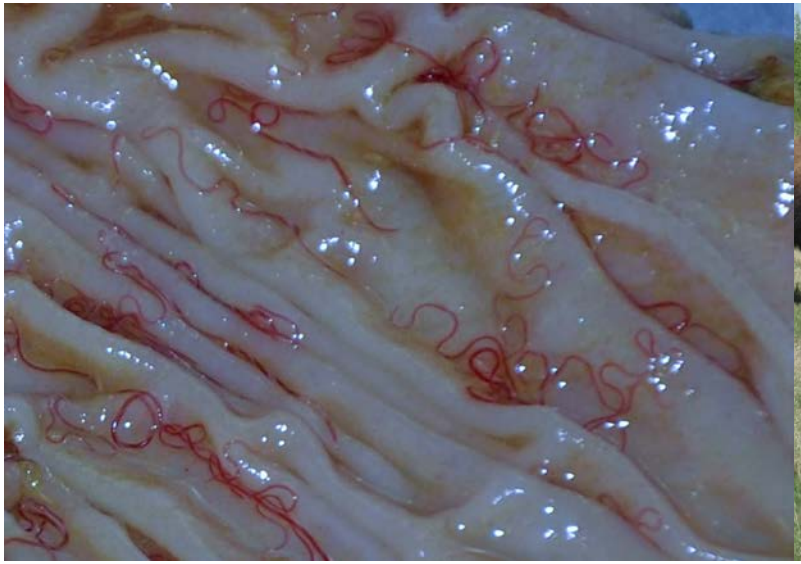
Gastro-intestinal Parasitic Nematodes (Roundworms) of Bison

Developing diagnostics to investigate parasite diversity and drug resistance



John Gilleard

Libby Redman, Russell Avramenko, Ana Bras, Claire Windeyer, Roy Lewis, Murray Woodbury

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ALMA
Alberta Livestock and Meat Agency Ltd.

NSERC CREATE
Host Parasite Interactions
University of Calgary

Parasitic Roundworms of Livestock in Western Canada

Cattle

Bison

Sheep

Clinical Disease

Sub-clinical Production Loss

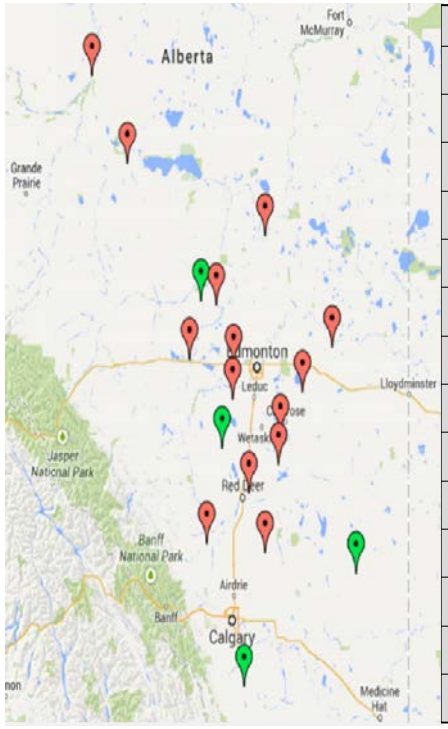
Invisible stealer of profit

Visible Disease Concern

Alberta Sheep Parasite Study

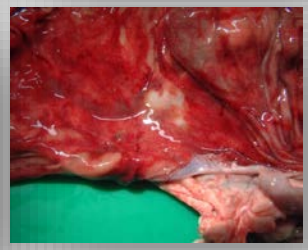
Sampling of ewes in Alberta 2014

ALMA Alberta Livestock and Meat Agency Ltd.



Fecal egg counts

Farm%	Mean%
1"	162"
3"	2748"
4"	458"
7"	563"
11"	392"
15"	700"
18"	8"
19"	0"
20"	2201"
21"	1092"
22"	1604"
23"	1861"
24"	4507"
25"	217"



	Fenbendazole		Ivermectin	
Farm	% Reduction	95% CI	% Reduction	95% CI
7	24%	0%-83%	77%	10%-94%
15	70%	13%-90%	41%	0%-79%
20	38%	0%-71%	78%	33%-93%
24	-94%	0%-22%	-33%	0%-48%

-Clinical Haemonchosis has become common in Alberta sheep flocks

-Resistance to ivermectin and fenbendazole is well established

Gilleard Research Group Program at UCVM

Anthelmintic Drug Resistance in Livestock Parasitic Nematodes

Basic Research

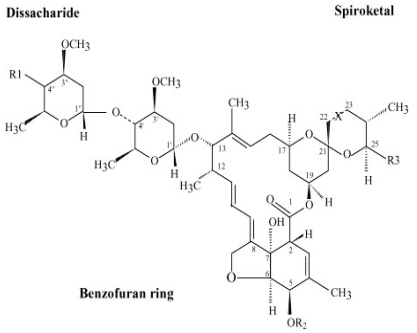
Genomic and genetic approaches to understand the mechanisms of drug resistance in parasites

Applied / Translational Research

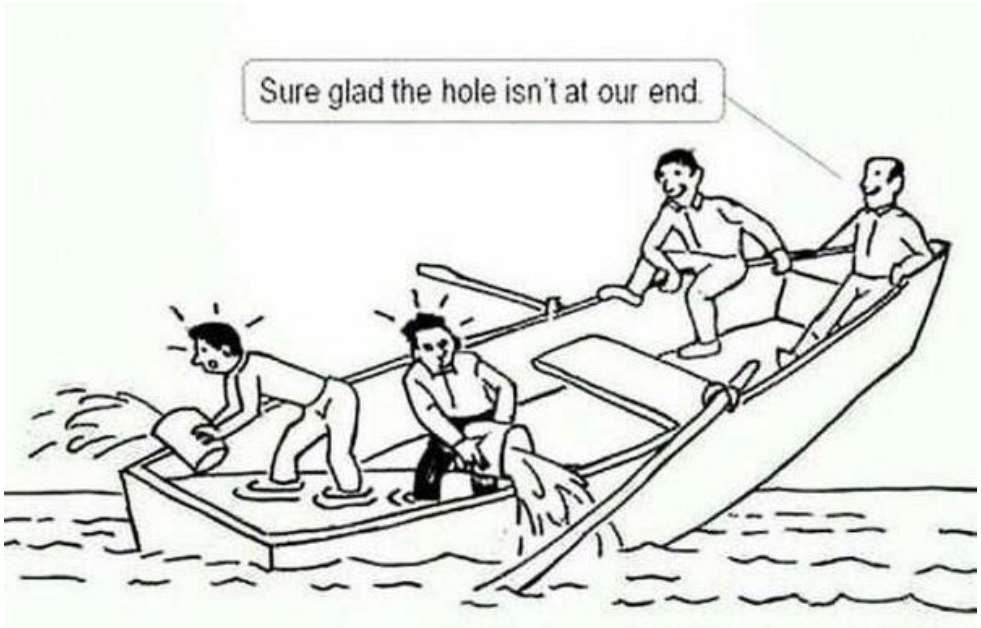
Improved Diagnostics and Development of Evidence-based Sustainable control

Parasite control in livestock has been dependent on highly effective and relatively cheap anthelmintic drugs for many years

Ivermectin



The emergence of anthelmintic drug resistance



Anthelmintic resistance – worldwide problem in livestock

Prevalence of farms with anthelmintic resistant parasites (%)

Cattle

	NZ	Brazil	US	W. Australia
ML	74	92	30	59
BZ	76	20	?	50
LEV	6	8	?	67

Sheep

	Australia	S.Africa	Brazil	UK
ML	60	73	13	30% +
BZ	90	79	90	70-80%
LEV	80	73	84	30% +

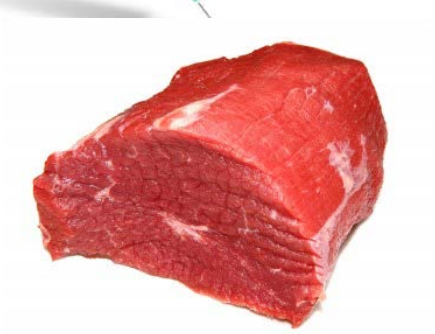
Bison ???

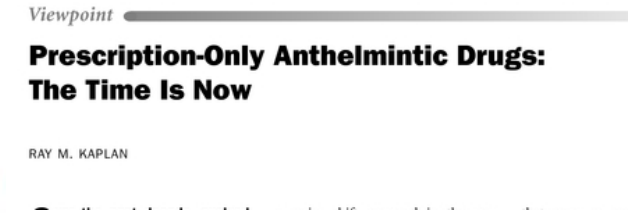
Veterinary Record (2005) 156, 105-109

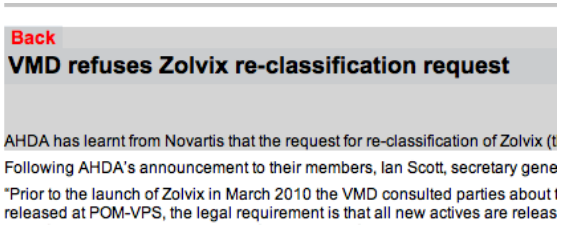
(Kaplan,R., Veterinary Parasitology 186 (2012) 70– 78)

(Cotter et al Vet Parasitol. 2015 Jan 30;207(3-4):276-84)

Consumer and Retailer Demands and Regulatory Issues







FVE recommends in order containing anthelmintic resistance that:

1. **<u>All anthelmintic products for food producing animals</u> shall be only available upon <u>veterinary prescription</u>,** so that their use is conditional on appropriate veterinary advice; FVE calls EU legislators to make this change in the revision of Directive 2001/82/EC

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Parasites control in livestock : time to rethink

Current approaches to livestock parasite control are:

- Unsustainable (anthelmintic resistance)
- Increasingly incompatible with modern consumer attitudes

Immediate Research Priorities

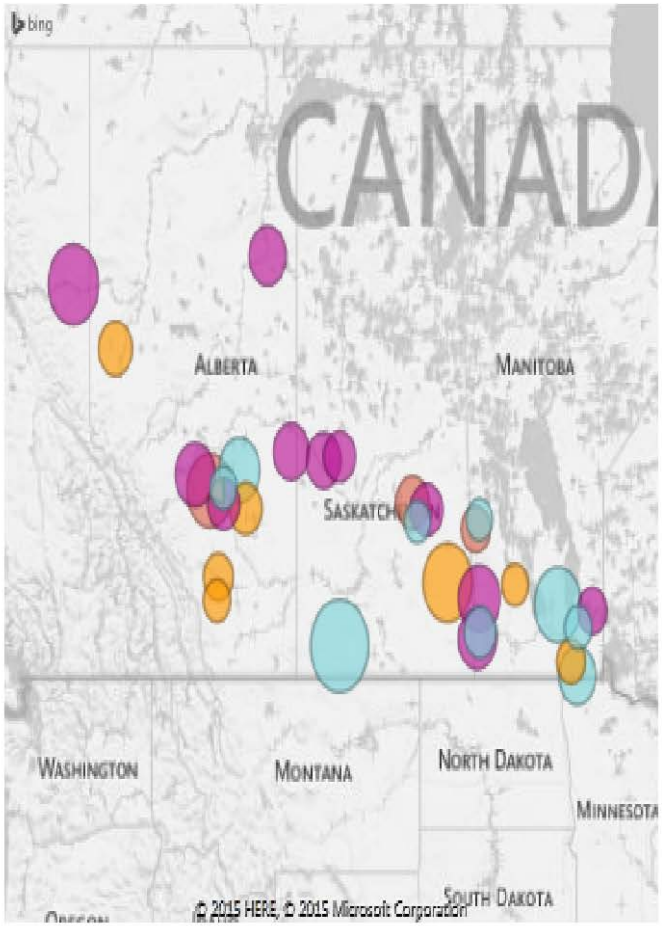
- Better diagnostic tests
- Better understanding of epidemiology
- Exploring evidence-based control strategies

Long Term Research Prioirties

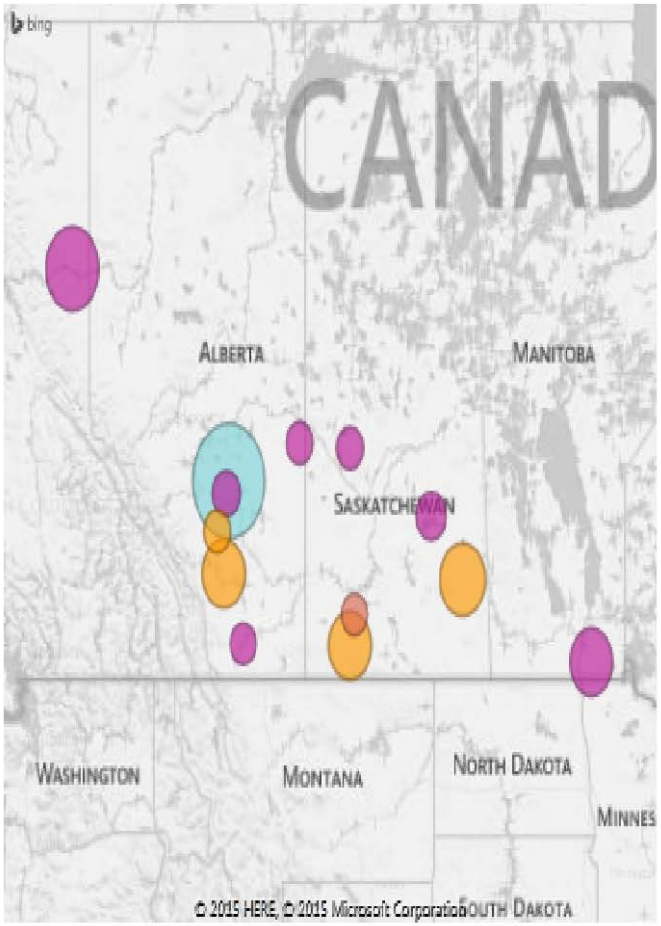
-New drugs and vaccines

Summary of Bison Herd Locations, fecal egg counts and anthelmintic class used 12 months previous to sampling

Cow-Calf



Yearlings /Feeders



FB
IVM
NT
UNKNOWN

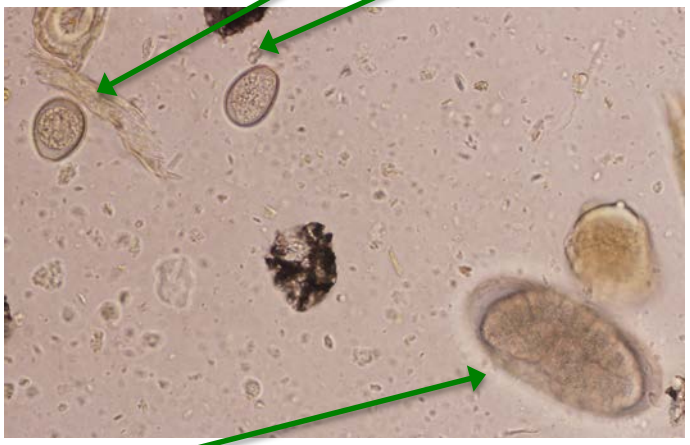
Samples collected Oct 2014-Jan 2015

ALMA
Alberta Livestock and Meat Agency Ltd.

Parasites found in Bison feces

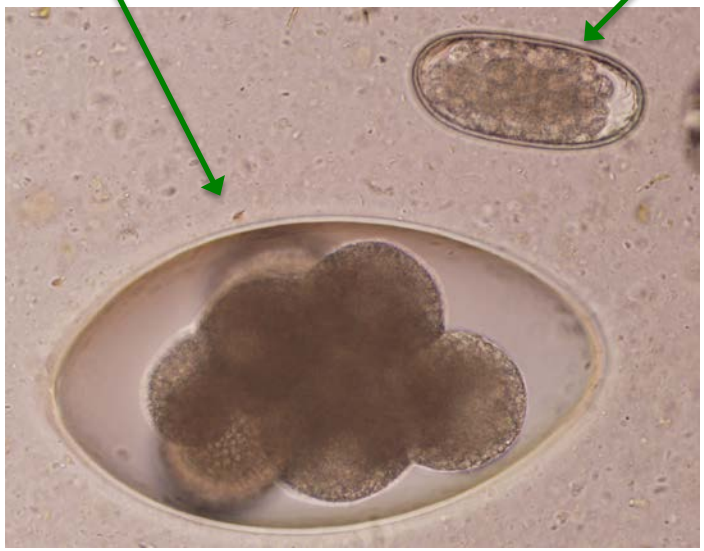
Capillaria

Eimeria



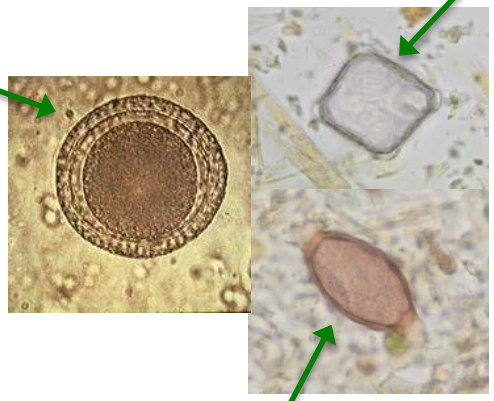
Trichostrongyles

Nematodirus



Monieza

Toxocara



Trichuris

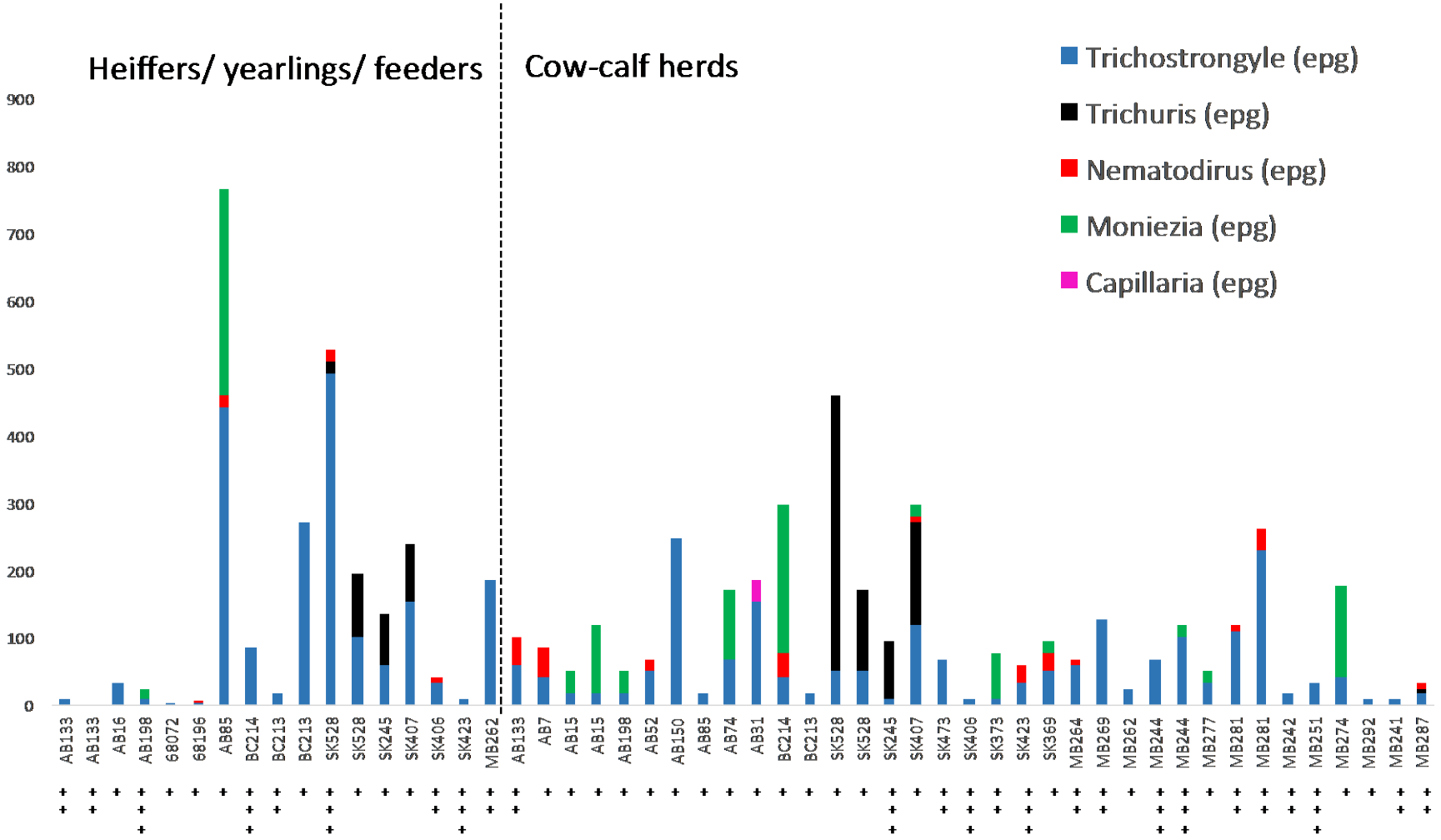
100 µm

Herd Prevalence of Major Parasite Groups

	This study Oct 2014- Jan 2015	Woodbury study Jun-Aug 2012*
Trichostrongyle sp. (stomach worms)	94.15%	100%
Eimeria sp. (coccidiosis)	82.35%	95%
Moniezia sp (tapeworm)	23.53%	72%
Capillaria sp. (Hairworm)	14.71%	13%
Nematodirus sp	29.41%	13%
Trichuris sp. (Whipworm)	11.76%	5%
		0

* Woodbury et al 2014, Canadian Veterinary Journal (2014) ,55, 870-874. 98 Bison farms in Manitoba and Saskatchewan

Fecal egg counts from Bison herds sampled across Alberta, Saskatchewan and Manitoba



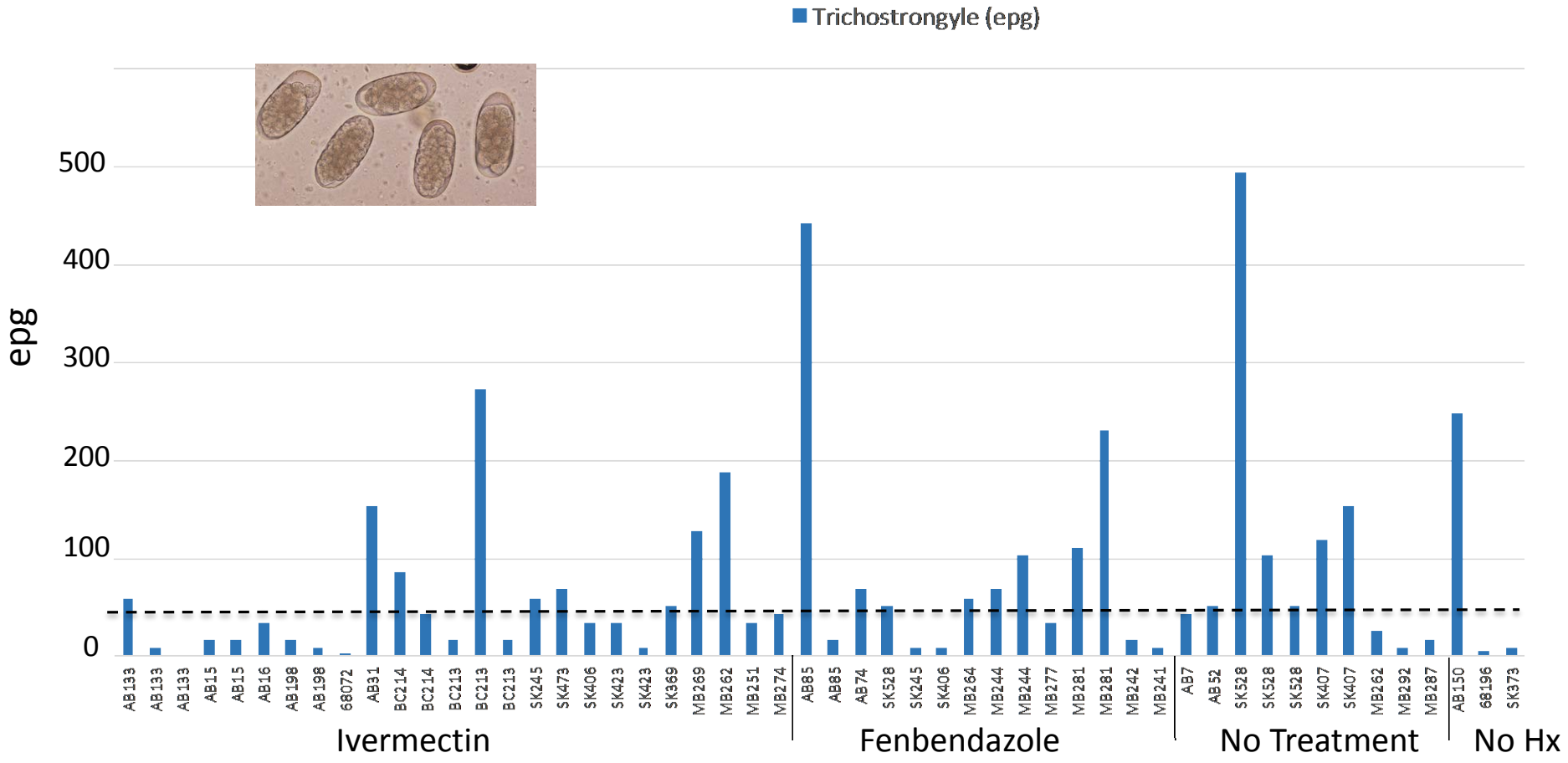
Eimeria oocysts/g

+ 1-400

++ 400-1000

+++ >1000

Strongyle egg counts from Bison herds across Alberta, Saskatchewan and Manitoba



Samples ordered by last anthelmintic drug treatment

Summary of producer questionnaire on parasite control 1

Major operation type, n (%)	
Cow-calf to finish	19 (39%)
Cow-calf that sells breeding stock or yearlings or calves	14 (28%)
Feedlot and Background	7 (14%)
Feedyard	9 (19%)

# times per year animals dewormed, n (%)	
No deworming	7 (14%)
Once per year	26 (53%)
Twice per year	16 (33%)

Done any fecal testing in last 5 years, n (%)	
Yes	34 (69%)
No	15 (31%)

Reasons to deworm, n (%)	
FEC and preventive measure	2 (5%)
FEC + preventive measure + thin or doing poorly	6 (14%)
Preventive measure	23 (51%)
Preventive measure + thin or doing poorly	13 (30%)

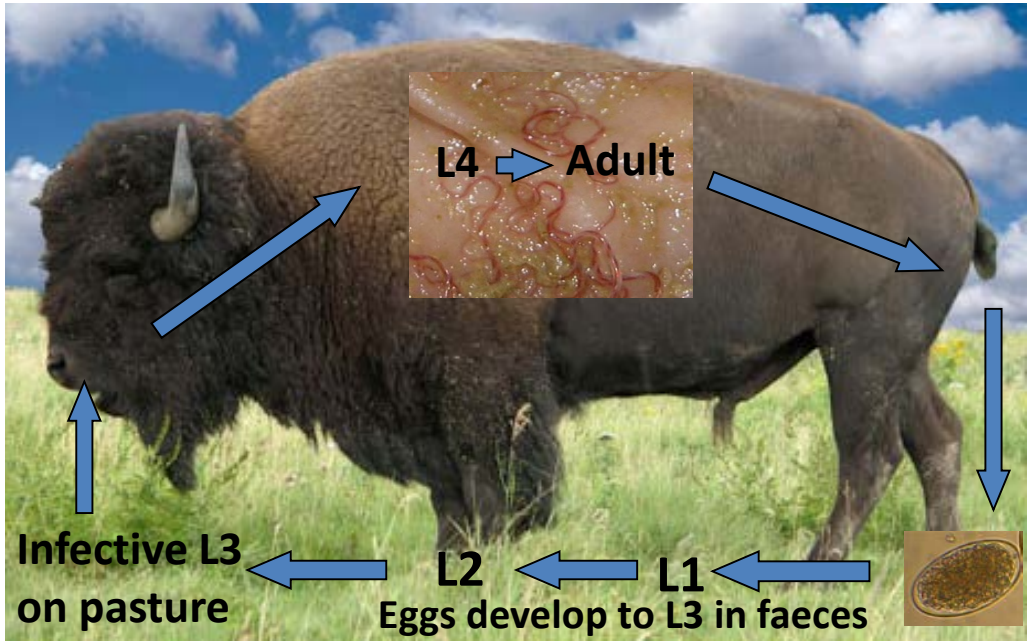
Calculate dewormer dose by: n (%)	
Average weight of group	30 (68%)
Individual weight of bison	14 (32%)

Summary of producer questionnaire on parasite control 2

Dewormer used annually, n (%)	
None	7 (14%)
Diatomaceous	1 (2%)
Safeguard	6 (12%)
Ivomectin	2 (4%)
Ivomectin injectable	9 (18%)
Ivomectin inj + Safeguard	8 (16%)
Ivomectin inj + Safeguard + Diatomaceous	1 (2%)
Ivomectin inj + ivomectin pour-on	1 (2%)
Ivomectin pour-on	8 (16%)
Ivomectin pour-on + Safeguard	4 (8%)
Ivomectin + Safeguard	2 (4%)

Gastro-intestinal trichostrongyle nematodes of Bison

Life Cycle



Many species!

Abomasum

Haemonchus placei

(Haemonchus contortus)

Ostertagia ostertagi

Trichostrongylus axei

Orlaffia bisonis

Small Intestine

Cooperia oncophora

Cooperia punctata

Cooperia pectinata

Trichostrongylus spp

Nematodirus helvetianus

Large Intestine

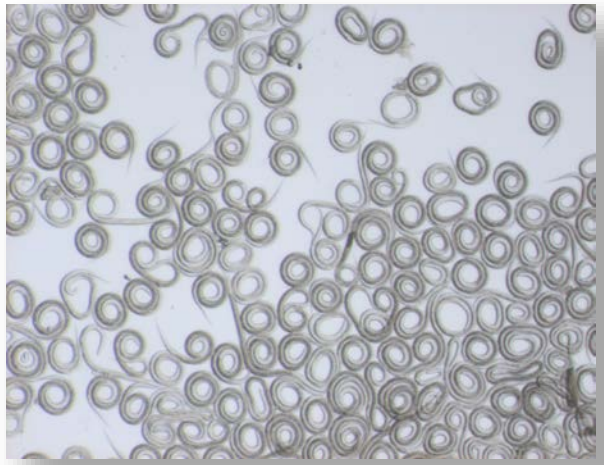
Oesophogostomum radiatum

Improving Livestock Parasite Diagnostics

Traditional Approach

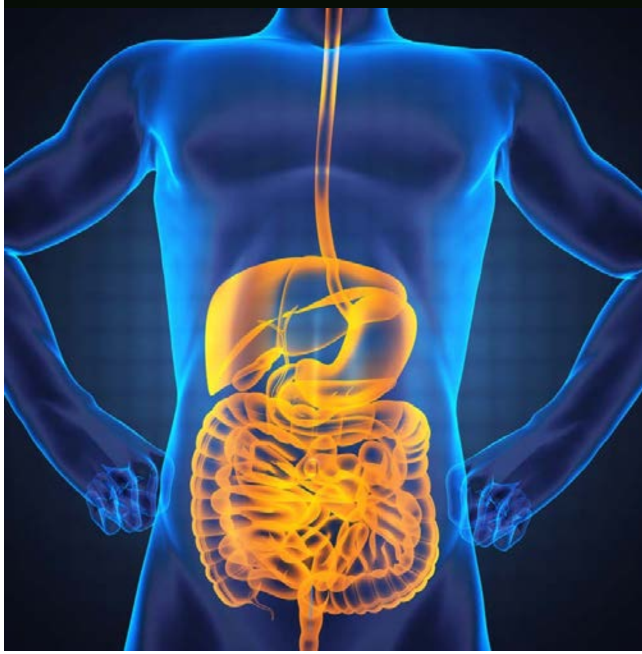
Count eggs in feces

2 weeks incubation



Microscopically identify individual larvae

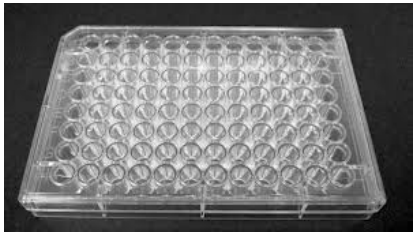
- Laborious
- Requires Specialist Expertise
- Subjective
- Low throughput


microbiome
performed
mention
many
published
different
pregnant
paper
show
levels
link
differently
environment
may
homes
important
connection
also
health
article
gut
immune
stress
study
women
vaginal
research
microbiomes
scientists
authors
non-pregnant
Furthermore
new
system
home
researchers
showed
studies
project
inflammation
anxiety
changes
bacteria
pregnancy
gender
review
mice


Antimatter-Sparked SUPERNOVAE
Building a MACHINE BRAIN
The Rough Road to FUSION ENERGY
SCIENTIFIC AMERICAN
Your Inner Ecosystem
In your body, bacteria outnumber your own cells 10 to 1.
Who's in control?

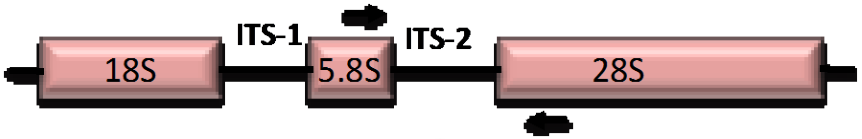

Deep amplicon sequencing assay to study the "Nemabiome"

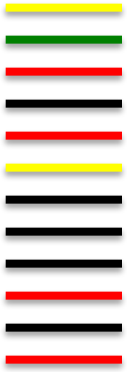
Culture feces and extract larvae from each sample



Prepare DNA from several thousand larvae from each sample

Amplify rDNA ITS-2 PCR from each DNA prep





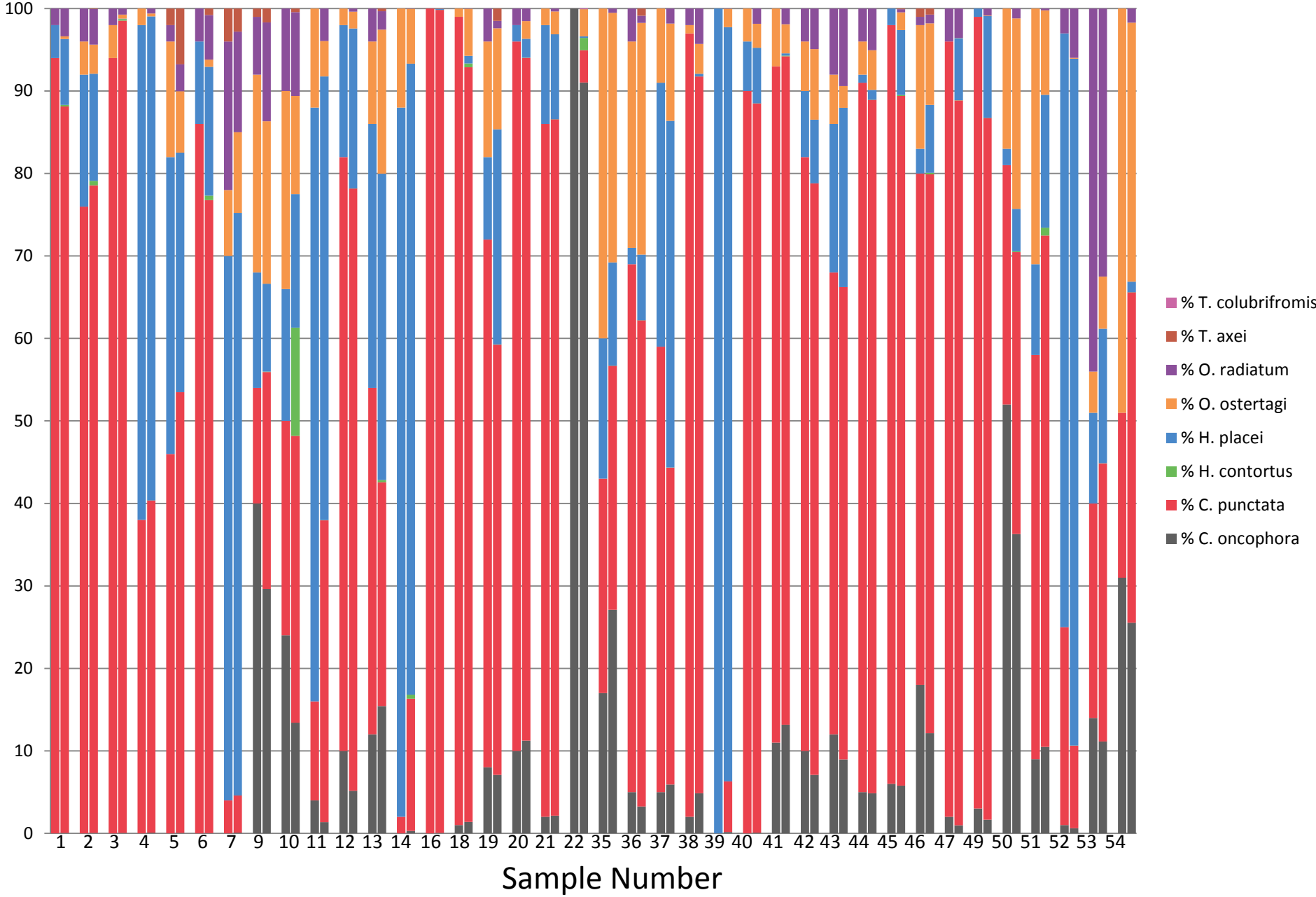
ITS-2 sequences different for each species

Sequence 10,000s of amplified rDNA ITS-2 molecules from each sample

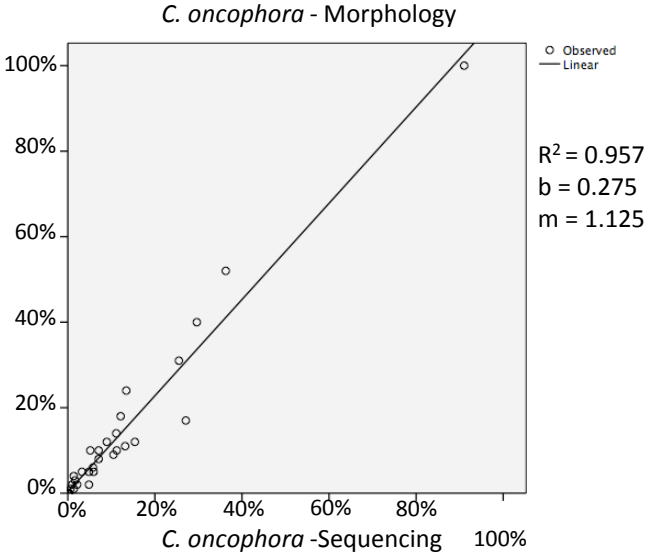
Bioinformatic analysis to cluster and count sequences from each species in each sample

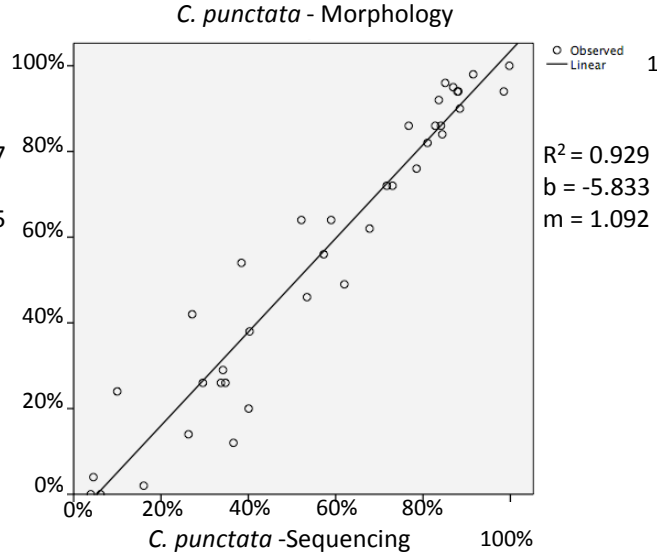
Validation of deep amplicon sequencing by comparison to morphological identification

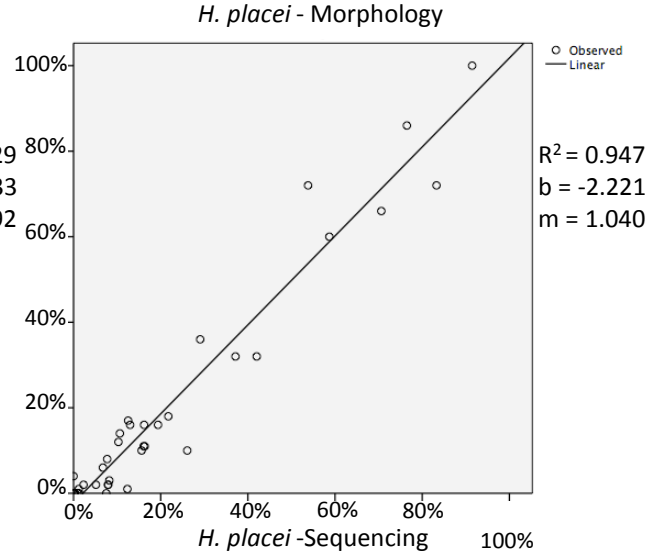
Beef cattle samples from Arkansas, Oklahoma and Nevada (Tom Yazwinski)

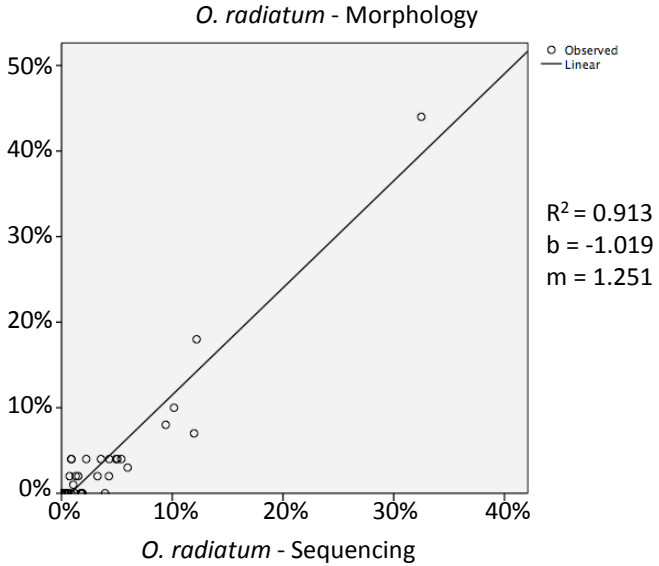


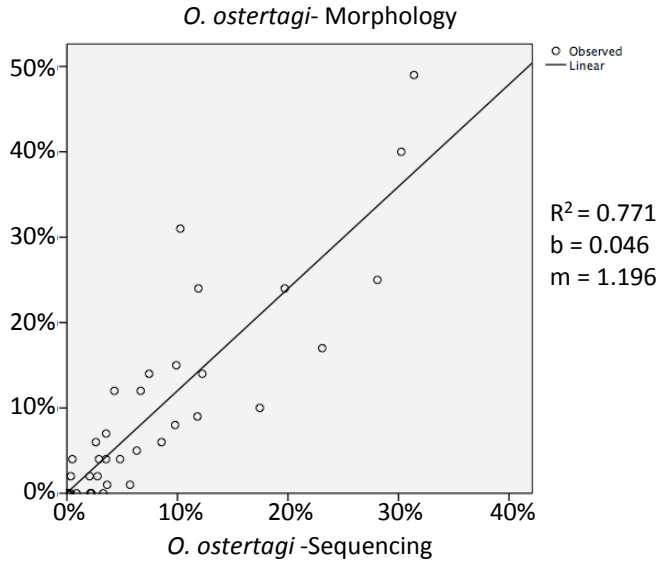
Regression analysis shows good aggreement between morphological identification and deep amplicon sequencing to quantify species composition of parasite communities





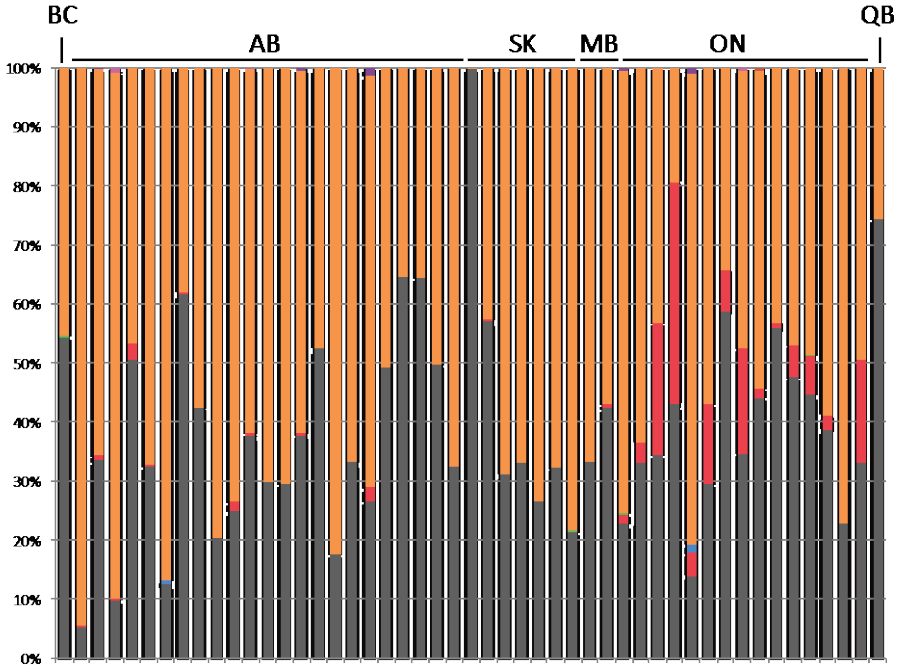




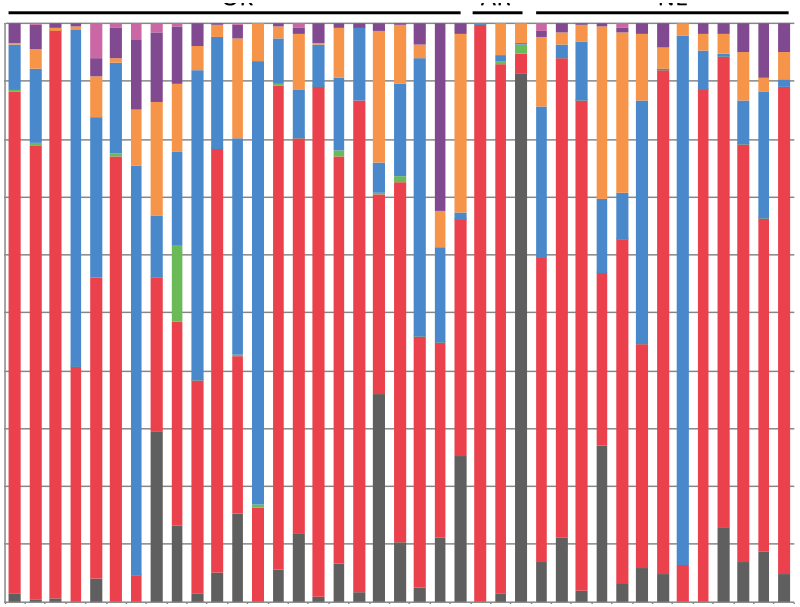


Comparison of trichostrongylid nematode species diversity in CATTLE fecal samples from Canada vs mid-west USA

Canada



USA



Trichostrongylus spp

Nematodirus helvetianus

Oesophagostomum radiatum

Ostertagia ostertagi

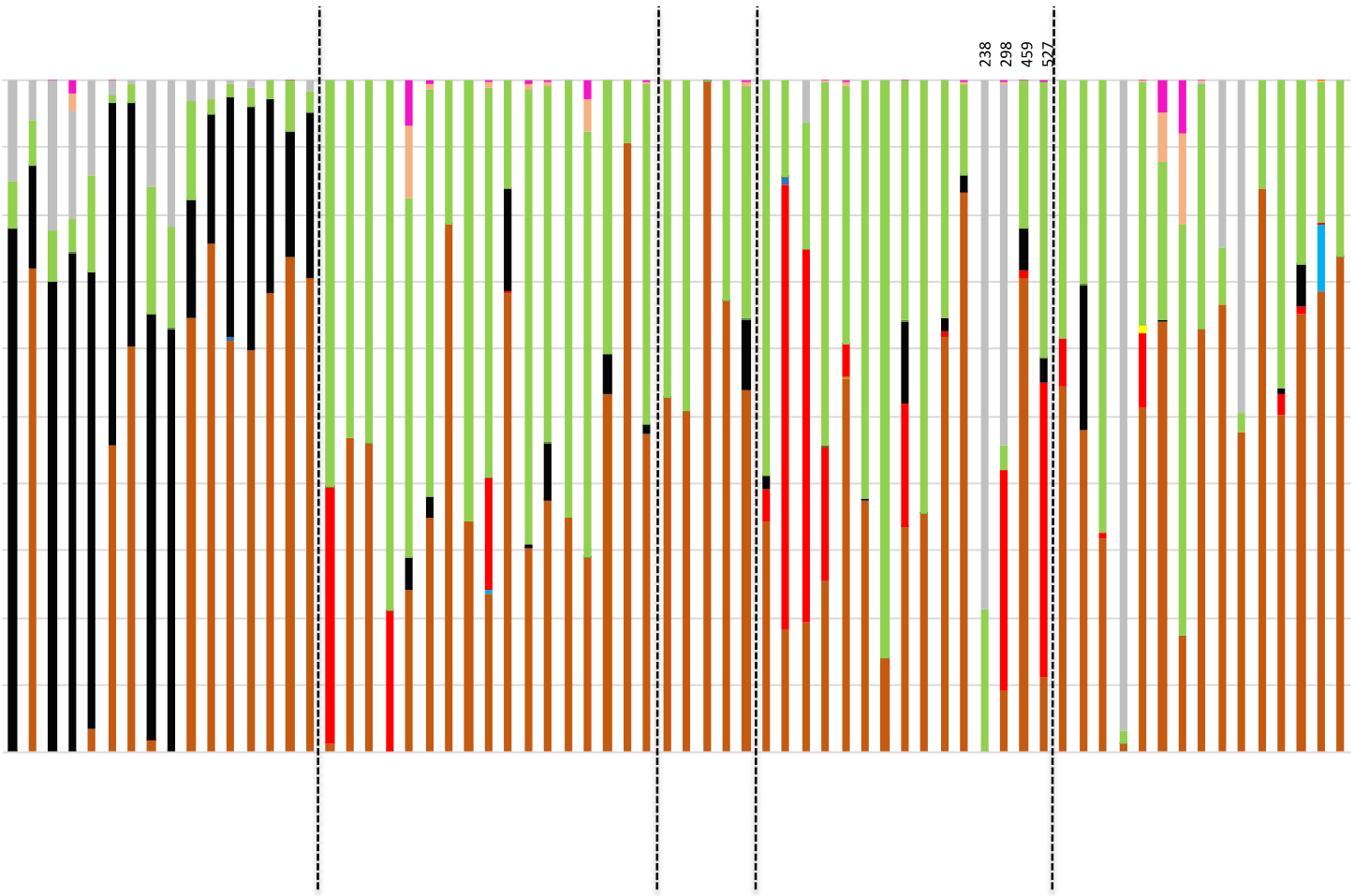
Haemonchus placei

Haemonchus contortus

Cooperia punctata

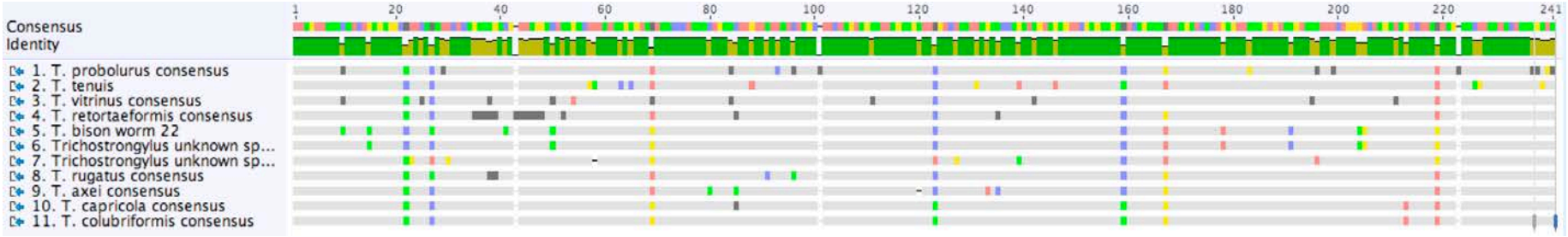
Cooperia oncophora

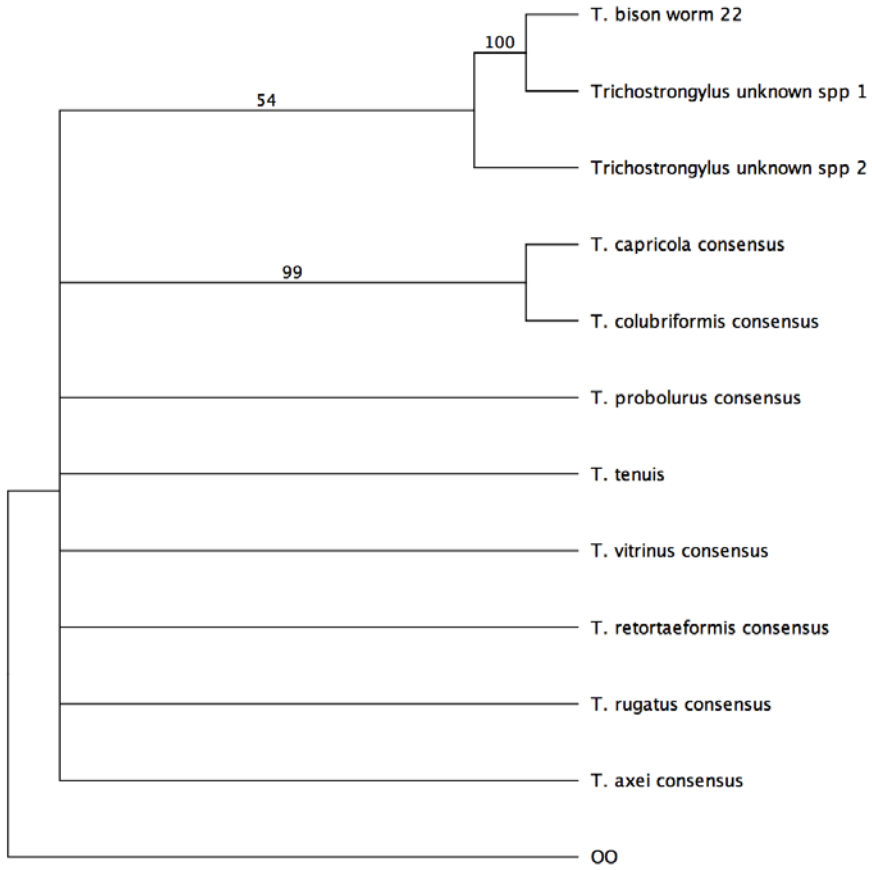
Trichostrongyle species diversity in Bison fecal samples across Western Canada



Samples ordered by province and by FEC rank

Trichostrongylus species





Conclusions

- GI trichostronglyle parasites are not well controlled in many Western Canadian Bison herds
- Many producers perceive parasites produce negative impacts
- Varied anthelmintic control programs in use which are not always effective
- Main trichostrongyle species are *Ostertagia ostertagi* and *Cooperia oncophora* as in cattle
- *Haemonchus placei* present at high frequency in a number of Bison herds
- Trichostrongylus axei present at high frequency in a few herds.
- New “next-gen” sequencing diagnostic test for relative quantitation of trichostrongyle species in Bison fecal samples

Future work

- **Investigation of reasons for poor control**

- **Determine extent of anthelmintic resistance in Bison parasites**

  - Pre and Post-Treatment sampling (Fecal egg count reduction test)

  - Molecular tests to determine frequency of resistance mutations in parasite populations

- **Better understanding of epidemiology, prodcution and health impact of the different parasite species in Bison herds**

Acknowledgements

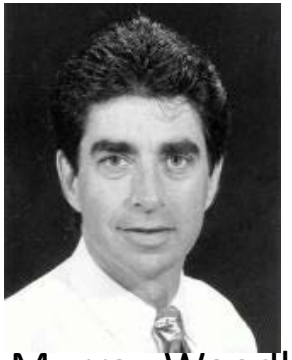

Dr Libby Redman


Russell Avramenko


Ana Bras


Thom Oostenbrug


Dr Roy Lewis (Merck)


Murray Woodbury


Claire Windeyer


Tom Yazwinski

All the producers involved in the studies!

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