

Blood on Filter Paper for Detecting *Brucella* in Caribou: Validation and Herd Investigation

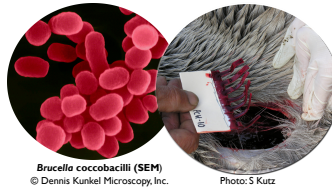
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BACKGROUND

Brucella suis biovar 4

- A zoonotic bacterium that causes 'brucellosis' in caribou (*Rangifer tarandus*)
- Effects in caribou: abortion, orchitis, joint illness, other chronic disorders¹
- Passed to humans by i) eating raw/undercooked infected meat
ii) skin cuts during butchering of an infected animal
- Population impacts? – Caribou herd declines² may involve *Brucella* and other pathogens; however, wildlife disease surveillance in the Arctic is a challenge



Filter-paper blood testing has been used in human medicine since the 1960s.³ While very practical in the field, validation for wildlife work is lacking.

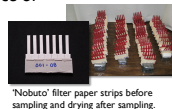
AIM: To evaluate and apply a practical diagnostic tool, blood on filter paper, for widespread monitoring of disease exposure in caribou by hunters, biologists and others.

- **Assess efficacy** for detecting *Brucella* antibodies in *Rangifer*.
- **Use serum and filter paper samples** to test circumpolar caribou herds.

METHODS

Validation: Paired filter paper and serum samples (n=185) were collected from a barren-ground caribou herd known to have high (>45%) prevalence of antibodies (seropositivity) to *Brucella* spp. Sample pairs were tested simultaneously after **2 months, 1 year and 2 years of storage**.

Herds: Serological testing of samples collected from circumpolar herds during International Polar Year is underway.



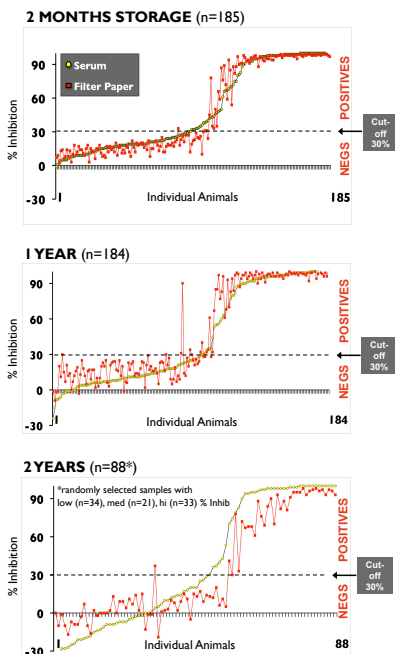
Test: Competitive enzyme-linked immunoassay (cELISA)

cELISA wells:
Pale = Positive

- Colour-tagged antibodies for *Brucella* are added to the sample in a plastic plate well containing pathogen ('antigen') - if the animal has been exposed to *Brucella*, its natural antibodies will have no colour tag - the natural and tagged antibodies compete to bind antigen
- After a period of binding, any unbound antibodies are rinsed away and chemicals are added causing the **colour-tagged** antibodies to appear - colour density is measured, '% Inhibition' is calculated)
High % Inhibition (pale colour) = **POSITIVE** (exposed or infected)

RESULTS

I. Filter paper vs serum (the 'gold standard')



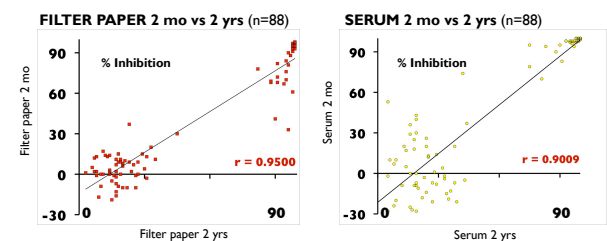
SUMMARY: Filter paper performance

(all values %)	STORAGE TIME		
	2 mo	1 yr	2 yr
Sensitivity ^a (95% CI)	88.5 (82.2-94.9)	96.5 (92.5-100)	85.0 (73.9-96.1)
Specificity ^{ab}	98.9 (96.7-100)	92.9 (87.9-98.0)	97.9 (93.9-100)
True prevalence ^c	51.9 (44.7-59.1)	46.2 (39.0-53.4)	—
Apparent prevalence ^{cd}	46.5 (39.3-53.7)	48.4 (48.1-55.6)	—
Predictive value + test	98.8 (96.6-100)	92.1 (86.5-97.7)	97.1 (91.6-100)
Predictive value - test	88.9 (82.7-95.1)	96.8 (93.3-100)	88.7 (80.1-97.2)

^a % true (+) with filter paper ^c prevalence with serum
^{ab} % true (-) with filter paper ^{cd} prevalence with filter paper

INTERPRETATION:
Relative to serum, filter-paper *Brucella* cELISA remains sensitive and specific to 2 yrs of sample storage. Sensitivity is more variable after 1 yr. Predictive values also remain high to 2 yrs of storage. Filter paper testing tends to underestimate seronegatives in a population with approximately 50% *Brucella* seroprevalence.

II. Variability over time (comparison within individuals)



III. Herd study

- Samples and data were collected by the Circum-Arctic Rangifer Monitoring Network during 2007-09
- Serum or filter paper samples from eight herds are now being tested for *Brucella* antibodies (cELISA)

Herd	Sample Size
Porcupine	33
Tuktoyaktuk Peninsula	14
Bluenose West	78
Bathurst	145
Rivière-aux-Feuilles	147
Rivière George	144
Kangerlussuaq (Greenland)	50
Akia-Manitsoq (Greenland)	47
Total:	658



Refs: 1. Forbes 1991. CVJ 32:686-688; 2. Vars & Boyce 2009. Glob Chg Biol 15(11):2626-33; 3. Mei et al 2001. J Nutr 131(5):1631-6



Special thanks to all CARMA collectors, the hunters of Coral Harbour, NU, Jonathan Pameolik, Linda Kelly (CFIA), Jane Harms. Thanks also to Nathalie Parenteau for lending her beautiful artwork to this science.

