

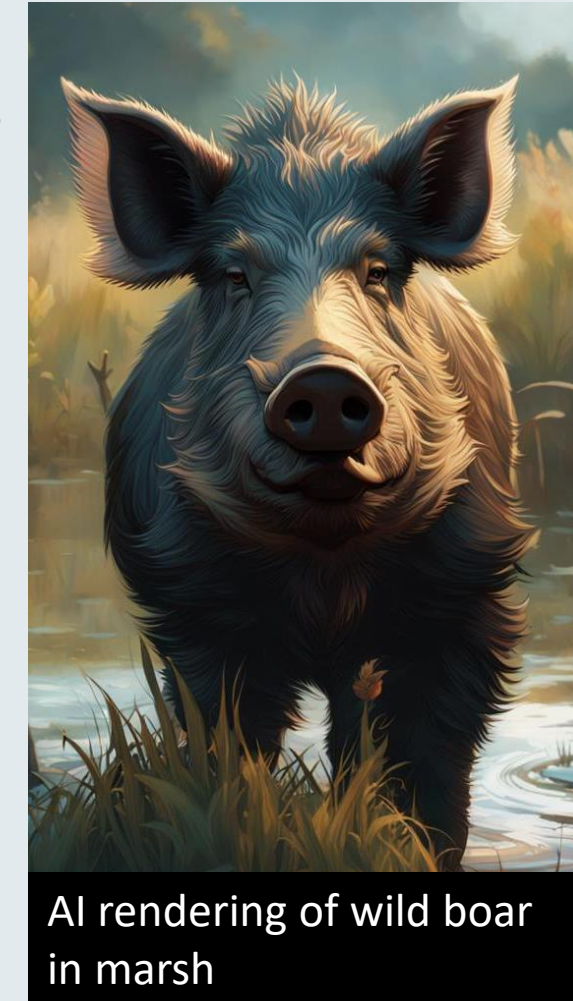
Environmental DNA for the Detection of Wild Boar in Alberta

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Background

- Wild boar threaten natural habitats and agricultural lands.
- Population control requires knowledge of sounder location.
- Environmental DNA (eDNA) detects genetic material in environmental samples (i.e., water, soil).
- Potentially, more sensitive than established methods at low population densities.
- eDNA results must be interpreted considering sample characteristics.
- How much DNA degradation is possible/likely?
- Is contamination possible/likely?
- Could DNA have moved from its point of origin?
- Off-target detections possible – only a small fraction of taxa have been fully sequenced.



AI rendering of wild boar in marsh



Rooting Damage; Photo: Gov. of Alberta



Wild boar damage to oat field; Photo: Gov. of Alberta

Goals

- Validate and refine USDA assay using local samples.
- DNA-based assays can be sensitive to genetic differences across populations.
- Need to balance sensitivity and specificity.
- Evaluate sampling techniques.
- InnoTech filtration sampling – large amount of DNA can be collected but can be time consuming.
- USDA grab-sampling – quick but may yield limited amounts of DNA.
- Evaluate rapid-detection kits for identifying boar scat in the field.
- Rapid-detection kits for pork protein (albumin) have been developed to test for the presence of illicit pork in foodstuffs.
- Sensitivity to wild boar albumin unknown.
- Suitability for evaluation of scat unknown.

Tissue and Scat Samples

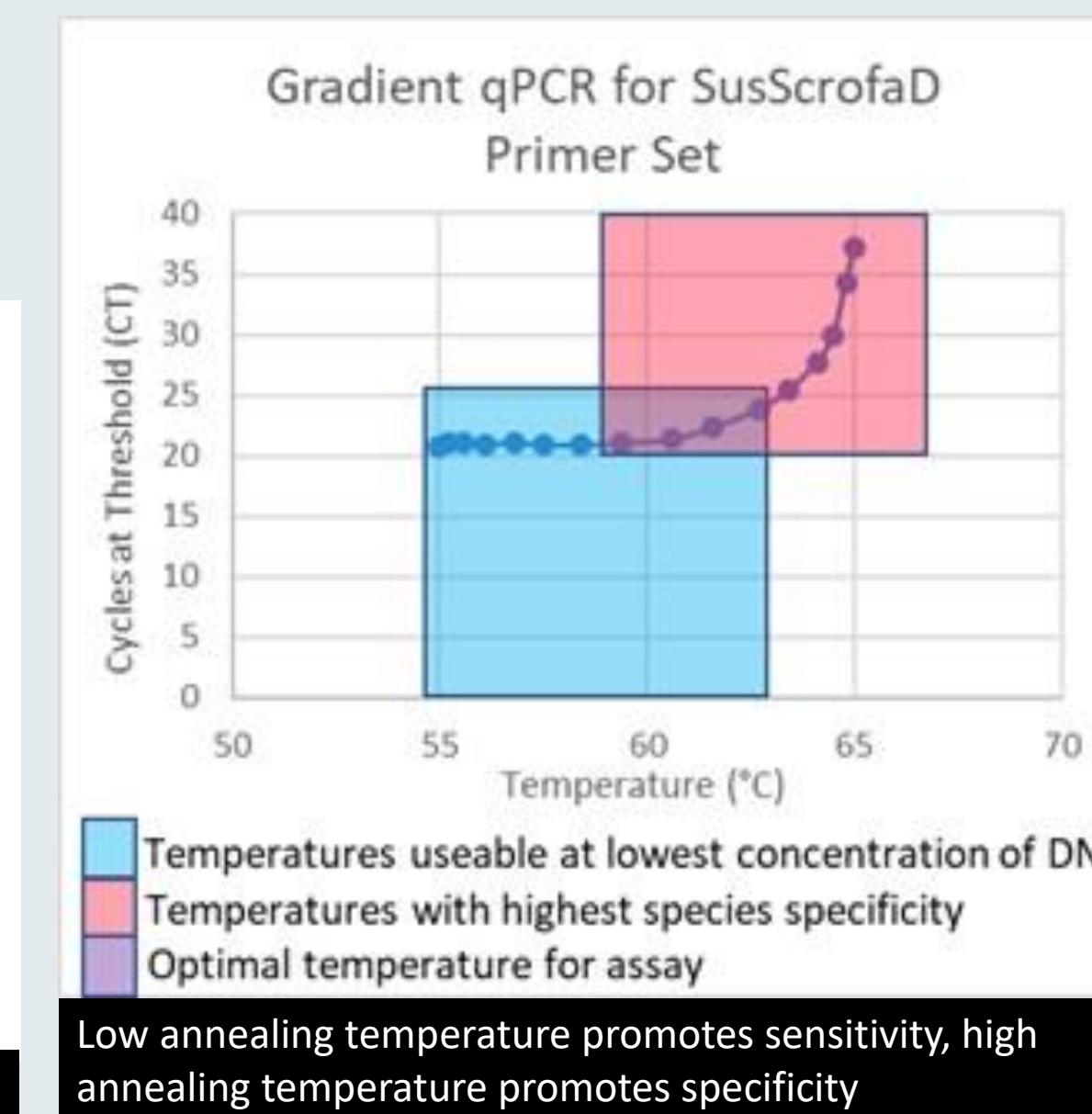
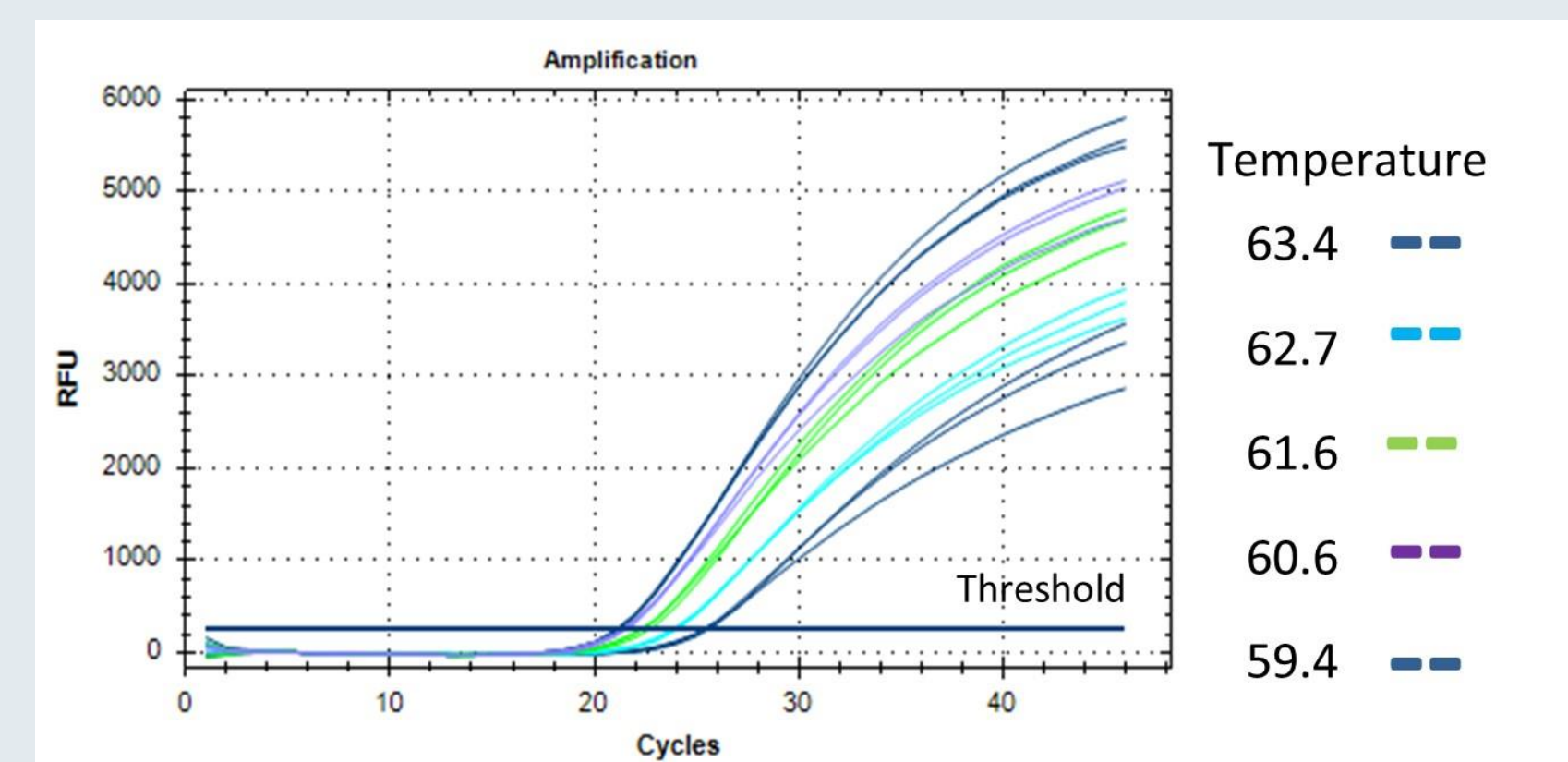
Species	Tissue	Scat
Wild Boar	4	9
Domestic Pig	4	3
Black Bear	3	3 ^A
Wolf	3	3
Coyote	3	3
Fox		1
Domestic Dog		5 ^B
Moose	3	2
Elk	3	3
Mule Deer	3	3 ^A
Whitetail Deer	3	
Cow	1	1
Chicken	1	

^ASpecies cannot be precisely determined for some scat samples - identified only as bear or deer

^BThree of these samples came from dog that had recently consumed pork, two from dogs which had not

eDNA assay development

- Tested USDA qPCR assay with locally-sourced tissue and scat samples.
- Optimal performance: annealing temp. of 63°C, CT cutoff of 35 cycles.
- Positive result for 8/8 porcine tissue samples, 11/12 porcine scat samples.
- Negative result for 22/23 non-porcine tissue samples, 20/24 non-porcine scat samples.
- False positives:
 - Beef sample cut with pork-contaminated knife (expected outcome).
 - Scat from dogs which had recently consumed pork (expected outcome).
 - One coyote scat sample (unexpected outcome).
- False negative:
 - One boar scat sample (unexpected outcome).

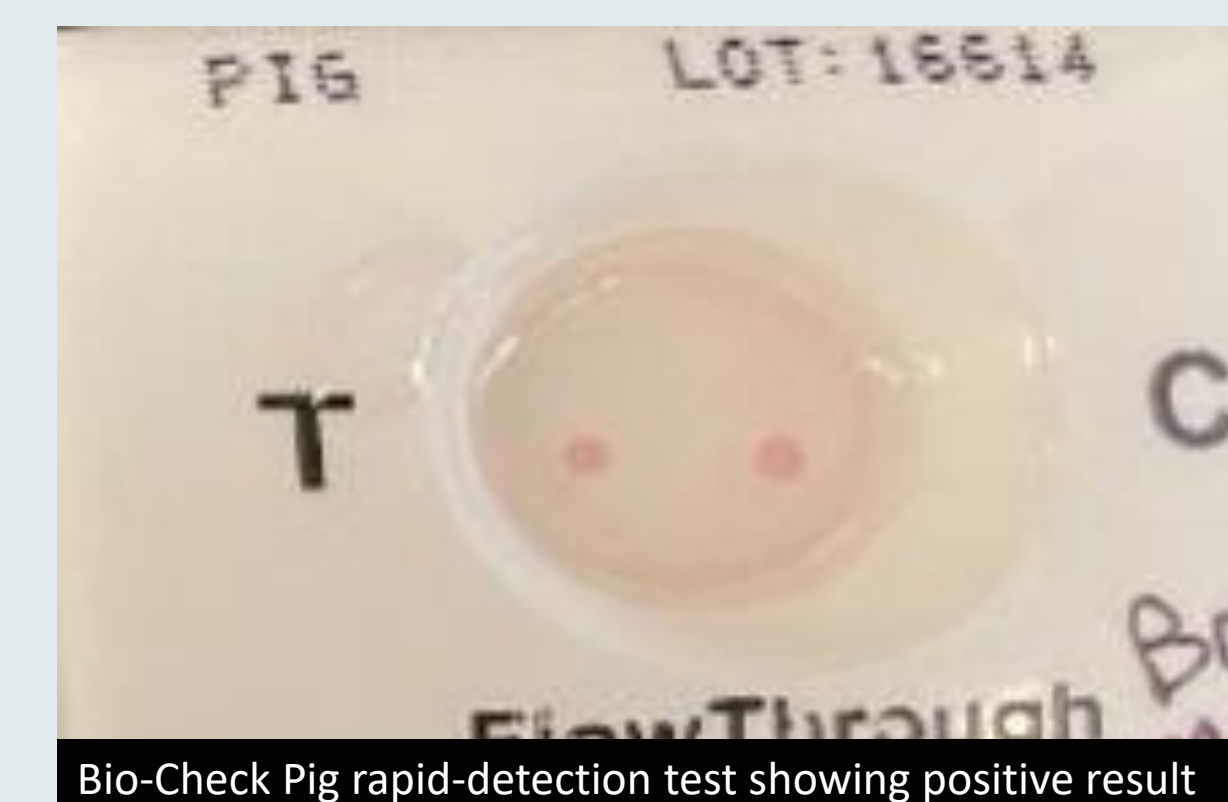


Rapid-detection kits

- Tested using same tissue and scat samples as for eDNA assays.
- Attempted detection using BioCheck Pig Flow Through kits.
- Tissue
 - Porcine tissue: strong positive.
 - Wolf and coyote tissue: weak positive.
 - Other tissue: negative.
- Scat
 - Porcine scat produced negative to weakly positive results – increasing sample amount and extraction time did not help.
 - Non-porcine scat produced variable results.



Wild boar scat; Photo: Government of Alberta



Bio-Check Pig rapid-detection test showing positive result

Sampling

- 12 field sites identified for water sampling.
 - 3 where boar known to be present.
 - 3 where boar known to be absent.
 - 6 where boar presence uncertain.
- Paired filtration and grab samples collected at each site.
 - 3 sampling locations at each site (~ 50 meters separation between samples).
- Samples of soil or mud collected from suspected trackways or wallows.
- Laboratory and statistical analysis of samples continues in 2024.



InnoTech filtration technique – inflow tubing deployed via extendable pole



InnoTech filtration technique – drill drives peristaltic pump



InnoTech filtration technique – filter membranes preserved by desiccation

Conclusions

- DNA-based assays can reliably distinguish porcine tissue and potentially distinguish porcine scat from that of other species.
 - Scat samples can produce false positives if the animal has consumed domestic pig or wild boar tissue.
 - Samples from domestic pigs and wild boar produce similar results.
- Protein-based rapid-detection kits are not reliable for identifying wild boar scat in the field.

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