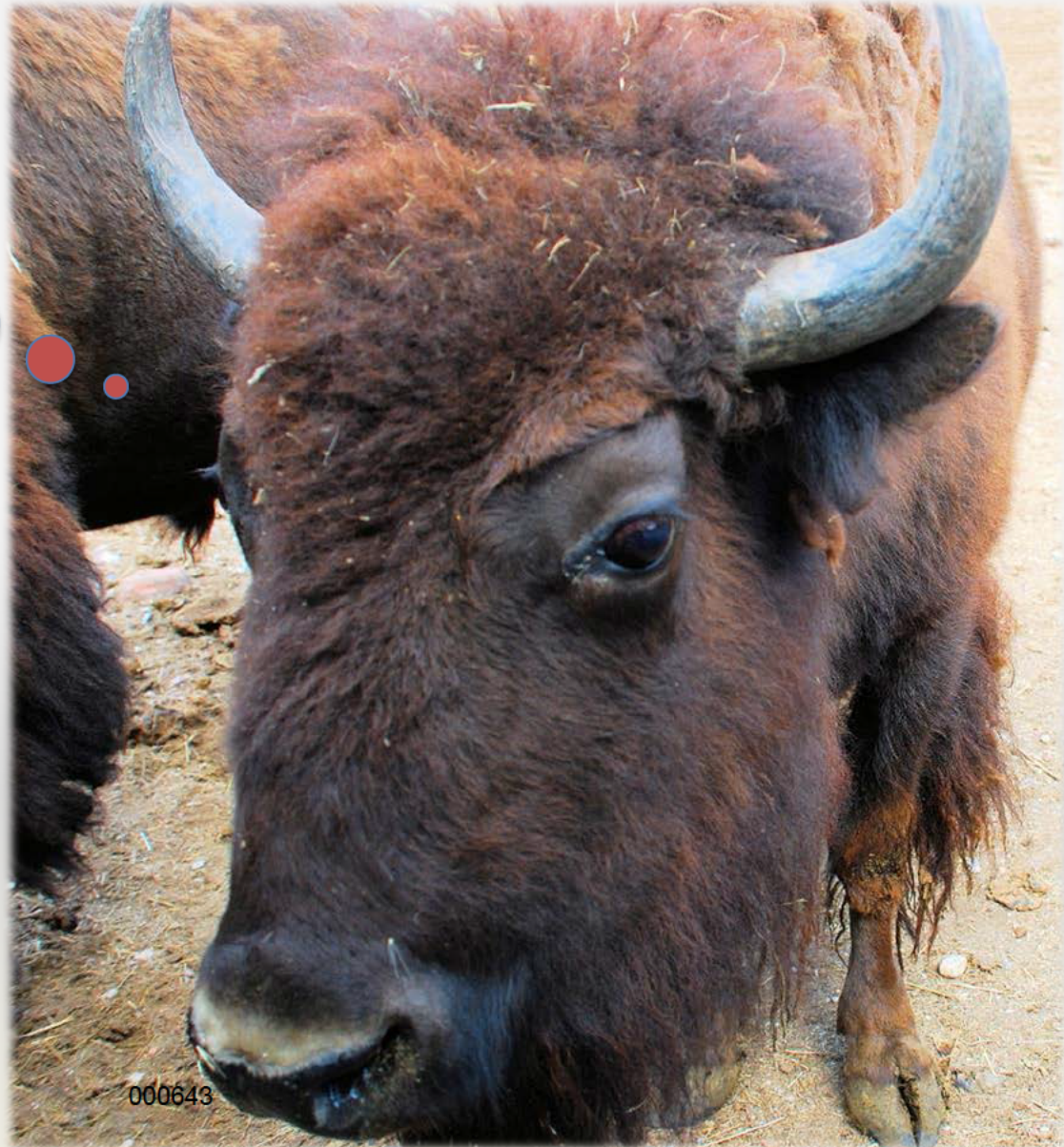
Colorado State University  
Greg Farrand, DVM  
Wildlife Conservation Society





United States Department of Agriculture

Questions?





# Wildlife/Livestock Disease Investigations Team (WiLDIT)

“Developing science-based solutions to disease problems at the wildlife/domestic animal/human interface”

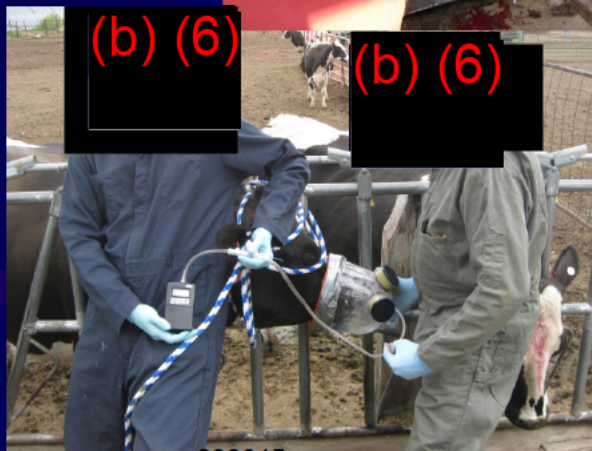
Pauline Nol  
Matt McCollum  
Karl Held  
Jack Rhyan



# WiLDIT Activity Areas

- ❖ Developmental work
- ❖ Diagnostics/  
surveillance
- ❖ Consultation/liaison

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# WiLDIT Research Facilities

- ❖ APHI/APHIS Wildlife Research Facility
  - Fort Collins, CO

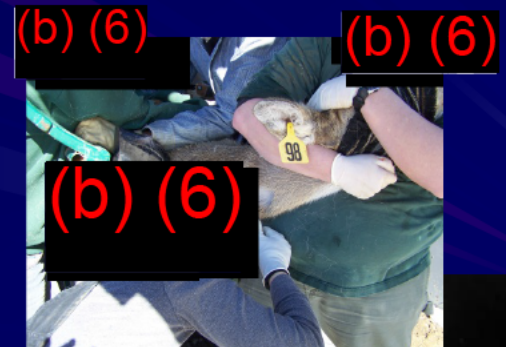


- ❖ Bison Quarantine Facility
  - Corwin Springs, MT



# Major interface diseases engaged in since 2000

- ❖ Brucellosis in GYA and swine
- ❖ TB in deer and feral swine
- ❖ CWD in cervids
- ❖ FMD threat in North American wildlife





# Bison Quarantine Feasibility Study

- ❖ Graduated 148 adults and offspring
- ❖ No latent infections detected on 3 annual tests on first group and 1 test on second group



# WiLDI Brucellosis in the GYA

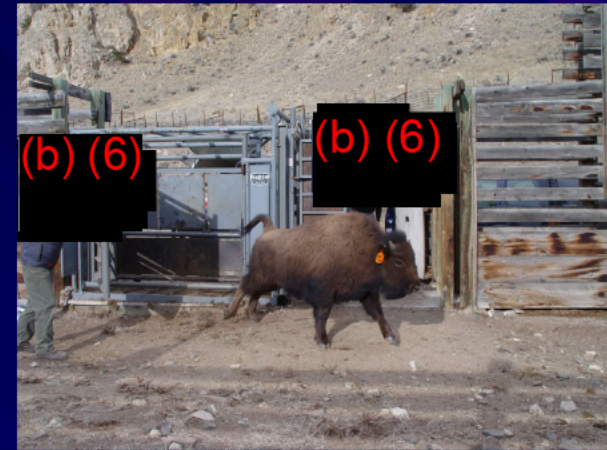
Develop nonlethal strategies to eradicate brucellosis from GYA bison and elk

## ❖ Bison

- Contraception
- Sustained release antibiotics
- Therapeutic vaccination

## ❖ Elk

- Vaccine
- Contraception



# WiLDIT-Brucellosis

- ❖ Elk/bison/cattle: ecology of disease
- ❖ Bison: venereal transmission
- ❖ Feral swine: oral vaccination for *B. suis*
- ❖ Diagnostics
  - Bison: detection of brucellosis by breath analysis for VOCs
  - Multispecies: lipid antigens uniquely produced by individual *Brucella* spp.



# WiLDIT Bovine TB Work

- ❖ Development of oral TB vaccines:
  - Michigan deer
  - Molokai feral pigs
- ❖ Detection of VOCs for the screening/diagnosis of TB in wildlife and livestock





# FMD Susceptibility & Transmission in North American Wildlife

- Experimental infections in bison, elk, pronghorn, & mule deer.



# Current/Future Work – Wildlife Disease Surveillance and Control

## ❖ Development and demonstration of techniques for wildlife disease surveillance

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# Wildlife/Livestock Disease Investigations Team (WiLDIT)

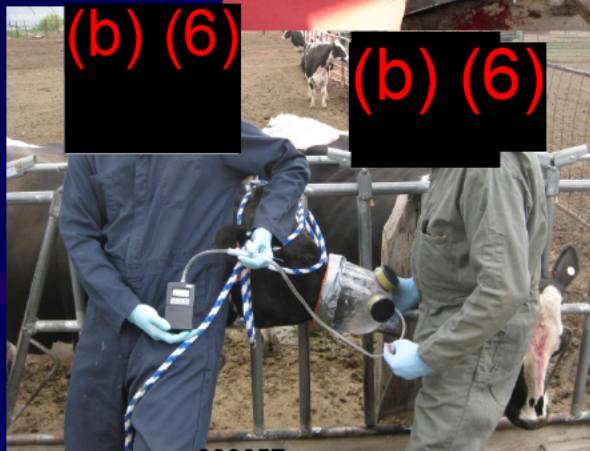
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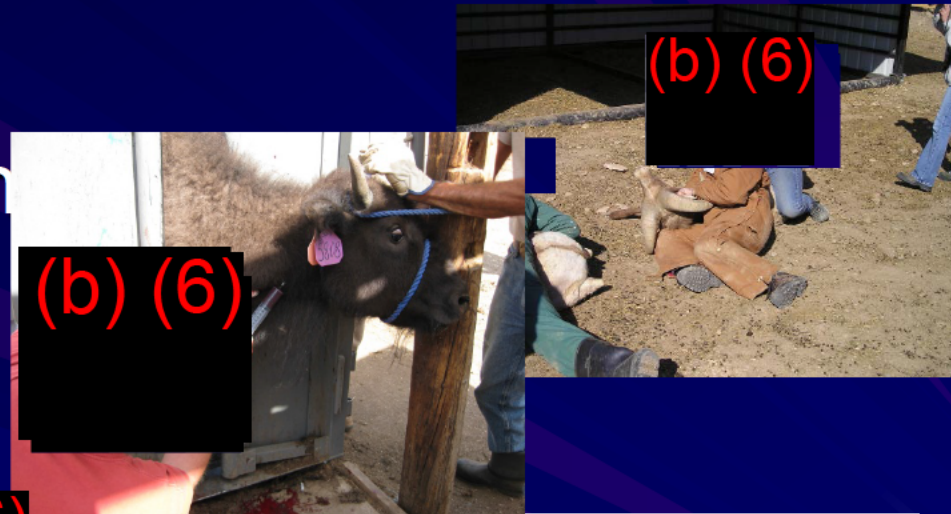


# WiLDIT Activity Areas

- ❖ Developmental work
- ❖ Diagnostics/  
surveillance
- ❖ Consultation/liaison
- ❖ Training



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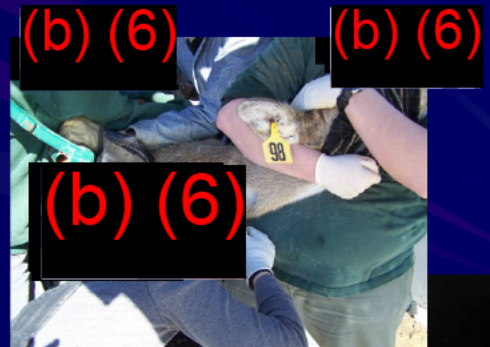


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- Therapeutic vaccination

## ❖ Elk

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- Contraception



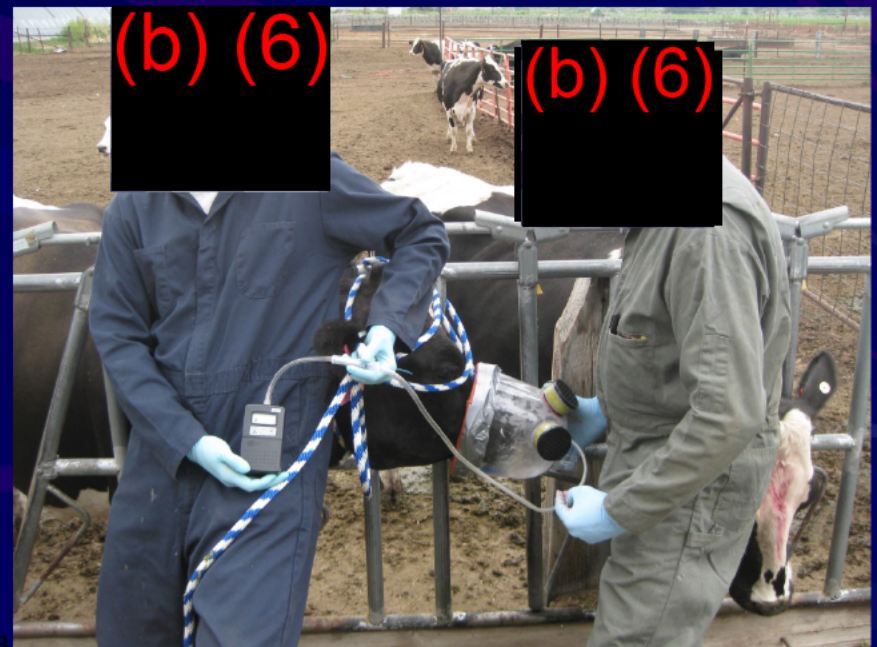
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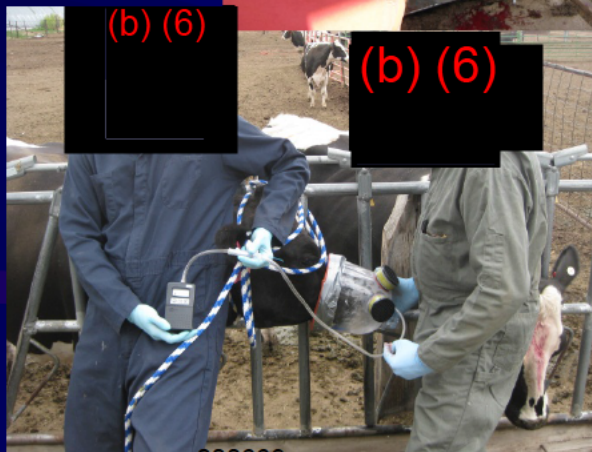
“Developing science-based solutions to disease problems at the wildlife/domestic animal interface”

Pauline Nol  
Matt McCollum  
Karl Held  
Jack Rhyan



# WiLDIT Activity Areas

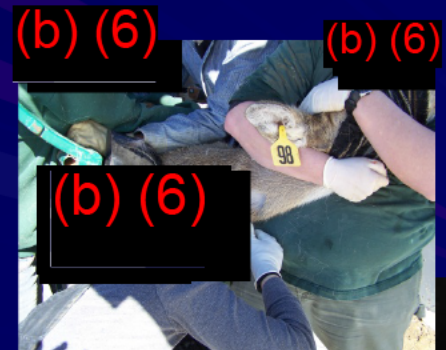
- Developmental work (collaborating with ARS, NWRC, YNP, CSU, others)
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- Training





# Major interface diseases engaged in since 2000

- Brucellosis in Greater Yellowstone Area wildlife
- Brucellosis in feral swine
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- FMD threat in North American wildlife

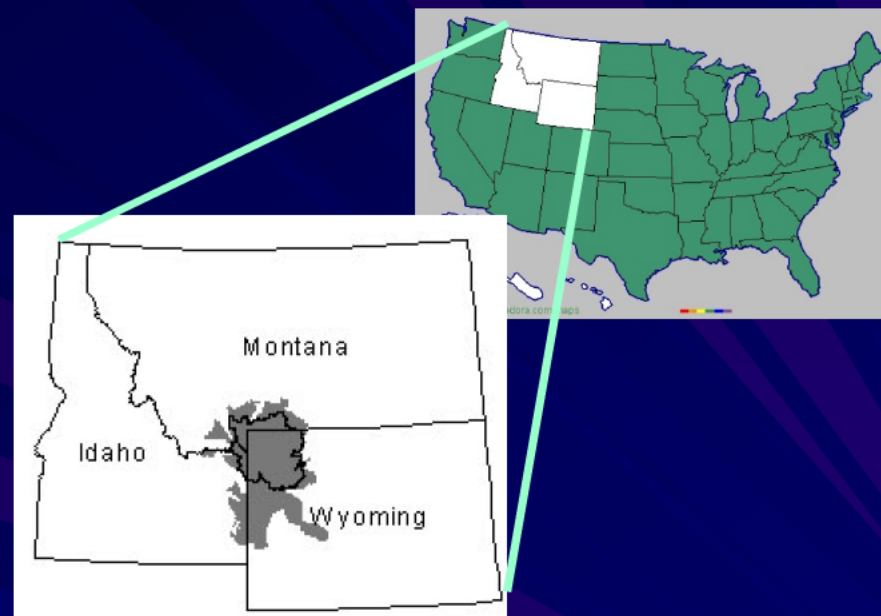


# Brucellosis in Greater Yellowstone Area

## Elk and Bison



- Introduced early 1900's
- Bison: 50-80% seropositive
- Elk: 3.7-40% seropositive
- Bison: *B. abortus* biovars 1 and 2
- Elk: *B. abortus* biovars 1 and 4
- Elk implicated in most cattle herd cases





# Brucellosis in Greater Yellowstone Area Elk and Bison

## Yellowstone Bison Quarantine Feasibility Study

- Determine feasibility, efficacy, and associated risks of using the USDA's Uniform Methods & Rules to Eradicate Brucellosis protocol to qualify bison from Yellowstone National Park as free of brucellosis
- 2005-2012: Graduated 148 adult bison from quarantine



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# WiLDI Brucellosis in the GYA

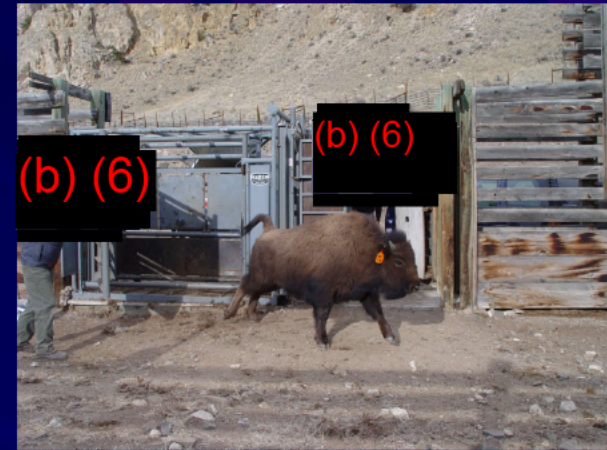
Develop nonlethal strategies to eradicate brucellosis from GYA bison and elk

## ■ Bison

- Contraception
- Sustained release antibiotics
- Therapeutic vaccination

## ■ Elk

- Vaccine
- Contraception



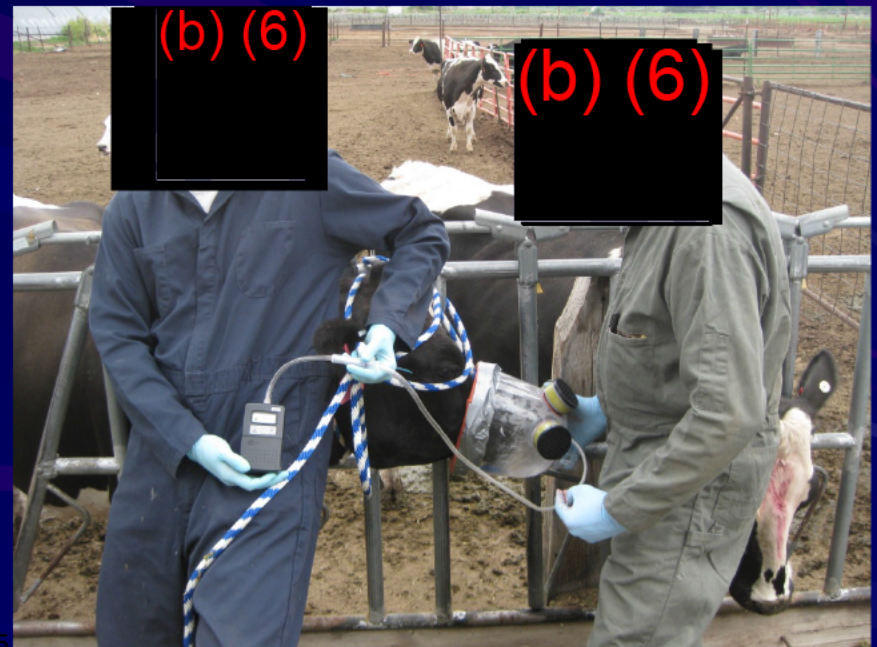
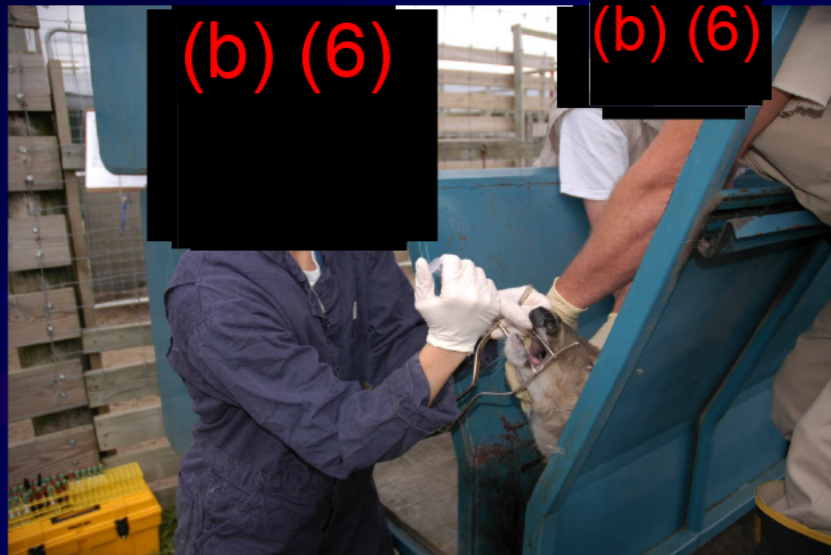
# WiLDIT-Brucellosis

- Elk/bison/cattle: ecology of disease
- Bison: venereal transmission
- Feral swine: oral vaccination for *B. suis*
- Diagnostics
  - Bison: detection of brucellosis by breath analysis for VOCs
  - Multispecies: lipid antigens uniquely produced by individual *Brucella* spp.



# WiLDIT Bovine TB Work

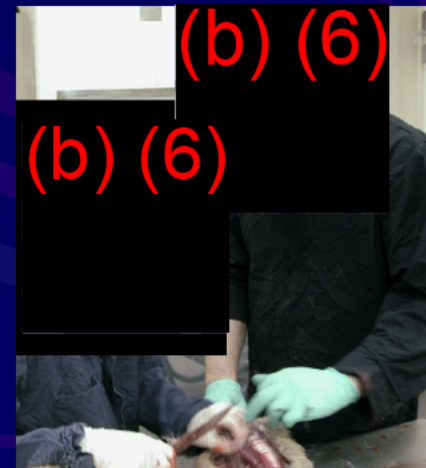
- Development of oral TB vaccines:
  - Michigan deer
  - Molokai feral pigs
- Detection of VOCs for the screening/diagnosis of TB in wildlife and livestock





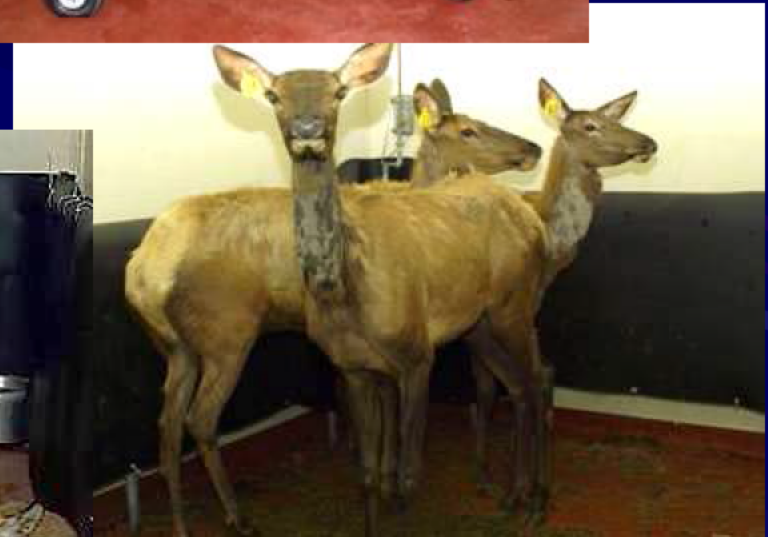
# TB Results

- Oral BCG vaccine shown efficacious in white-tailed deer and wild boar (IREC-Spain)
- Current studies evaluating safety and tissue clearance
- Unique VOCs and VOC profiles demonstrated in breath of TB infected cattle



# FMD Susceptibility & Transmission in North American Wildlife

- Experimental infections in bison, elk, pronghorn, & mule deer.



# Current/Future Work – Wildlife Disease Surveillance and Control

## ■ Development and demonstration of techniques for wildlife disease surveillance

- Use of drones for census and disease detection
- Thermal imaging to conduct census and detect febrile animals





# Current/Future Work – Feral Swine

- Techniques for feral swine (develop and prove 4 point program using temporary feeding sites)
  - Implement feeding sites to keep feral swine from leaving area
  - Motion-triggered infrared imaging at feeder site for disease monitoring (fever or lesions)
  - Detect disease-specific VOCs
  - Using pig-specific feeder: kill, contraceptive or vaccinate (*B. suis*, TB, swine flu, CSF, FMD, etc.)





# APHI/APHIS Wildlife Research Facility



- On CSU land south of NWRC – shared between APHIS and CSU-APHI
- Currently housing bison and feral pigs



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# Wildlife/Livestock Disease Investigations Team Team (WiLDIT)

USDA APHIS Veterinary Services

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

Jack Rhyan  
Matt McCollum  
Karl Held  
Pauline Nol



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# WiLDIT Activity Areas

- Developmental work (collaborating with ARS, NWRC, YNP, CSU, others)
- Diagnostics/surveillance
- Consultation/liaison
- Training

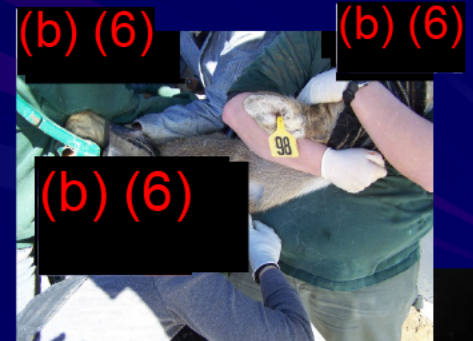


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# WiLDIT Diseases Focus

- Brucellosis in Greater Yellowstone Area wildlife
- Brucellosis in feral swine
- TB in deer and feral swine
- CWD in cervids
- FMD threat in North American wildlife

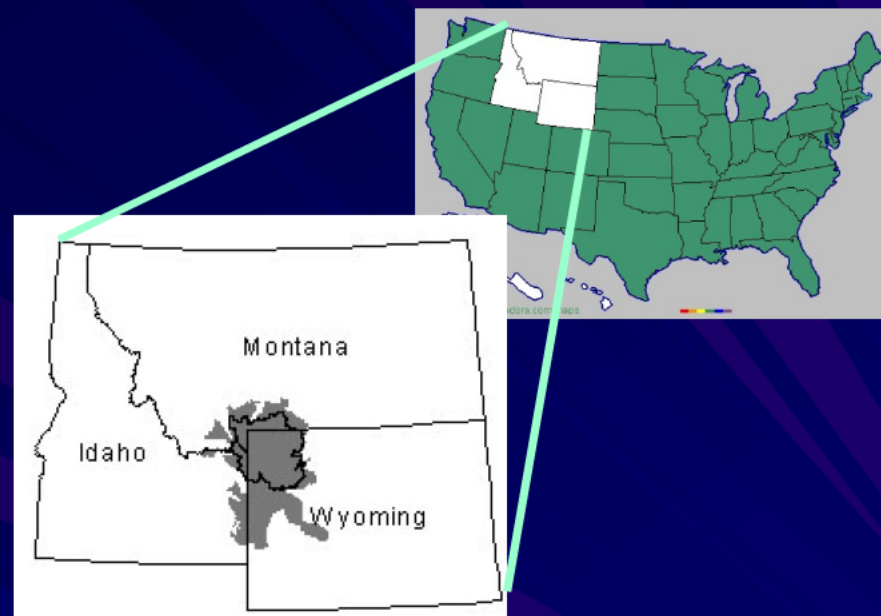




# Brucellosis in Greater Yellowstone Area Elk and Bison



- Introduced early 1900's
- Bison: 50-80% seropositive
- Elk: 3.7-40% seropositive
- Bison: *B. abortus* biovars 1 and 2
- Elk: *B. abortus* biovars 1 and 4
- Elk implicated in most cattle herd cases



# Brucellosis in Wildlife



# Brucellosis in Greater Yellowstone Area Elk and Bison

## Yellowstone Bison Quarantine Feasibility Study

- Determine feasibility, efficacy, and associated risks of using the USDA's Uniform Methods & Rules to Eradicate Brucellosis protocol to qualify bison from Yellowstone National Park as free of brucellosis
- 2005-2012: Graduated 148 adult bison from quarantine



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# WiLDI Brucellosis in the GYA

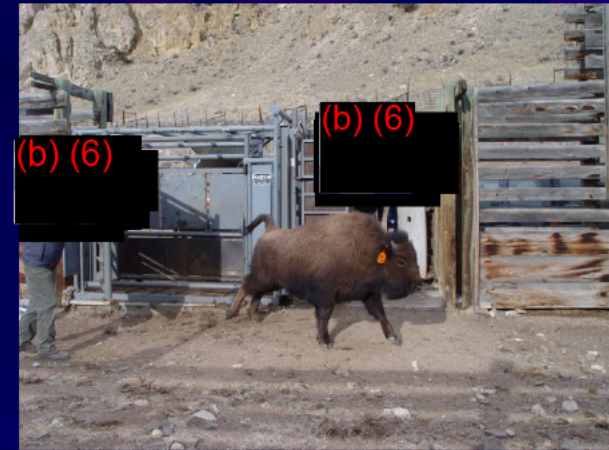
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## ■ Elk

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# WiLDIT Bison Contraception Studies

- Does use of contraceptive vaccine decrease shedding of *B. abortus*? Montana-44 bison
- Duration of infertility southern Colorado - 20 bison

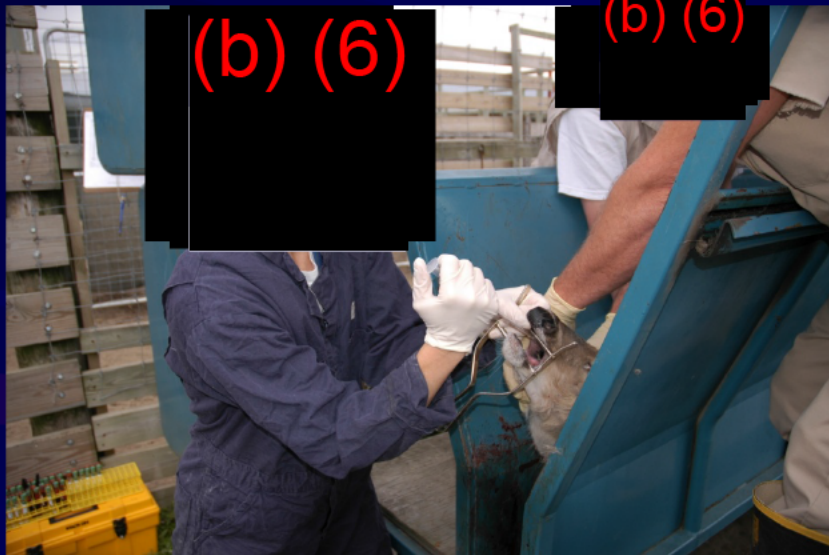


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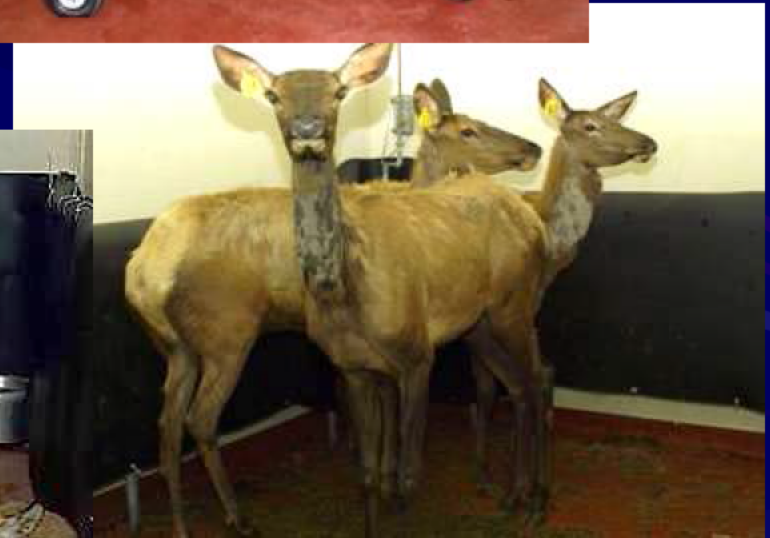
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