Effect of breed on the abundance and expression of *Escherichia coli* Shiga toxin genes in the recto-anal junction of feedlot beef cattle

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Introduction

- Shiga toxin producing *Escherichia coli* (STEC) cause one of the most important foodborne disease.
- Many human infections are attributed to beef consumption, or to food contamination by cattle. Cattle are the main reservoir of STEC with rectal-anal junction (RAJ) as the main colonization site.

Knowledge Gap

- Many studies focused on STEC O157, however, non-O157 strains produce Stx and can cause severe human disease.
- Our previous study found differential gene expressions between super shedders (SS shed >10^4 CFU *E.coli* O157, super-shedder) and non-SS cattle. However, whether the abundance of *Stx* genes in RAJ can be influenced by cattle breed is unknown. It is also unknown if host gene expression correlates with *Stx* expression.

Methods

- In total, rectal tissue and contents were collected from 143 beef steers composed of three breeds (Angus, Charolais and Kinsella Composite) in 2014 and 2015.

q-PCR, qRT-PCR for *Stx* gene and four host genes (*MS4A1, CCL21, CD19,LTB*)

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PROC MIXED model: the effect of breed, sampling year, sample type

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Correlation analysis: relation between the expression of *Stx* gene and the relative expression of host genes

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The random forest model and Boruta method: the predictiveness of host genes for *Stx* gene expression.

Results

Abundance of *stx1* and *stx2* using q-PCR for samples collected from rectal tissue and contents in 2014 and 2015

Year	Breed	AN		CH		KC		P-Value		
	Tppe	T	C	T	C	T	C	Breed	Type	Breed*Type
2014	*Stx1* Mean	N/D	4.09	N/D	1.73	N/D	1.40	<0.0001***	<0.0001***	<0.0001***
	Stx1 SE	0	5.20	0	5.79	0	5.47			
2014	*Stx2* Mean	6.02	4.92	5.31	5.91	5.70	1.00	<0.0001***	<0.0001***	<0.0001***
	Stx2 SE	0.08	1.01	0.05	0.22	0.05	4.65			
2015	*Stx1* Mean	6.78	0.25	6.82	N/D	6.76	N/D	0.31	<0.0001***	0.28
	Stx1 SE	0.02	1.11	0.03	0	0.03	0			
2015	*Stx2* Mean	5.70	4.58	5.73	4.91	5.67	5.06	0.17	<0.0001***	0.12
	Stx2 SE	0.02	1.58	0.03	0.20	0.03	0.31			

Table1. The abundance analysis of *Stx1* and *Stx2* gene

*T means tissue sample, C means content sample. H-/L-RFI means low or high feed efficiency, respectively. We use $\log_{10}$ transformed copy numbers to represent the gene abundance.

- The abundance of *Stx1* and *Stx2* in each sampling year was significantly difference (P<0.01) and results were separated by sampling year.
- **For samples collected in 2014:**

The interaction effect between breeds and sample type was significant ($P_{Stx1}<0.001$, $P_{Stx2}<0.001$).

The Stx1 gene was not detected in rectal tissue.

The Stx1 gene was more abundant in breed AN compared to CH and KC (P<0.001) in colonic contents.

The abundance of *Stx2* gene was not significant in tissue.

The *Stx2* gene was more abundant in contents of AN and CH cattle compared to KC steers (P<0.0001).

- **For samples collected in 2015:**

The interaction effect between breeds and sample type was insignificant ($P_{Stx1}=0.28$, $P_{Stx2}=0.12$).

The abundance of Stx1 or Stx2 gene was insignificant among three breeds, respectively ($P_{Stx1}=0.31$, $P_{Stx2}=0.17$,).

The abundance of *Stx1* and *Stx2* genes in tissue samples were higher compared to that in contents ($P_{Stx1}<0.001$, $P_{Stx2}<0.001$), respectively.

Correlation analysis between relative expressions of host genes and *Stx gene* expression

		Stx2RNA	*MS4A1*	*CD19*	*CCL21*	*LTB*
Stx2RNA	R-Value	1.00	-0.56	0.51	-0.44	0.60
	P-Value	0.00	0.05*	0.08	0.13	0.03*
MS4A1	R-Value		1.00	-0.55	0.39	-0.56
	P-Value		0.00	0.05*	0.19	0.05*
CD19	R-Value			1.00	0.19	0.98
	P-Value			0.00	0.53	0.00***
CCL21	R-Value				1.00	0.09
	P-Value				0.00	0.78
LTB	R-Value					1.00
	P-Value					0.00

Table2. The correlation analysis of the gene expression of *Stx2* gene and host genes

*Previous study showed differential expressions of *MS4A1, CCL21, CD19, LTB* in SS compared to non-SS.

- Expression of *Stx1* gene was not detected in samples collected in 2014 or 2015.
- *Stx2* gene expression was found in only 13 samples.
- *Stx2* gene was negatively correlated with the relative expression of *MS4A1* (R=-0.56, P=0.05) and positively correlated with the relative expression of *LTB* (R=0.60, P=0.05).

The predictiveness of host genes serving as markers for *Stx2* expression using random forest model and Boruta method

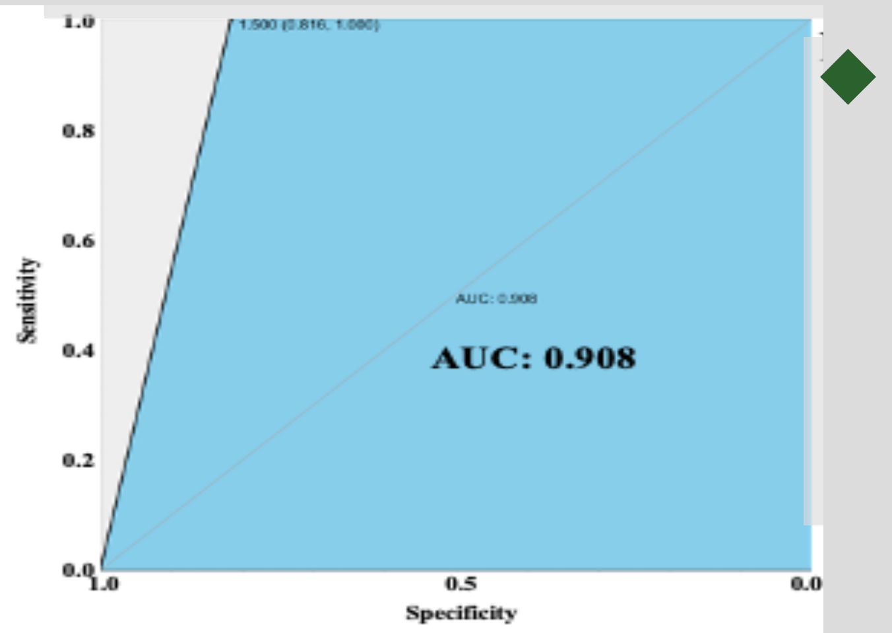

Figure1. The assessment of random forest model using ROC curve.

*Two-thirds of each group (Stx2 expressed vs non-expressed) was split to the training data, and the rest (one-third) was the validation data.

- The random forest model indicated selected host genes can serve as markers for *Stx2* with a prediction accuracy of *MS4A1>LTB>CD19>CCL21*.
- The assessment of the random forest model:

The accuracy of training data was 96.5% and 93.6% of validation data.

The area under ROC curve (AUC) was used to evaluate the robustness of this model with AUC equals to 0.908 indicating a good prediction model.

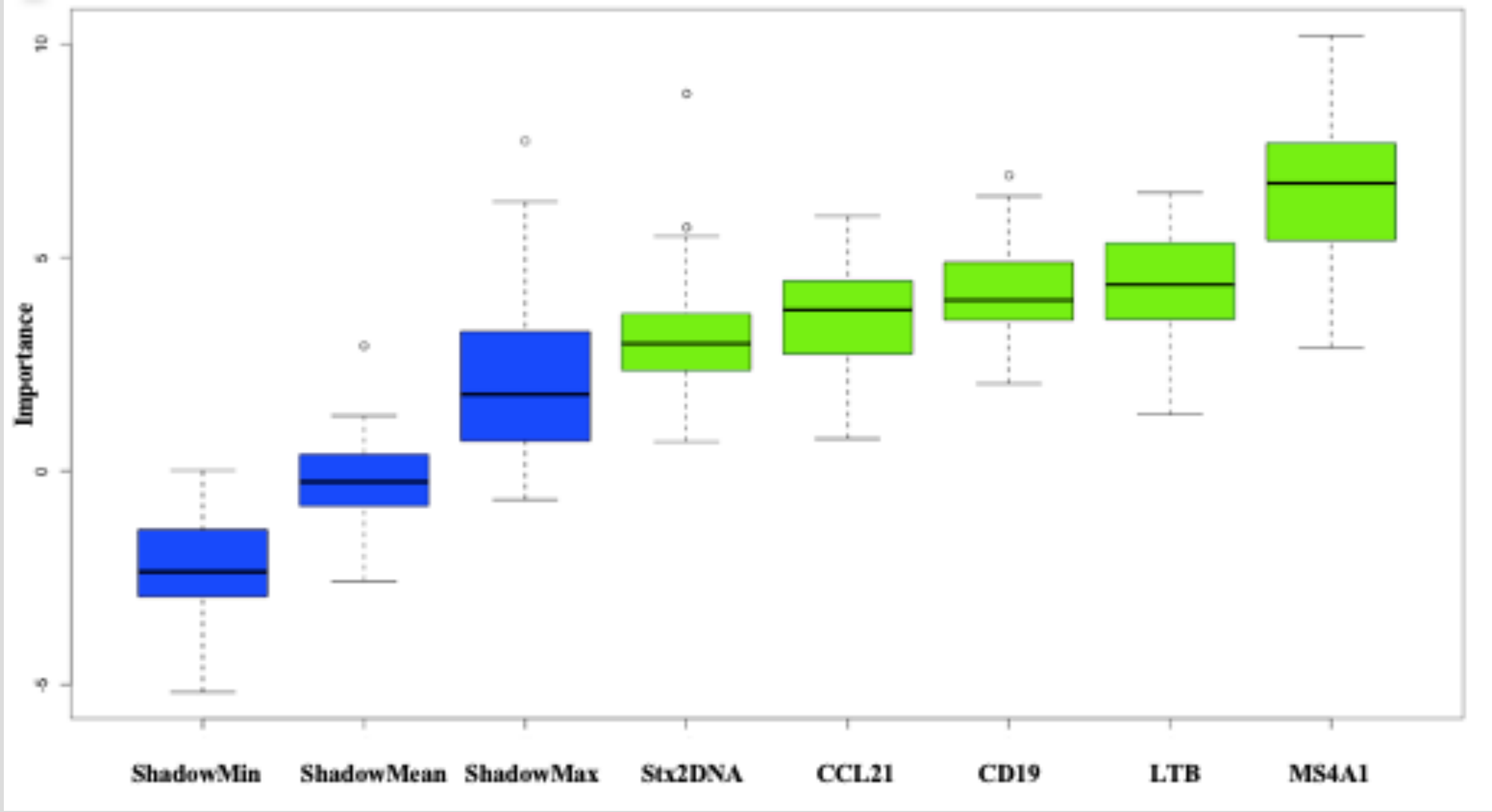

Figure2. The rank of host gene importance using Boruta approach.

- All selected host genes were attributes for identification of *Stx2* expression.
- The rank of host genes based on Boruta method was *MS4A1> LTB=CD19 > CCL21.*

Conclusions

- Cattle genetic background together with sample year effect could affect the abundance of *Stx1* and *Stx2* gene.
- We established the preliminary predicting model using host gene expressions as markers to forecast *Stx2* expression. However, further validation will be needed to support our model.

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