



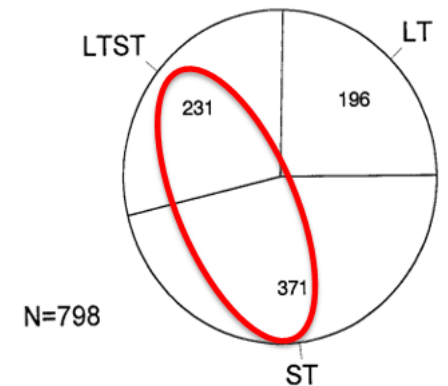
**ECMIS 2019 Ghent**

**Toxoid fusions STa<sub>toxoid</sub>-mnLT<sub>R192G/L211A</sub> induce neutralizing antibodies  
against STa but show little cross-reactivity with guanylin or  
uroguanylin**

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# Background

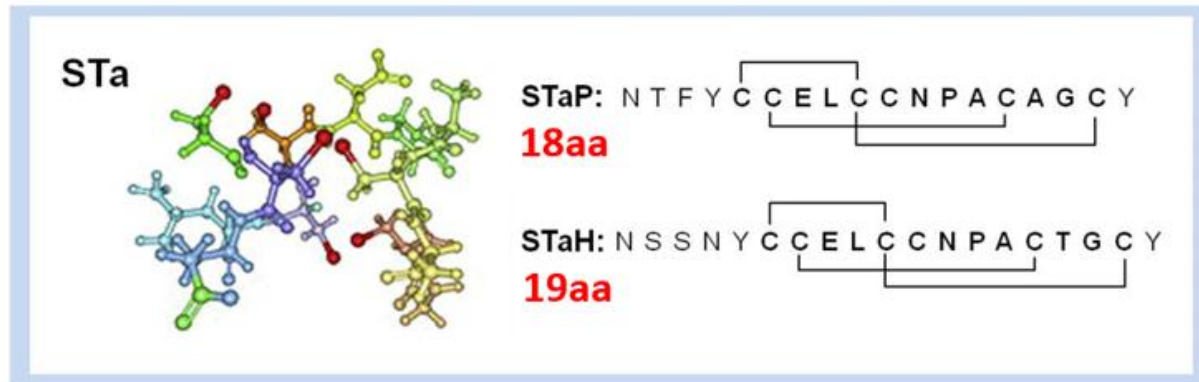
- Enterotoxigenic *Escherichia coli* (ETEC) is the leading cause of diarrhea in children (380-500 million), adult travelers (400 million), and also neonatal and post-weaning animals.
- ETEC strains expressing STa alone or together with LT, cause about 2/3 of ETEC diarrhea cases in humans.
- However, there is no licensed vaccines.



Toxin distribution of ETEC isolates in the database.

Wolf, 1997. *Clin. Microbiol. Rev.* 10 (4):569–584

# Background



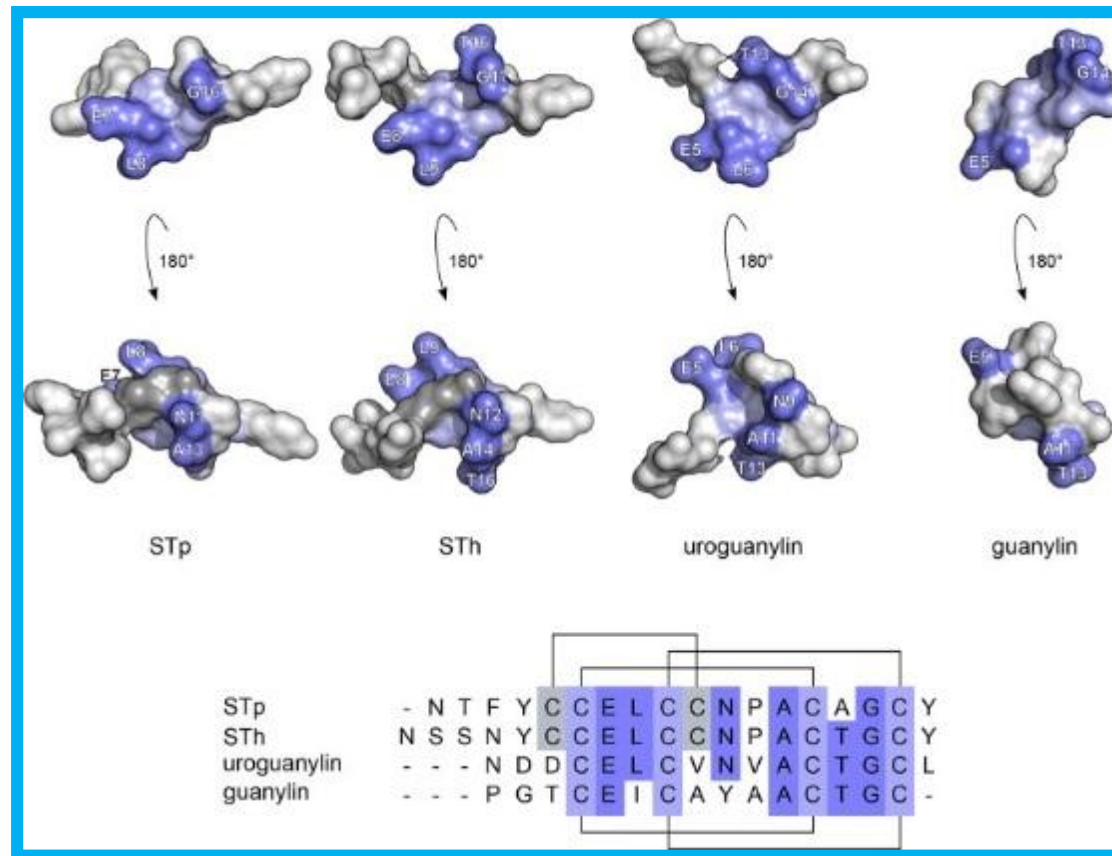
Small peptide;  
Three disulfide bonds;  
Poorly immunogenic;  
Potent toxin.

Nataro et al., 1998. *Clin. Microbiol. Rev.* 11(1):142-201.

**One key challenge in ETEC vaccine development is the inability to use safe STa antigens to induce neutralizing anti-STa antibodies.**

# Background

Guanylin and uroguanylin are endogenous GC-C ligands, and similar to STa in amino acid sequence, structure and function.

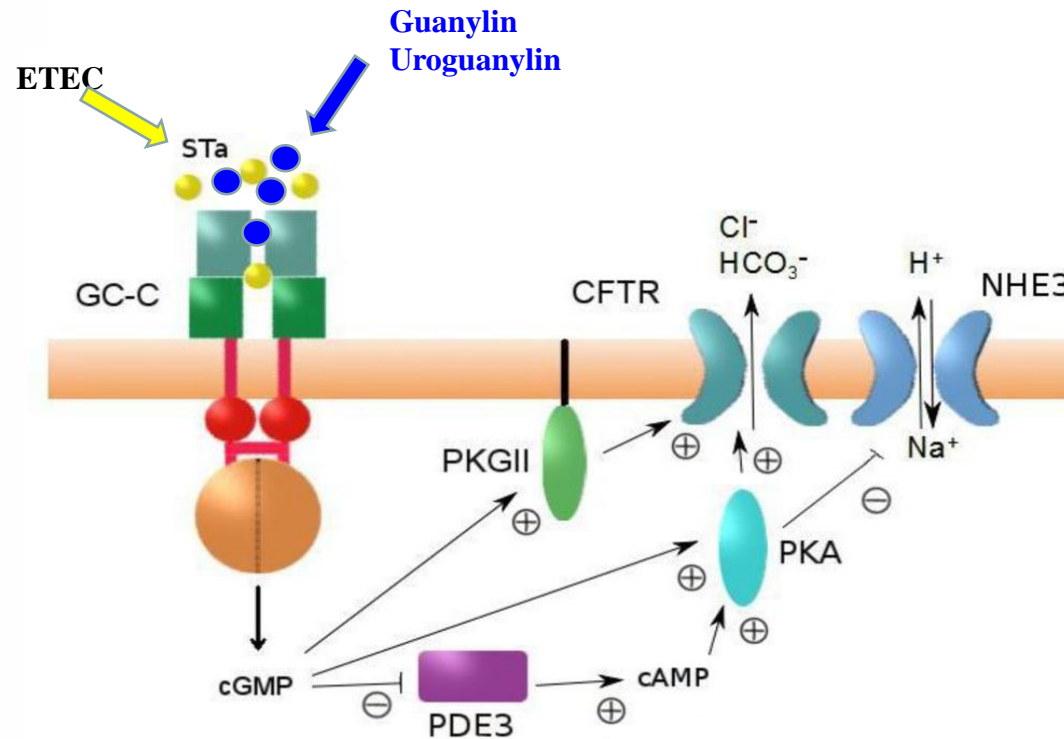


Structure and sequence comparisons of STp, STh, Guanylin and Uroguanylin

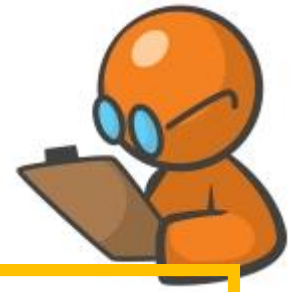
Taxt AM et al., 2014

# Background

## The molecular mechanism of STa and guanylin peptides



# Background



**Recent studies demonstrated that STa toxoids and toxoid fusions can induce neutralizing anti-STa antibodies.**



**However, anti-STa antibodies immunological cross-reactivity to the endogenous GC-C ligands guanylin and uroguanylin is a major concern because of the similar to STa in amino acid sequence, structure, and function.**

# Methods



- **Construction of the  $3 \times \text{STa}_{\text{toxoid}}\text{-mnLT}_{\text{R192G/L211A}}$  toxoid fusions**
- **immunization of mice with the fusion proteins and anti-STa and anti-LT IgG antibodies titration**
- **Mouse serum antibody *in vitro* neutralization activity against STa toxicity**
- **Mouse serum antibody cross-reactivity with guanylin and uroguanylin**



### 3×STatoxoid-mnLT<sub>R192G/L211A</sub> toxoid fusions were constructed

**3 × STa<sub>L9A/N12S</sub>-mnLT<sub>R192G/L211A</sub>; 3 × STa<sub>L9A/A14T</sub>-mnLT<sub>R192G/L211A</sub>;**

**3 × STa<sub>N12S/A14T-mnLT<sub>R192G/L211A</sub></sub>; 3 × STa<sub>L9A/N12S/A14H-mnLT<sub>R192G/L211A</sub></sub>**

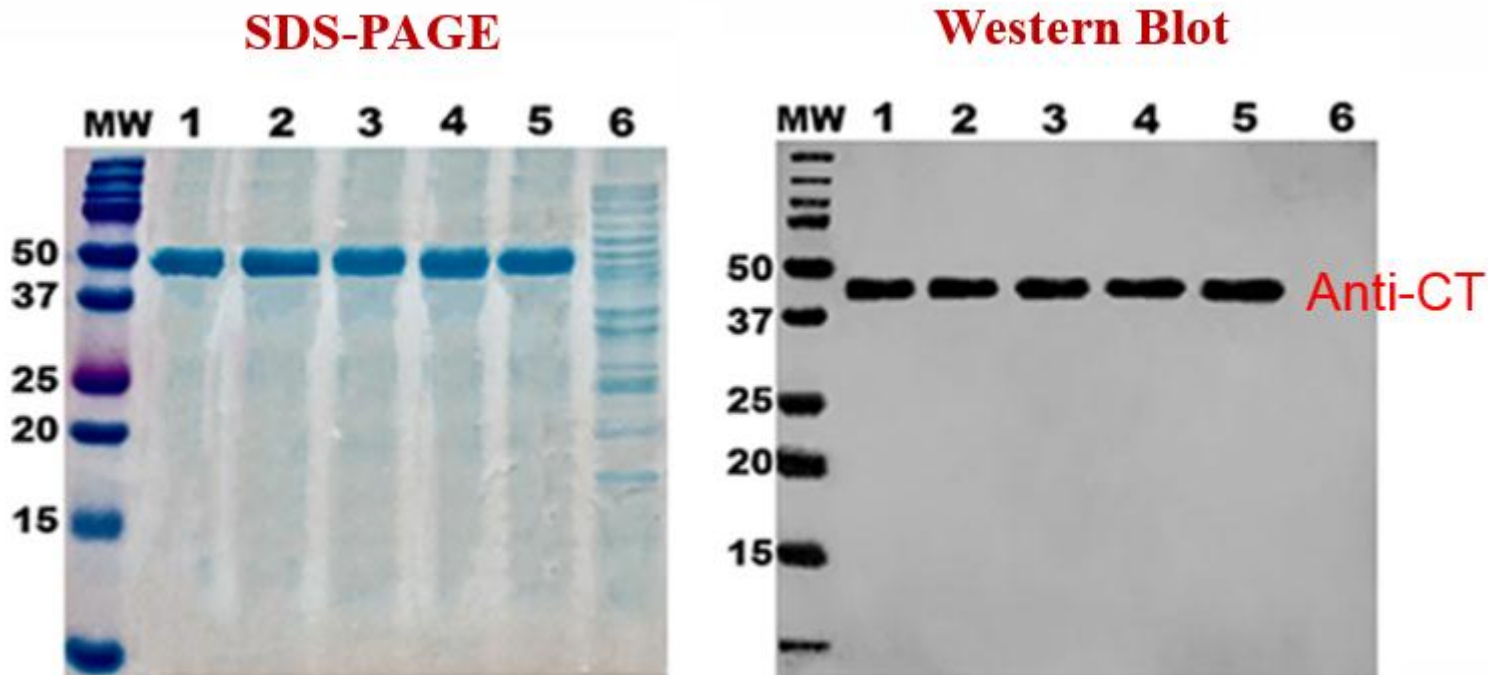
	N	S	S	N	Y	C	C	E	A	C	C	N	P	T/H	A	C	T	G	C	Y
STh																				
uroguanylin	-	-	-	N	D															
guanylin	-	-	-	P	G															





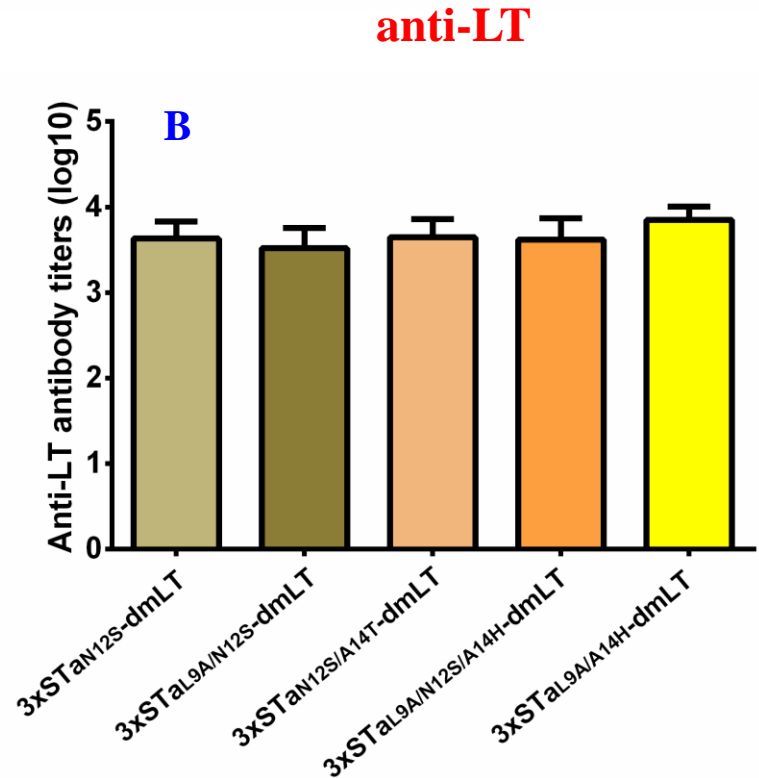
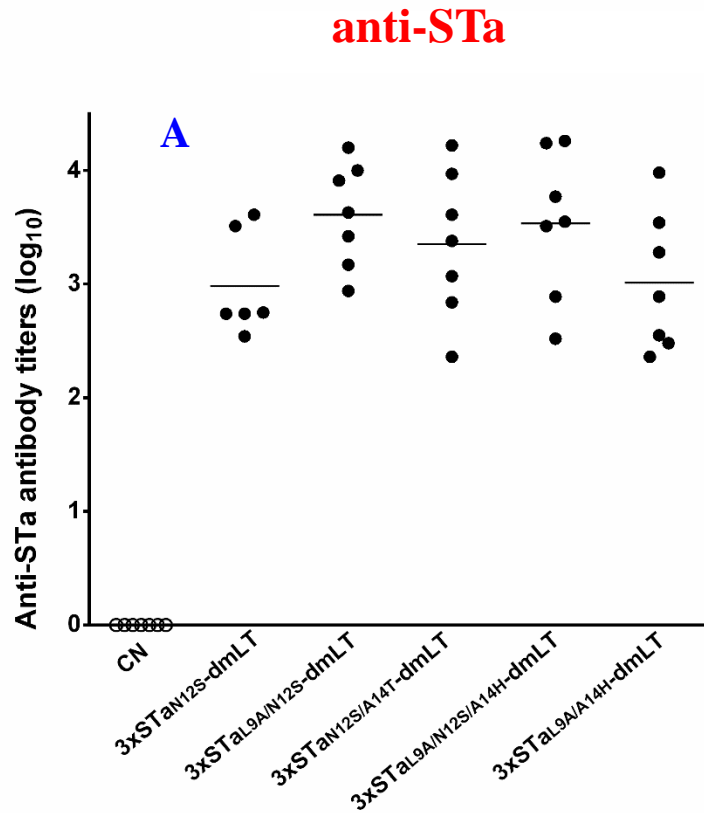
# Results

Fusion proteins were expressed & detected.



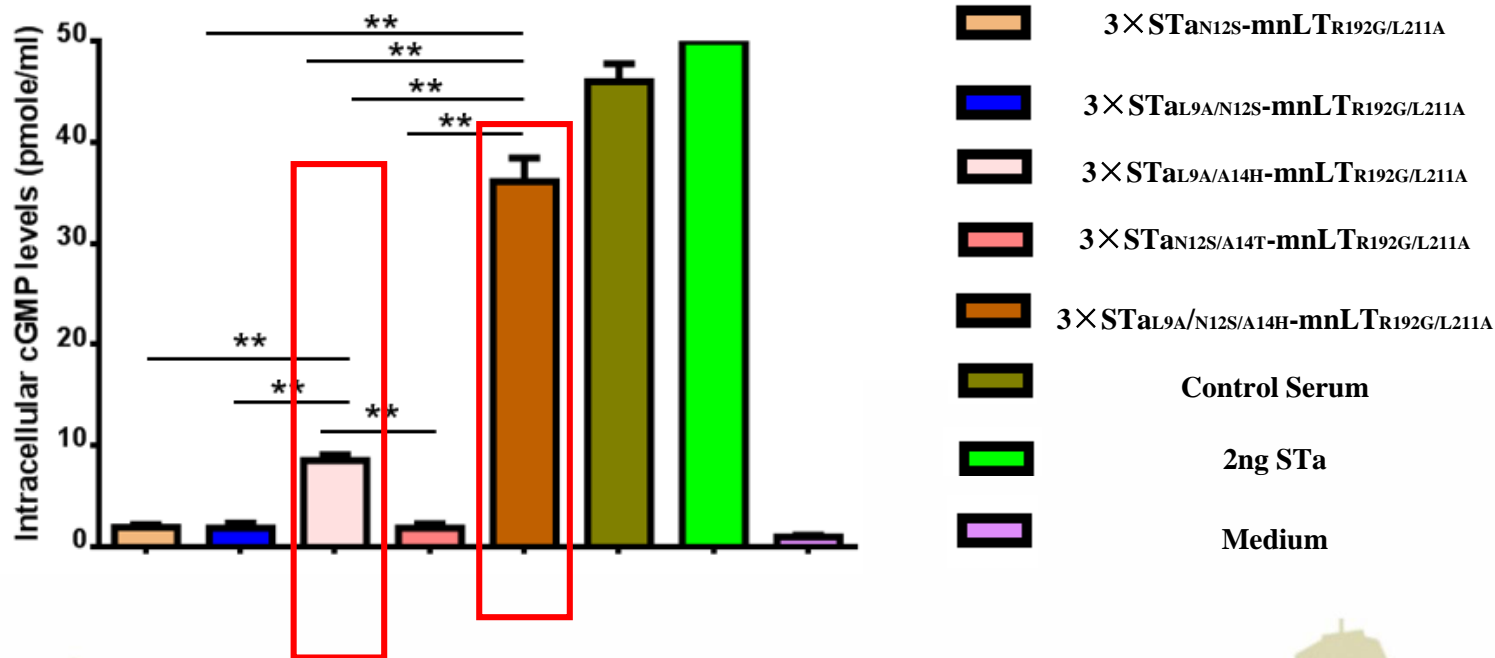
# Results

Toxoid fusions induced both anti-STa and anti-LT antibodies in mice



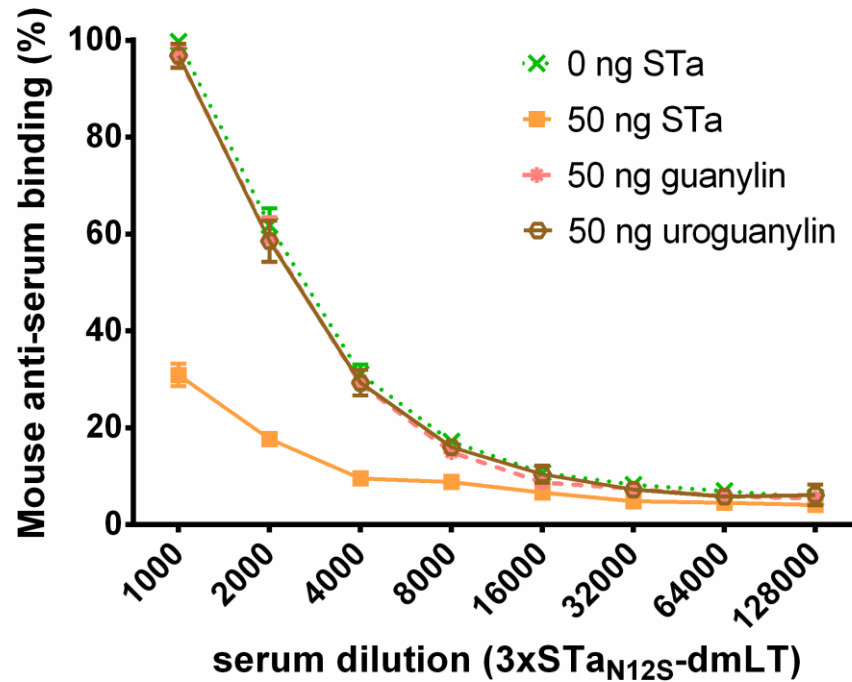
# Results

Serum samples of mice immunized with  $3 \times \text{STa}_{\text{N12S-mnLT}_{\text{R192G/L211A}}}$ ,  $3 \times \text{STa}_{\text{L9A/N12S-mnLT}_{\text{R192G/L211A}}}$ , or  $3 \times \text{STa}_{\text{N12S/A14T-mnLT}_{\text{R192G/L211A}}}$  neutralize against STa toxicity *in vitro*.

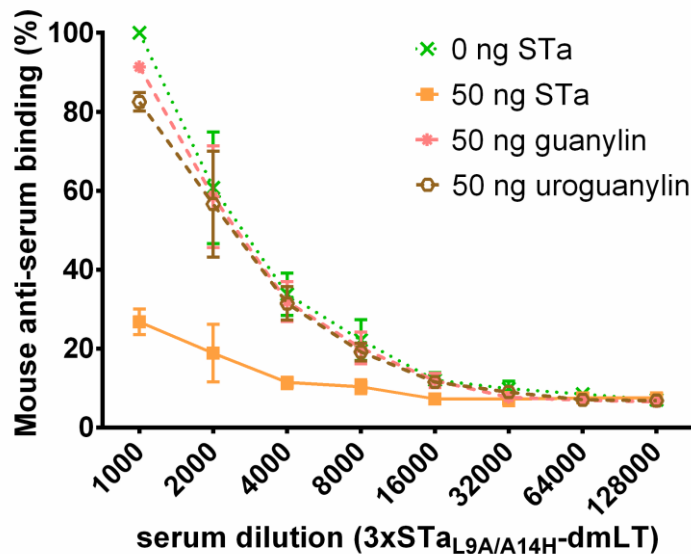
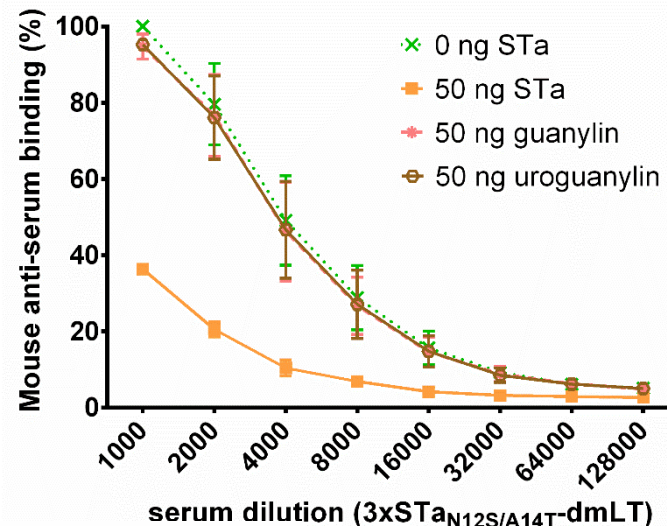
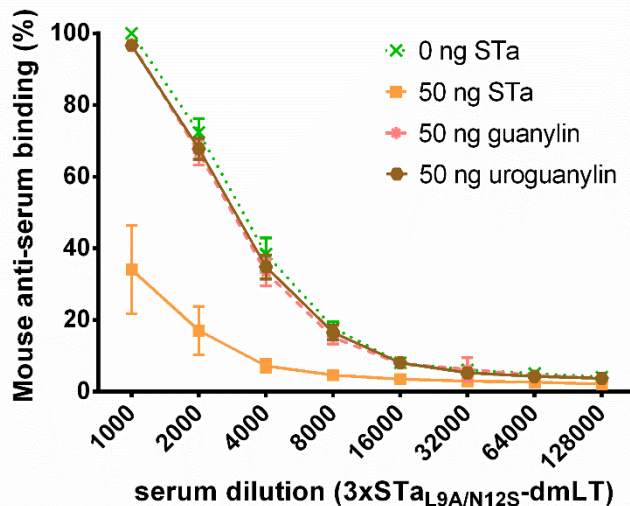


# Results

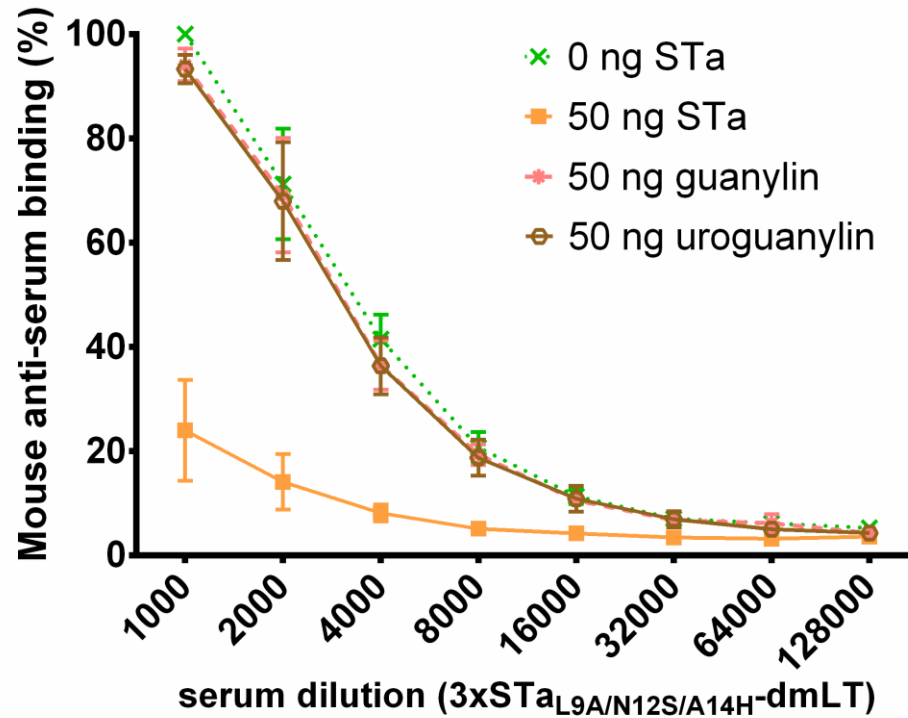
Serum antibodies from mice immunized with the toxoid fusion containing the single, double or triple STa mutants show little or limited cross-reactivity with guanylin or uroguanylin in a competitive STa ELISA



# Results



# Results



# Summary

- ❖ **STa single, double, and triple mutants STa<sub>N12S</sub>, STa<sub>L9A/N12S</sub>, STa<sub>N12S/A14T</sub>, STa<sub>L9A/A14H</sub>, and STa<sub>L9A/N12S/A14H</sub>, after being genetically fused to a monomeric LT mutant (mnLT<sub>R192G/L211A</sub>) is able to induce antibody responses to both STa and LT ;**
- ❖ **Antibodies derived from the toxoid fusions 3×STa<sub>N12S</sub>-mnLT<sub>R192G/L211A</sub>, 3×STa<sub>L9A/N12S</sub>-mnLT<sub>R192G/L211A</sub>, and 3×STa<sub>N12S/A14T</sub>-mnLT<sub>R192G/L211A</sub> neutralized STa *in vitro* and also did not cross-react with guanylin or uroguanylin;**
- ❖ **Our results indicated the above three toxoid fusions are potentially desirable antigens for developing safe ETEC vaccines .**



# Acknowledgements

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Dr. Guoqiang Zhu  
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Dr. Hyesuk Seo

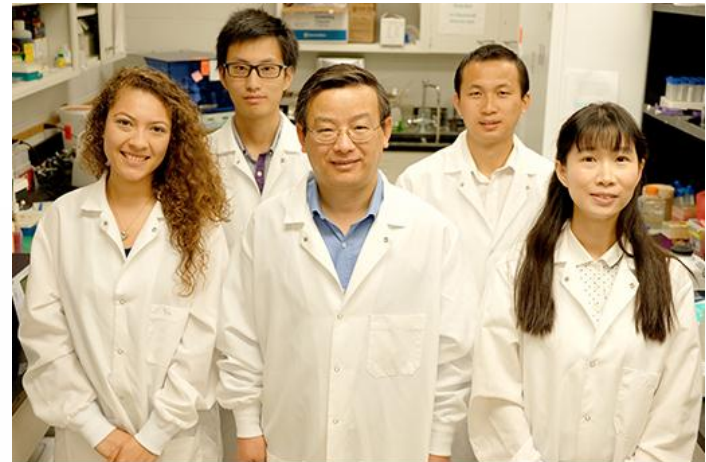


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PATH/EVI

Kansas State University College of Veterinary Medicine





A scenic view of a traditional Chinese garden. In the background, a white pagoda with a golden spire stands on a hill. In the middle ground, a pavilion with three green roofs is situated on a small island in a body of water. A willow tree with long, drooping branches is on the right. In the foreground, there are pink cherry blossoms. The text "Thank you!" is overlaid in the center in a large, bold, red font.

**Thank you!**