Surveillance of Johne's Disease in Northern New York Goat Herds

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Study Design

Goal 1

- **Describe** the goat herds of the region
  - Gather information from as many producers as possible via online survey
  - Distribute with NYSDAM, CCE and collaborating veterinarians

Goal 2

- **Identify** interested herds
  - Include questions about clinical signs and risk factors in online survey
  - Identify producers that are willing to participate in study with big enough herds

Goal 3

- **Perform** surveillance testing for Johne's disease
  - Sample participating herds for Johne's with blood and fecal samples
  - Include farms on NYSSGHAP and those with ≥10 does ≥2 years

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What Is Johne's Disease?

- A chronic enteric wasting disease of ruminants similar to Tuberculosis
  - Causing thickening of the GI track
  - Decreased absorption of nutrients
- Caused by *Mycobacterium avium subsp. paratuberculosis* (**MAP**)
- Infection most commonly establishes after exposure when young
  - Organism shed in feces by animals without symptoms
- Non-specific symptoms
- Long period before symptomatic (if ever)
- May shed organism even without clinical signs

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Why are goats at high risk for Johne's disease?

- Pasture Utilization
- Movement of bucks or other breeding stock
- Exposure of youngstock to adults
- Testing Cost and Logistics

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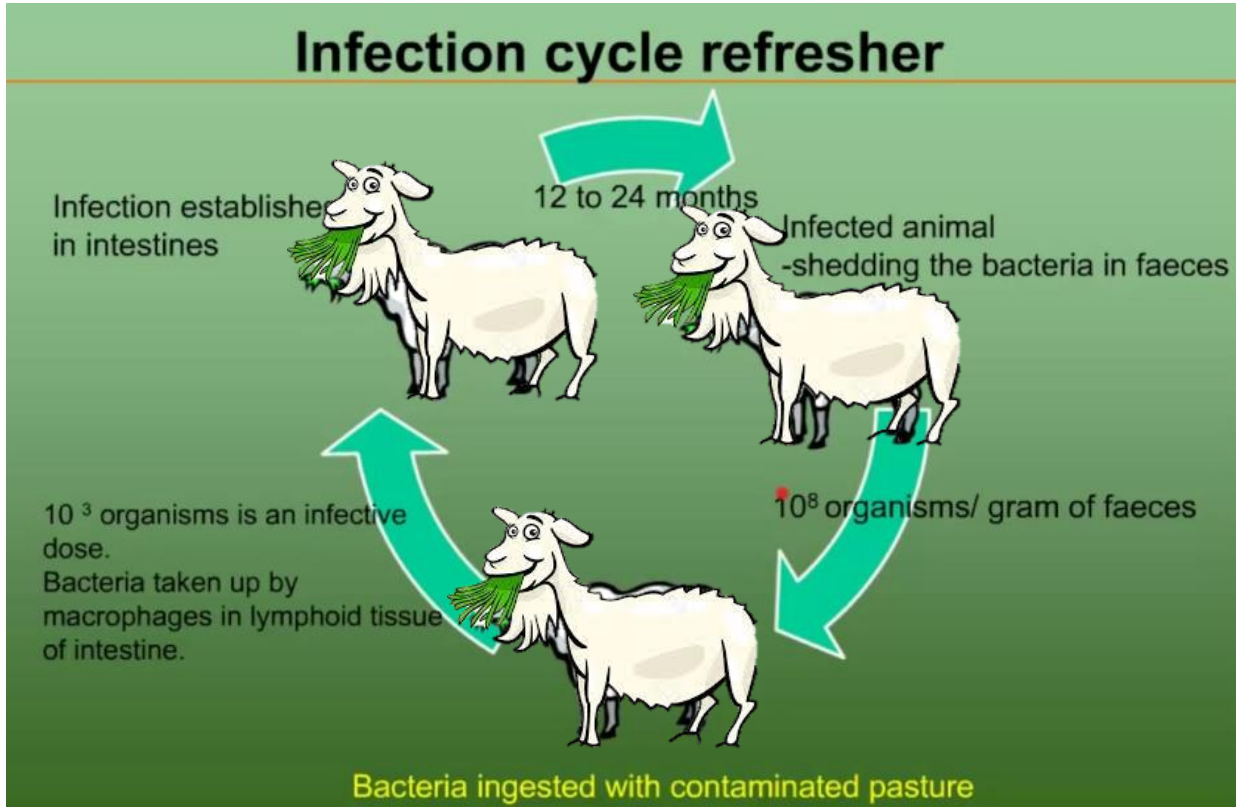
Cycle of Johne's Disease

- Enters goats predominantly via fecal-oral contamination
  - No clinical signs
  - May also pass in utero or via colostrum
- MAP infects the GI tract, mostly the small intestines
  - GI signs may be present at this time
  - May begin to shed organism into feces
- MAP infiltrates the bloodstream and begins living in the host's white blood cells



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Sheep versus Goats

- **Sheep** more likely to be infected with the **“S” strain**
  - These strains are difficult to culture and grow more slowly
  - Clinical animals are more likely to yield a positive result than a sub-clinical animal
- **Goats** more likely to be infected with the **“C” strain**
  - more commonly found in cattle
  - These strains are more easily cultured

Clinical Signs of Johne's Disease

- Clinical signs may not be present for years
  - Onset and severity may be related to dose and age at exposure
- Main signs:
  - Weight loss
  - Diarrhea (some animals)
  - Ventral and intermandibular edema (bottle jaw)
- Disease can lead to emaciation and death
- Non-symptomatic animals spread disease

A goat showing symptoms of Johne's disease.

AGDA.org

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Control of Johne's Disease

Biosecurity

- Purchase animals from herds with known disease status
- Test animals BEFORE purchase

or

- Quarantine and test animals upon arrival

Management

- Limit kid exposure to adults
  - Milk replacer if doe is known positive
  - Sanitary kidding areas
  - Separate pastures
- Routine testing for adults
  - Minimum: test suspects
- Minimize overall fecal contamination
  - Raised food and water troughs
- Cull known positives

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Financial Impact of Johne's Disease

- Decreased fertility –
  - Sub-clinically infected does that are older are more likely to be less fertile that uninfected animals of the same age (Kostoulas, et al, 2005)
- 23% lower profit efficiency when Johne's is present (Sardaro, et al, 2016)
  - Linear inverse relationship with Johne's prevalence
  - Decrease in milk income
  - Increased vet costs
  - Increased feed costs

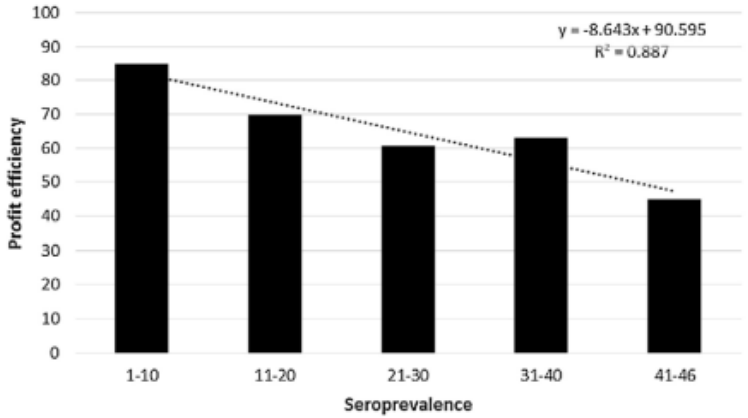

Fig. 1. Average profit efficiency per seroprevalence classes in the infected farms.

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Goat Demographic in the North Country

5 herds
61 goats

14 herds
370 goats

14 herds
384 goats

8 Herds
622 goats

2 herds
203 goats

4 herds
190 goats

47 herds

1906 goats Represented

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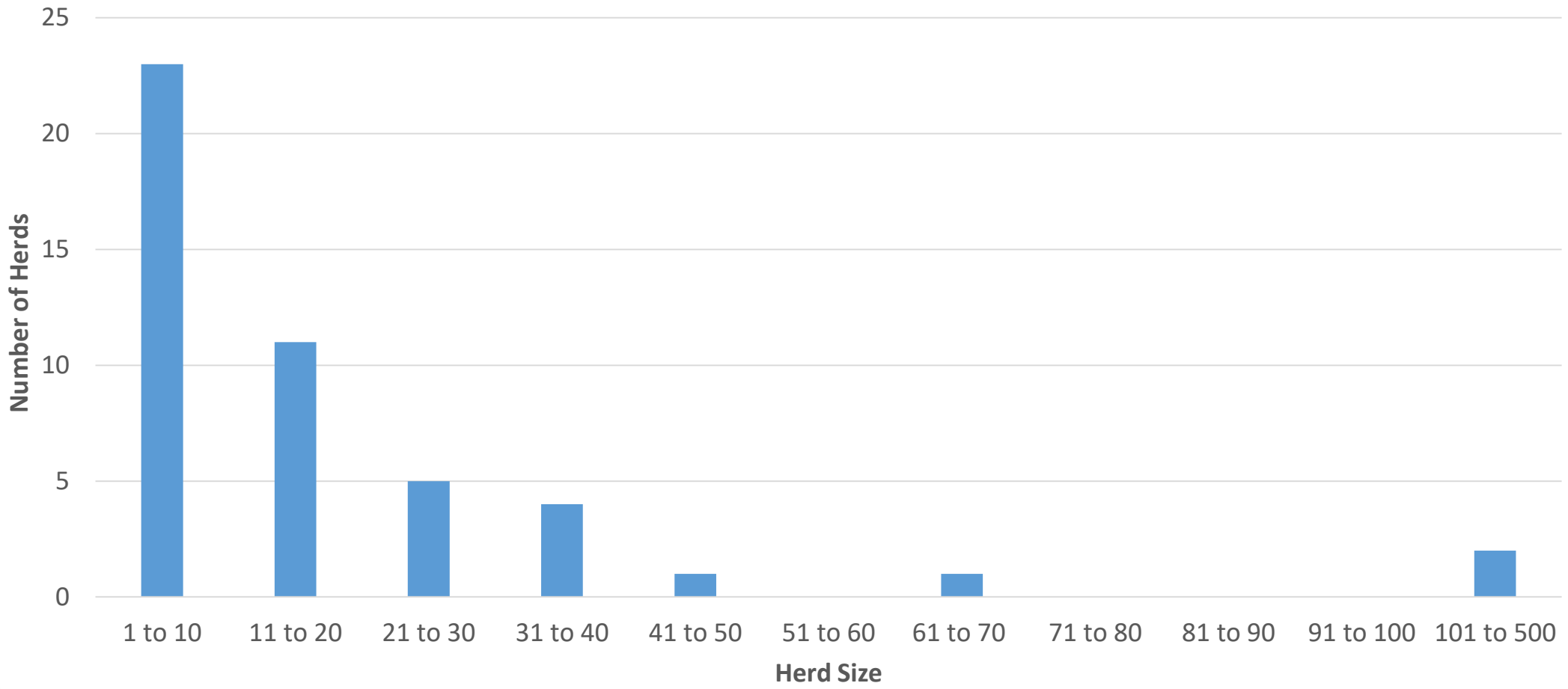
Herd size in the North Country



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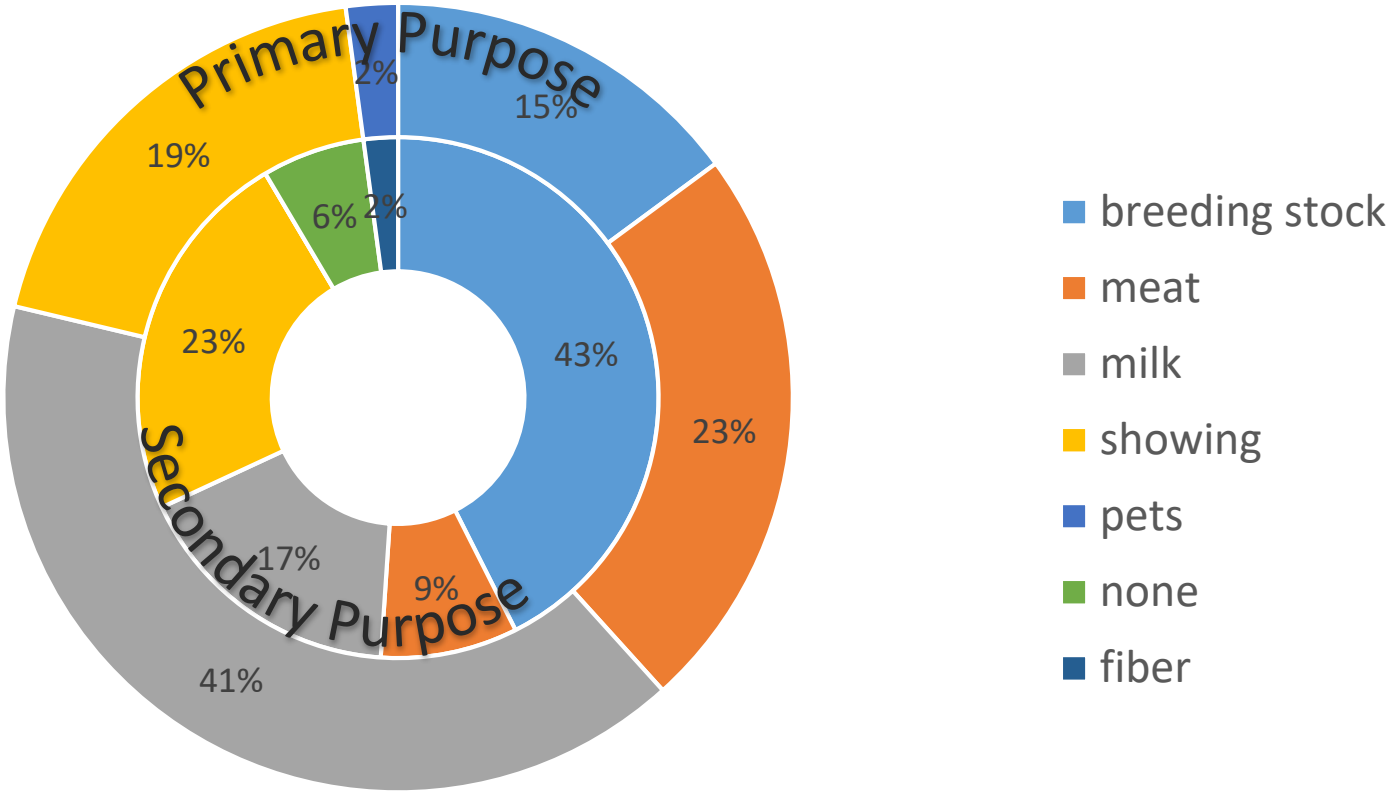
Goats Purpose in the North Country



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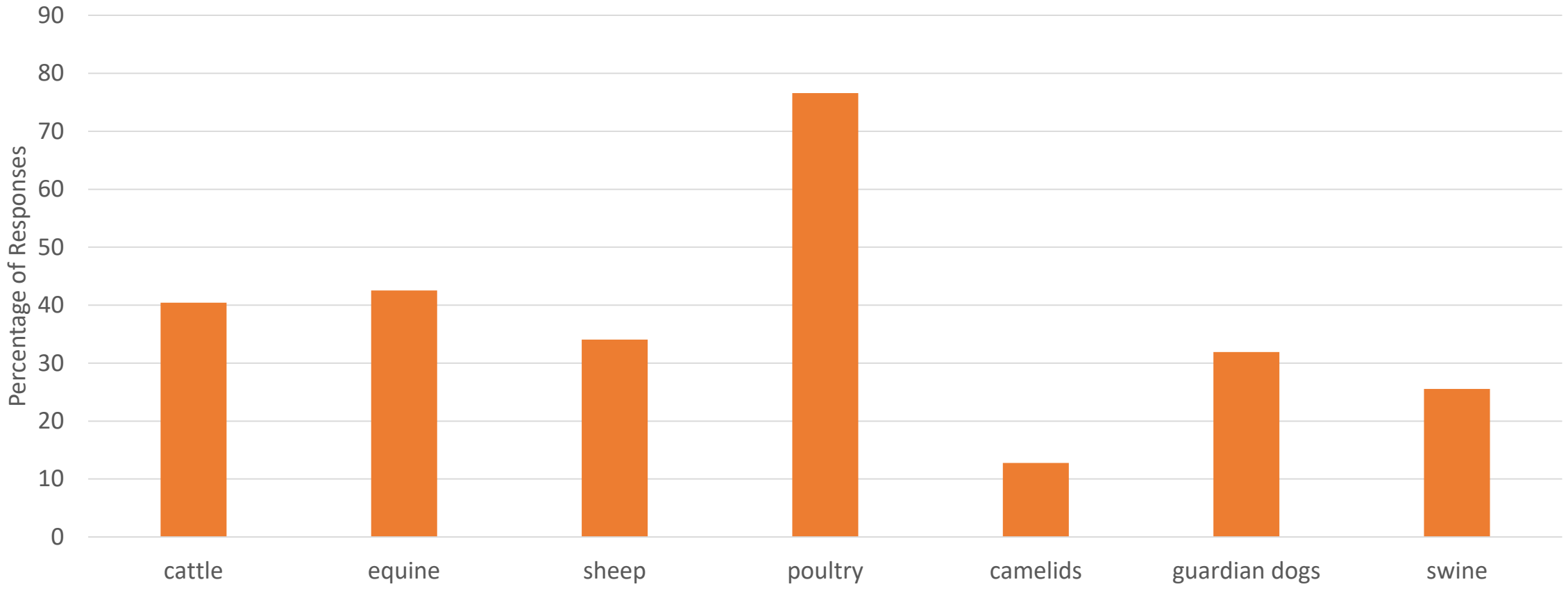
Other Species in the North Country



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Goats Breeds in the North Country

- Variety of Breeds
  - **Alpine**
  - **Boer**
  - LaMancha
  - Nubian
  - Sanaan
  - **Nigerian Dwarf**
  - Fainting/Myotonic
  - Cashmere
  - Oberhasli

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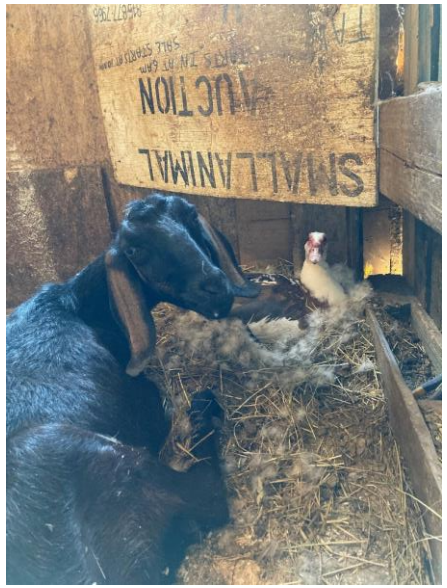
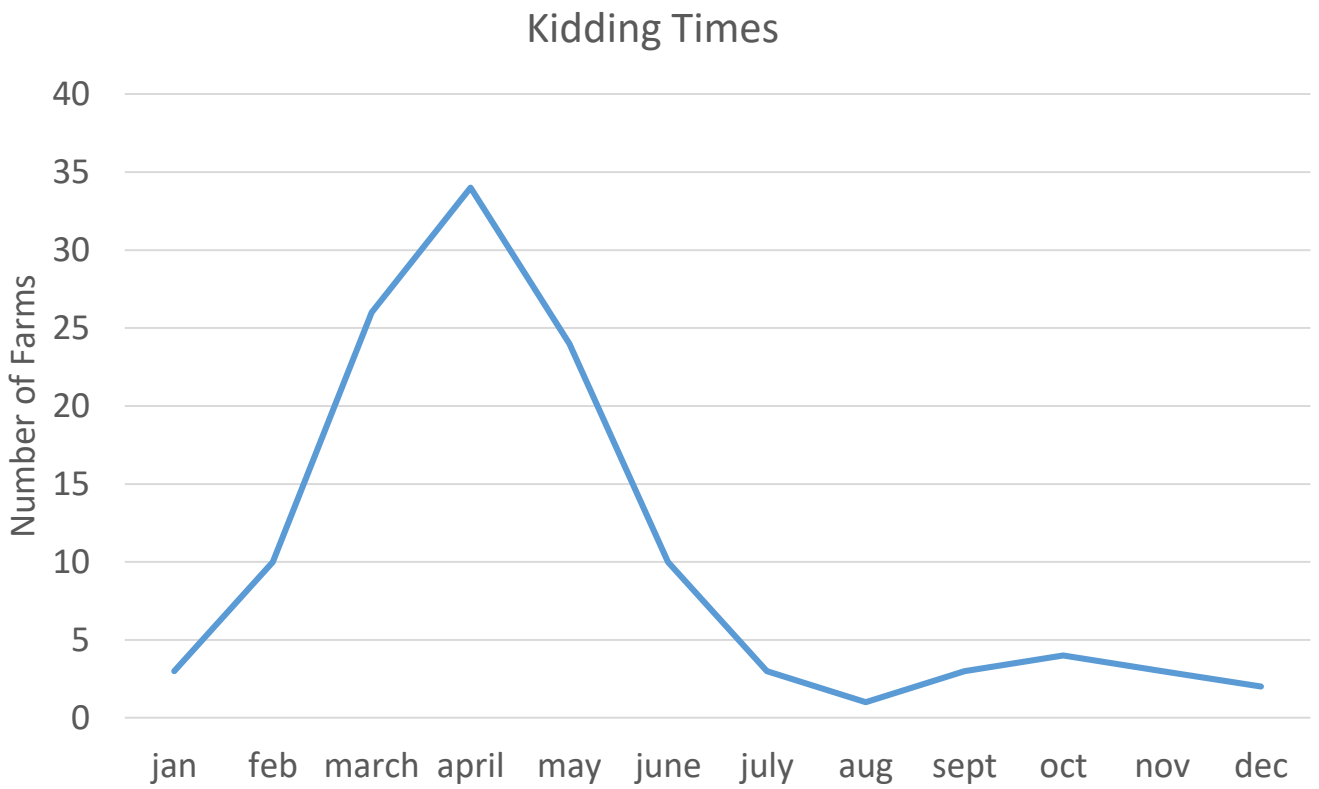
Goat Demographic in the North Country

- Pasture – 7/47 = 15%
- Group Pen – 17/47 = 36%
- Individual Pen – 21/47 = 45%
- No answer/other - 2/47 = 4%





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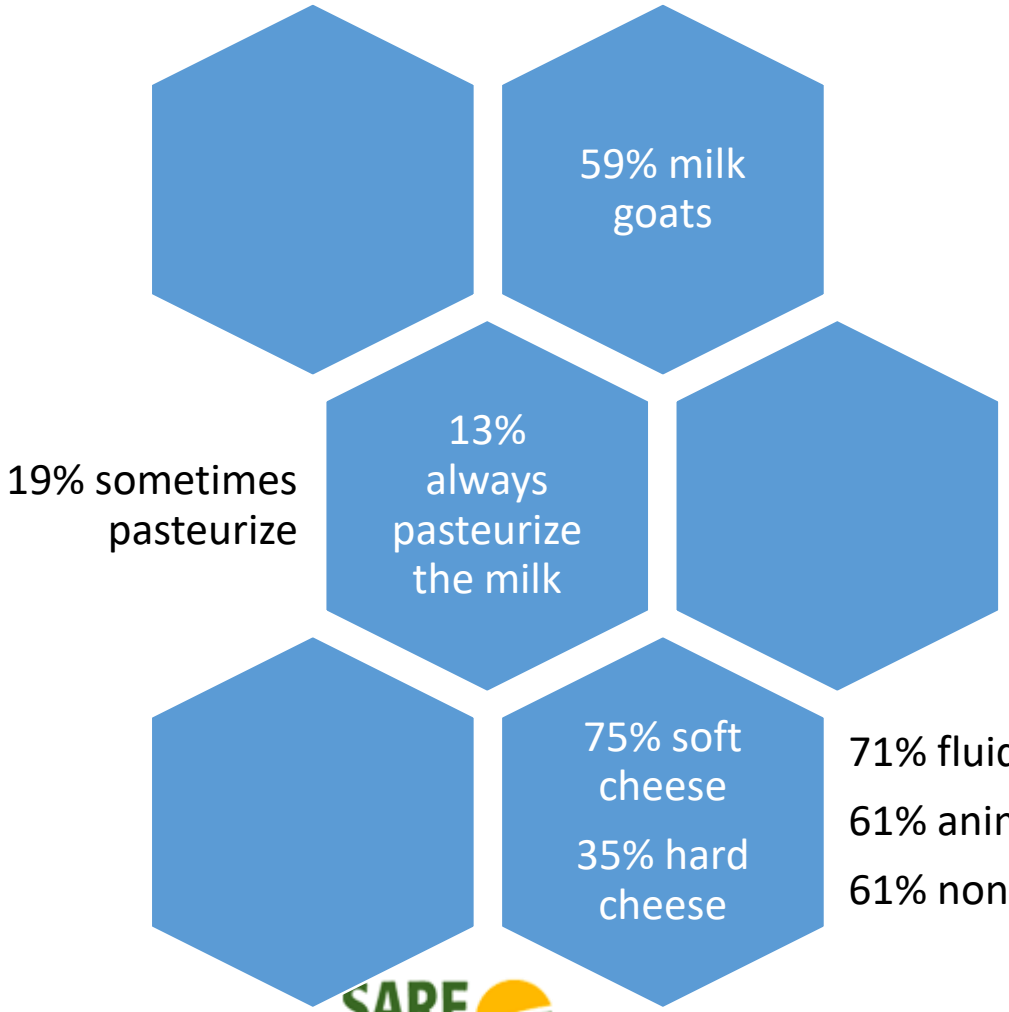
Goat Milk Consumption in the North Country



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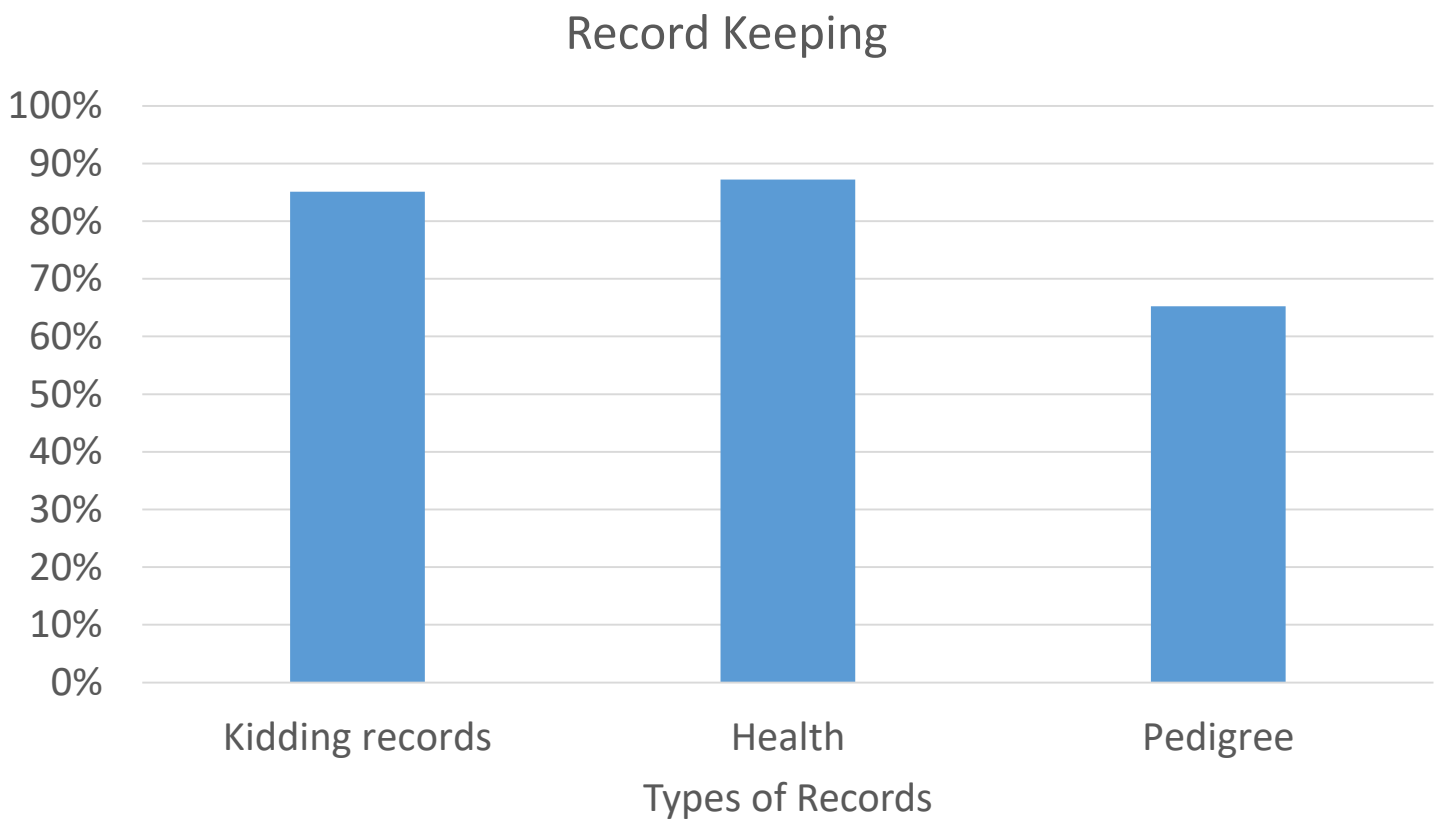
Goat Demographic in the North Country



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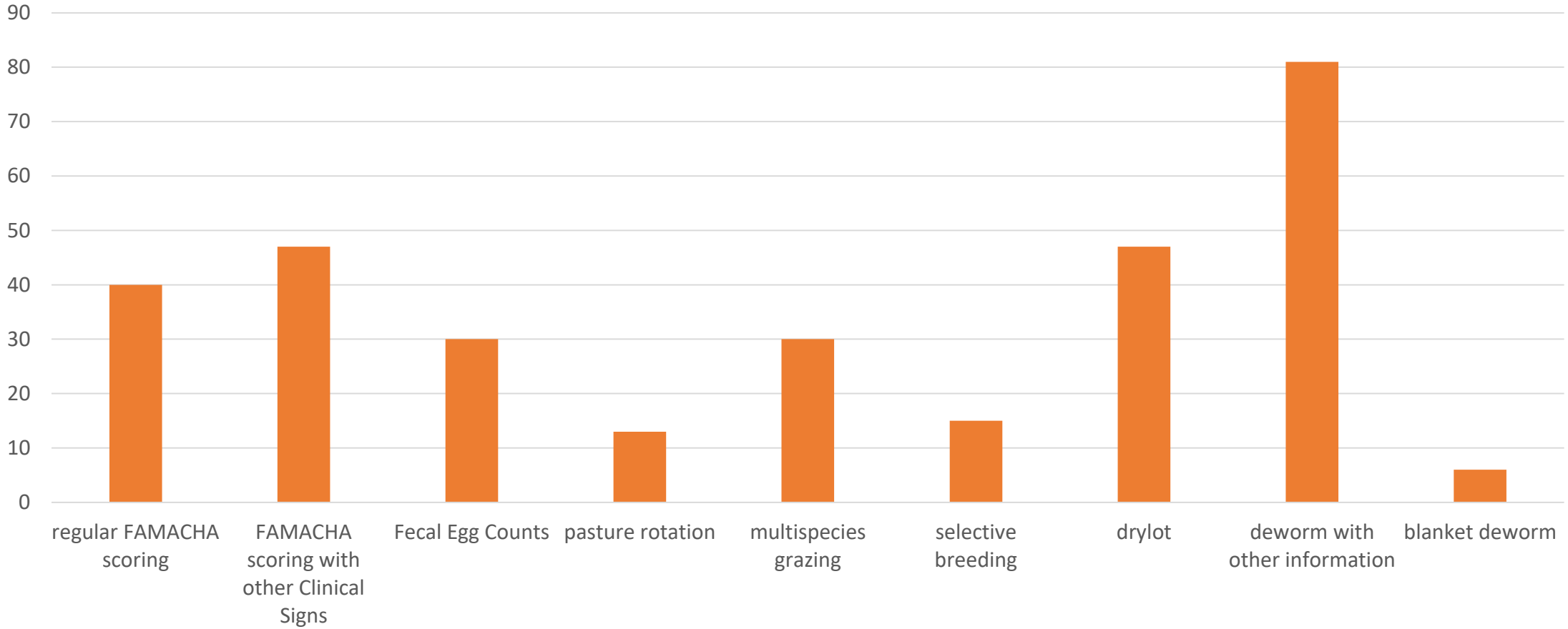

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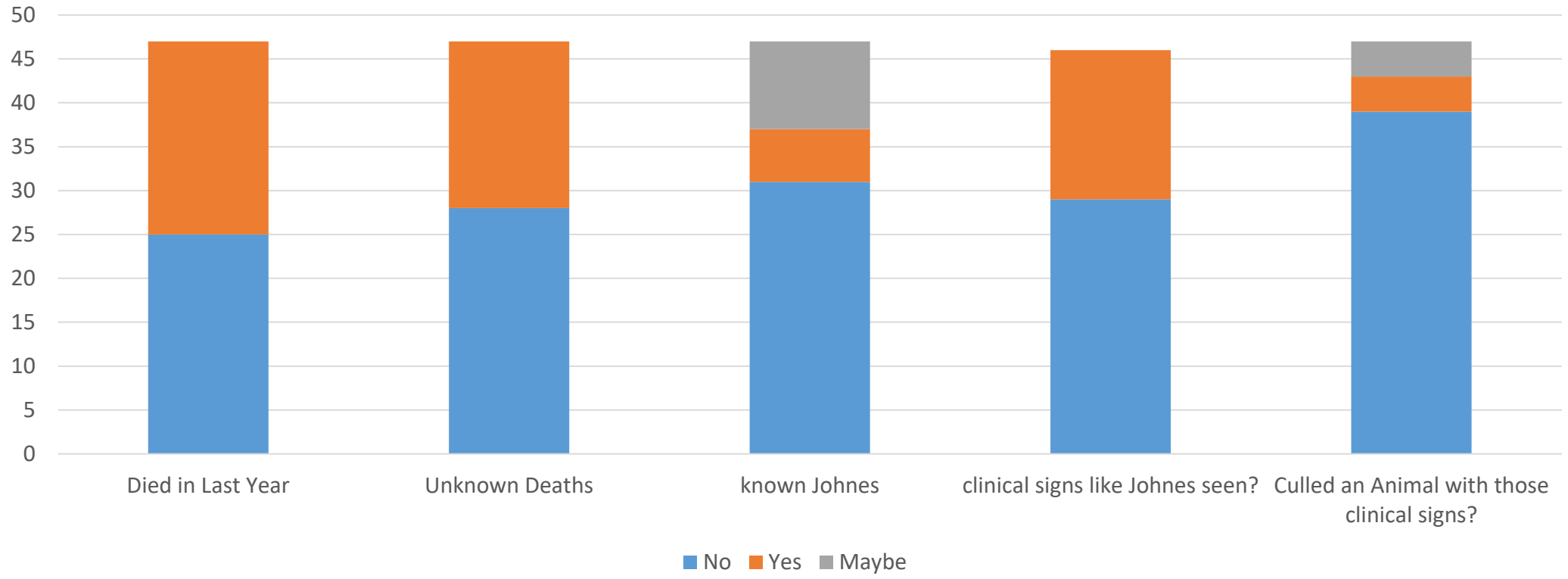
Johne's Disease Risk



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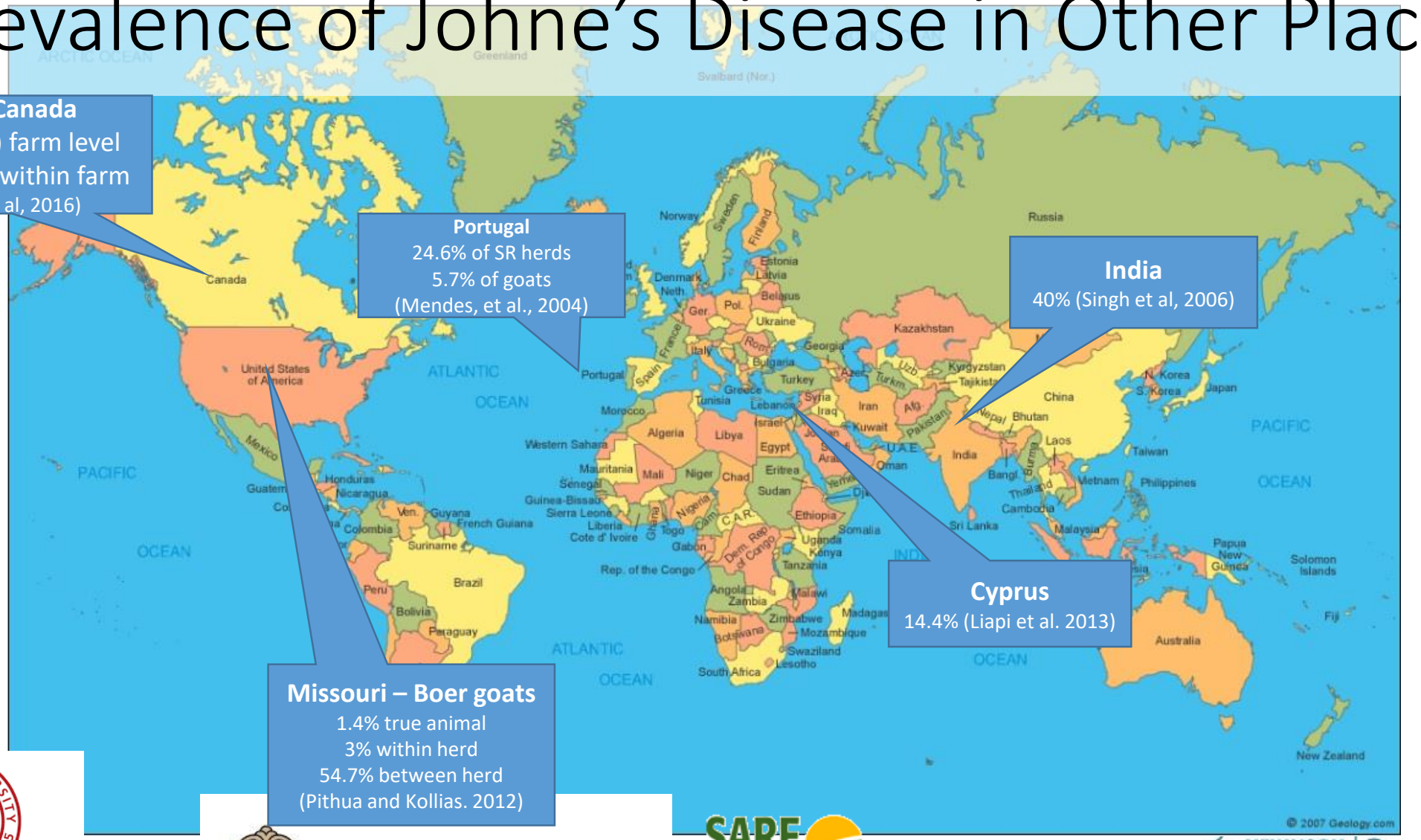
Prevalence of Johne's Disease in Other Places



Ontario, Canada
83% (62-98%) farm level
35% (23-50%) within farm
(Bauman, et. al, 2016)

Portugal
24.6% of SR herds
5.7% of goats
(Mendes, et al., 2004)

India
40% (Singh et al, 2006)

Cyprus
14.4% (Liapi et al. 2013)

to by Unknown

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Goals of Testing:

- Determine presence of disease in the herd
- Determine prevalence in the herd
- Help manage and reduce exposure to youngstock intended for breeding
- Accurate diagnosis of sick animals
- Reduce costs associated with treating “assumed” illness which may in fact be MAP
- Reduce the risk of bringing Johne’s into your herd

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Some Definitions

- **Sensitivity** (True Positive Rate) refers to the % who received a positive result on this test who are TRULY positive (accurate identification of disease)
  - how well a test can identify true positives
- **Specificity** (True Negative Rate) refers to the % who received a negative result on this test who are TRULY negative.
  - specificity is a measure of how well a test can identify true negatives

- For all testing, there is usually a trade-off between sensitivity and specificity - higher sensitivities will mean lower specificities and vice versa.

Testing

- Diagnostic tests generally have poor sensitivity (high false negatives)
  - (Whittington & Sergeant, 2001).
- Specificity of the tests is similar ~100% (few false positives)
  - (Sergeant, 2003a)
- Increasing accuracy as disease progresses (Whittington & Sergeant, 2001).
- Combination of tests help accurately diagnose Johne's (Carrigan & Seaman, 1990).
- Comparisons between tests can only be made when stage of disease is known.
  - (Whittington & Sergeant, 2001)

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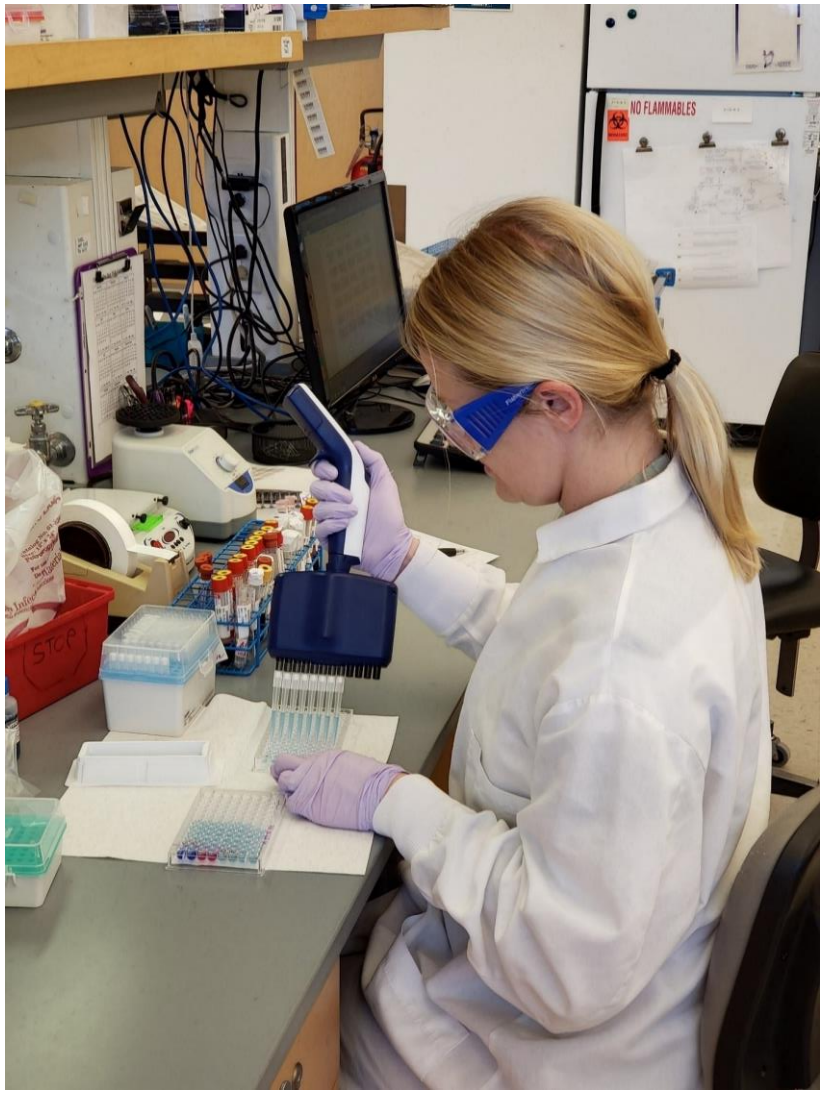
Testing for Johne's Disease

- Enzyme-Linked Immunosorbent Assay (ELISA)
  - Detects serum antibodies against MAP
  - Positive indicates exposure to Johne's
    - The body has begun to "defend" itself
  - As infection worsens, antibody levels increase
  - Positive/negative results are given along with a numerical result
    - Numerical result describes severity of infection



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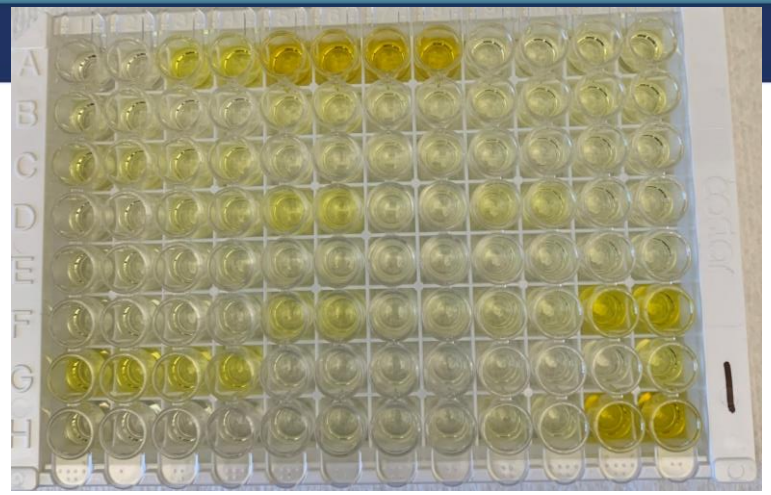
ELISA test characteristics



- PROS
  - Inexpensive
  - Pretty easy to get sample
  - Can use the same sample (blood) for multiple disease testing (CLA, CAE/OPP)
  - Yields numerical result which should correlate with certainty and level of infection
  - A positive does mean that the animal has mounted a response to MAP
    - Some risk of cross reaction with CLA in GOATS

- CONS
  - Designed to SCREEN large numbers of animals
  - Less sensitive than other types of tests
    - Only 30 to 50% of infected animals are identified, may miss MANY infected animals
    - **HIGH risk for a false negative!**

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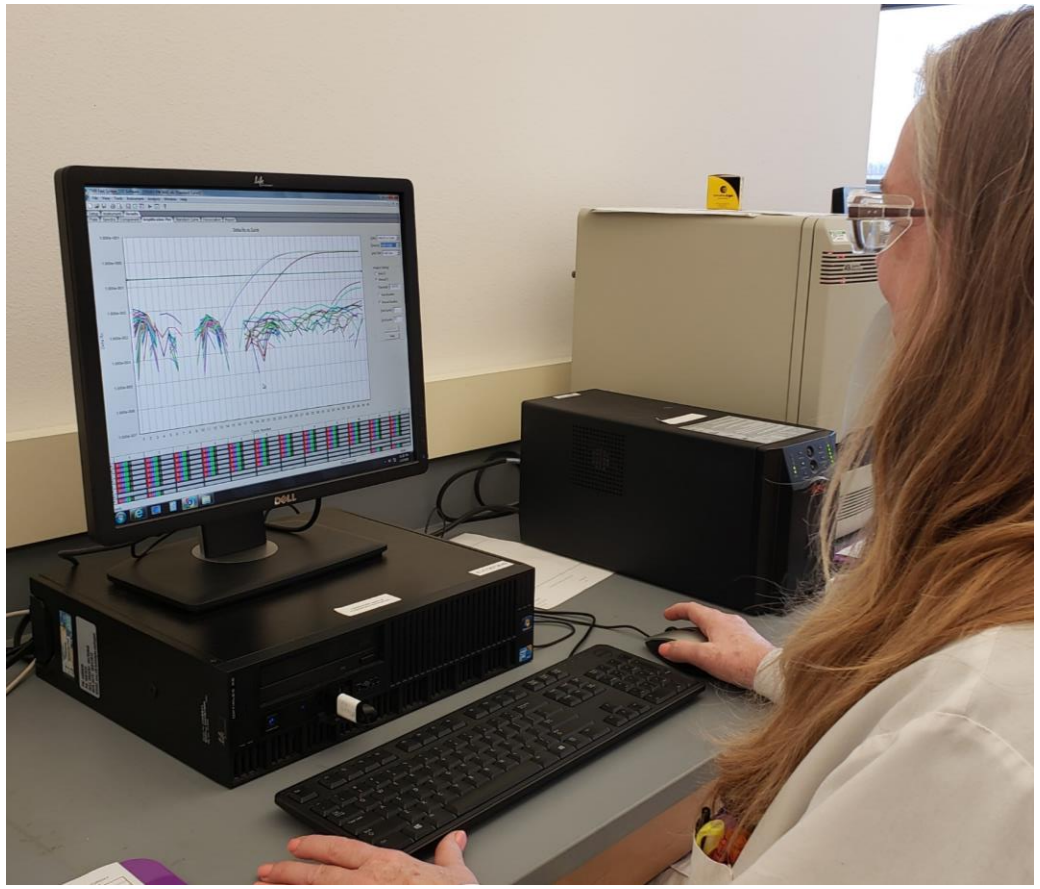
Table 1
Published estimates of the sensitivity (Se) and specificity (Sp) of the serum ELISA and the fecal culture (FC) for the diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in cattle, sheep and goats

Reference	Species	ELISA	FC
Collins et al. (1991), Ridge et al. (1991), McNab et al. (1991b)	Cattle	Se ~45% Sp > 90%	– –
Nielsen et al. (2002b)	Cattle	Se = 27–86%, Sp = 55–98%	Se = 20–73% Sp > 98%
Sockett et al. (1992)	Cattle	Se = 47–65% –	Se = 45–73% Sp > 99%
Sweeney et al. (1995)	Cattle	Se = 15–87%	–
Whitlock et al. (2000)	Cattle	Se = 15–75% –	Se = 42% Sp > 99%
Sergeant et al. (2003)	Sheep	Se = 25–73%; 10–47% Sp > 95%	– –
Whittington et al. (2003), Hilbink et al. (1994)	Sheep	– Sp > 97%	– –
Hope et al. (2000)	Sheep	Se = 35–54% Sp = 98.2–99.5%	– –
Dubash et al. (1995)	Sheep	Se = 48% Sp = 95%	– –
Rajukumar et al. (2001)	Goats	Se > 75% Sp > 90%	– –
Molina et al. (1991)	Goats	Se > 85% Sp > 93%	– –

Testing for Johne's Disease

- Polymerase Chain Reaction (PCR)
  - Detects MAP DNA in fecal samples
  - Can detect smaller amounts of the pathogen than other tests due to amplifying DNA segments
    - In some cases there may not be enough DNA to analyze, PCR is still able to detect, replicate and analyze those samples



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PCR test characteristics

- PROS
  - Can detect small amounts of bacteria DNA
  - Will yield positive with alive or dead bacteria
  - Quick test
- CONS
  - Cost
  - Need to ensure the feces submitted are from a specific animal

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Testing for Johne's Disease

- Fecal Culture
  - The most sensitive test available in live animals
    - A positive is a true positive
  - Has an extensive incubation period
    - 56 days

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Culture Test Characteristics

- PROS
  - Detects only live bacteria
  - If it's positive, it's a true positive
- CONS
  - May miss animals with low level shedding
  - Takes a long time to get results – how useful is it with the delay?
  - Cost
- Lower sensitivity of fecal culture in sheep MAP.
  - Sheep strains are difficult to grow (Collins et al. 1993).

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Testing for Johne's Disease

- Challenges of Testing
  - One test alone may not confirm presence or absence of infection
  - Extremely long dormancy period does not ensure clinical signs will be present in infected
  - To yield most accurate results all three tests must be ran, each needing various samples from each sheep tested
- Focal forms may represent latent and persistent lesions that developed when the animals were young, limited by the immune response.

(Perez et al., 1996)

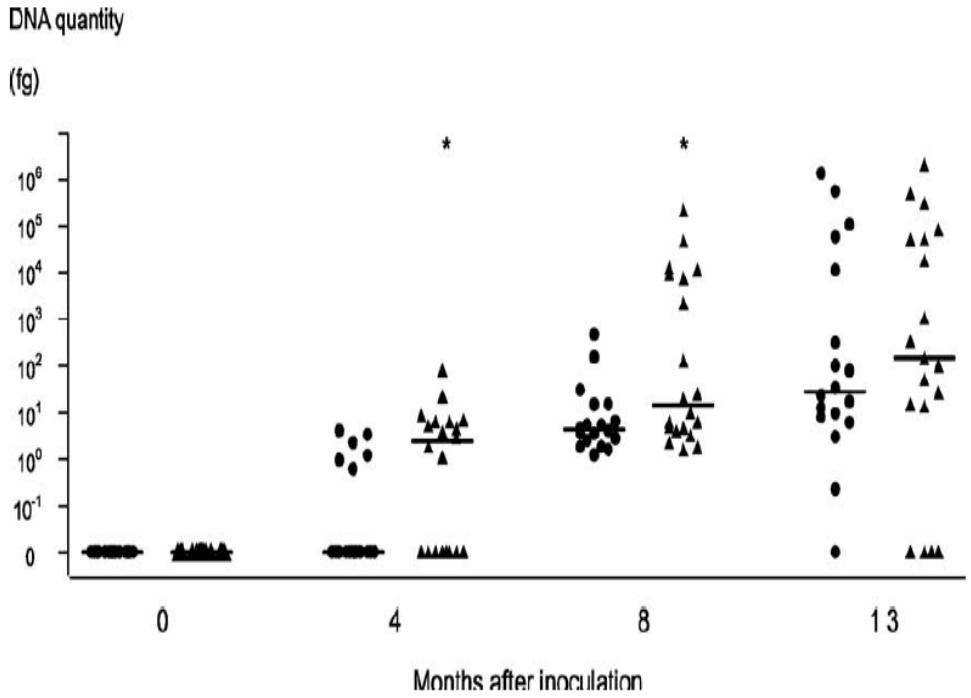

Fig. 6. Quantification of MAP DNA in the faeces of 38 sheep that were inoculated with MAP. DNA quantities (fg) calculated using data from the QPCR assay ●: Pure culture group, ▲: gut homogenate group. The horizontal bar indicates the median. Asterisks show differences between groups at that time point ir a Mann–Whitney test ($P < 0.05$).

Kawaji, et. al., 2011

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Necropsy

- True gold standard test
- Focus on intestinal lymph nodes and intestines
  - Lymphadenopathy
  - Prominent lymphatics
- Culture or PCR from tissue
- Histopathological changes can be suspicious of diagnosis

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Project Design

Screening Survey

Distribute survey

47 responses!

47 herds

Semi-convenient sample

Willing to Participate?

$$n = \frac{z_a^2 pq}{L^2}$$

Calculate sample size and control for clustering

28 herds

$$n' = n(1 + p(m - 1))$$

Unable to set minimum number of animals/herd

Already in NYSSGHAP or have >10 animals over 2y?

27 herds

Sample Collection

Visit all herds

Enroll in NYSSGHAP

NYSSGHAP

Collect blood and feces On up to 20 animals >2y

Ship to WI for ELISA, PCR and Culture

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Included Farms

27 herds (59%)
318 sampled
1197 goats Screened (60%)

2 herds
11 goats

6 herds
118 goats

10 herds
134 goats

4 herds
117 goats

2 herds
89 goats

3 herds
58 goats

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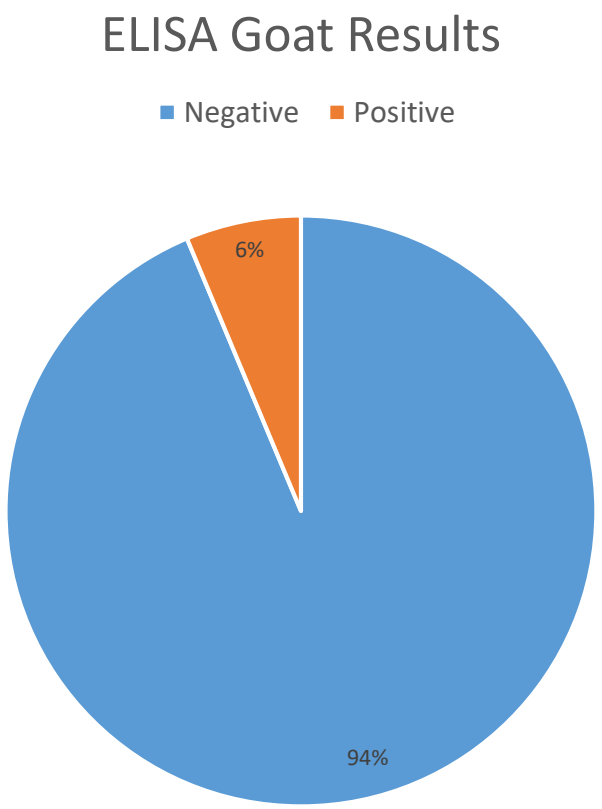
Preliminary Results - ELISA



- 20 goats identified as POSITIVE (6%)
- 12 FARMS identified with ≥1 positive (43%)

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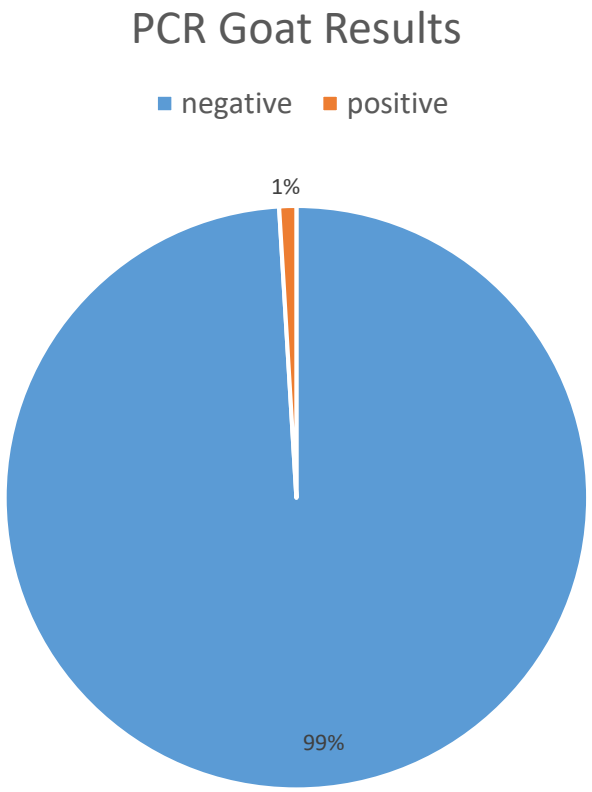
Preliminary Results - PCR



- 3 POSITIVE goats (1%)
- 3 FARMS with at least one positive goat (11%)

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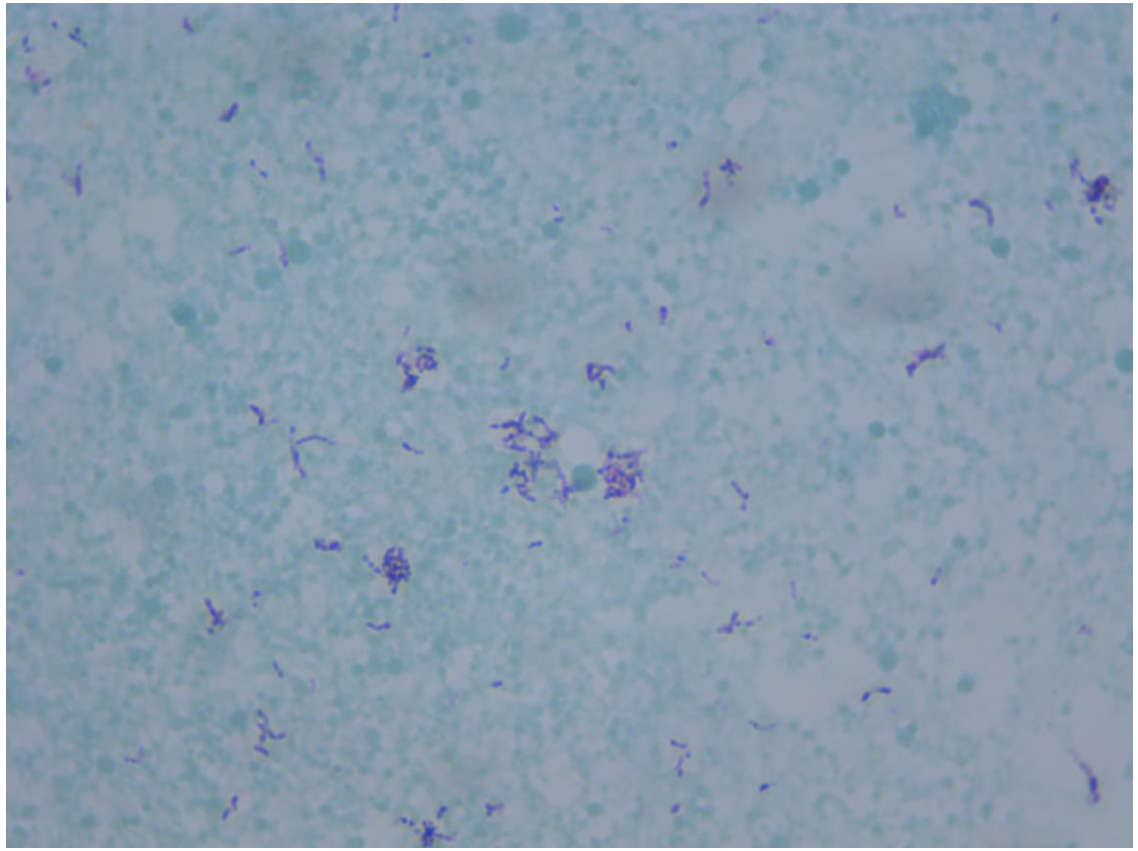
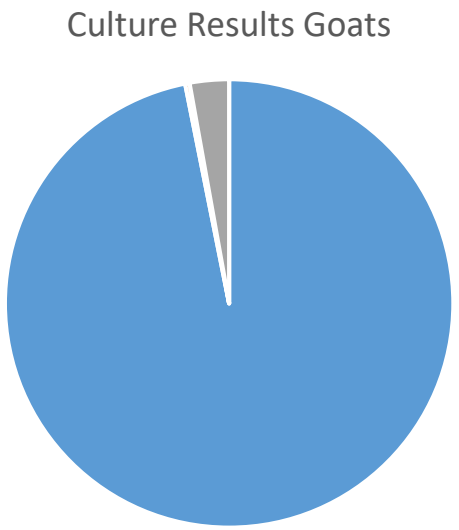
Preliminary Results - Culture

- 9 goats positive on culture (2.8%)
- 6 farms with at least 1 positive goat (21.4%)
- 1 goat with inconclusive culture





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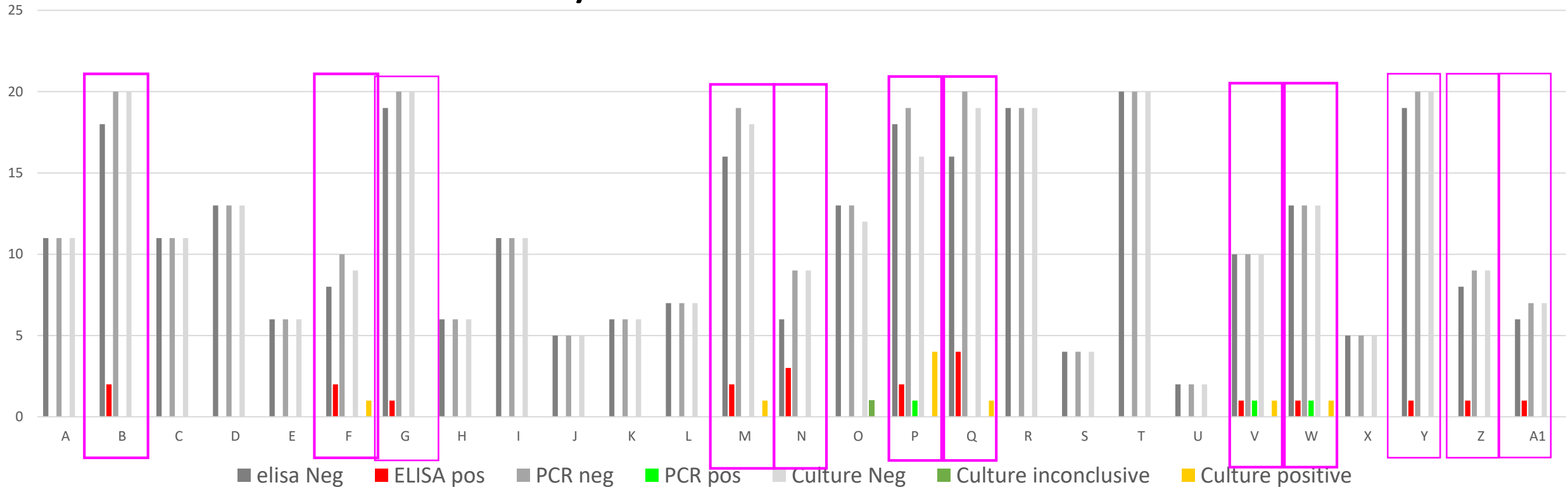
Overall results by FARM



12 FARMS with at least 1 positive animal **44%**

8 Farms with >1 positive animal **30%**

28 Goats identified on at least one test 8.8%

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Goats versus Sheep

	Individual GOATS	GOAT farms	Individual SHEEP	SHEEP farms
ELISA	20 (6%)	12 (43%)	**24 (7.5%)**	**12 (57%)**
PCR	3 (1%)	3 (11%)	**6 (1.9%)**	**5 (23.8%)**
Culture	**9 (3%)**	**6 (21%)**	0 (0%)	0 (0%)
Total	28 (9%)	12 (43%)	30 (9.4%)	13 (62%)

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Limiting Factors

- Goat demographics and distribution in NNY did not allow for a completely random sample
- Some herd sizes/management did not allow for random sampling within each flock
- Testing is not a guaranteed diagnosis
  - Although necropsy is a guarantee, it is not economically or ethically a sound test for a regional study such as this

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Conclusions

- Johne's disease is present in North Country goat herds
- A low level of disease may be present on the majority of premises
- Additional statistics to extrapolate the regional prevalence in process
- Some flocks did not seem interested in finding out their risk
- Sampling cost is prohibitive for most flocks to determine their true status

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- Herds that participated in the study
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Resources

- Johnes.org
- https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Control-and-Eradication/Johnes-Disease
- https://www.nj.gov/agriculture/divisions/ah/pdf/johnesgoatQ&Abooklet.pdf
- https://www.vet.cornell.edu/animal-health-diagnostic-center/testing/protocols/johnes-disease-program

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