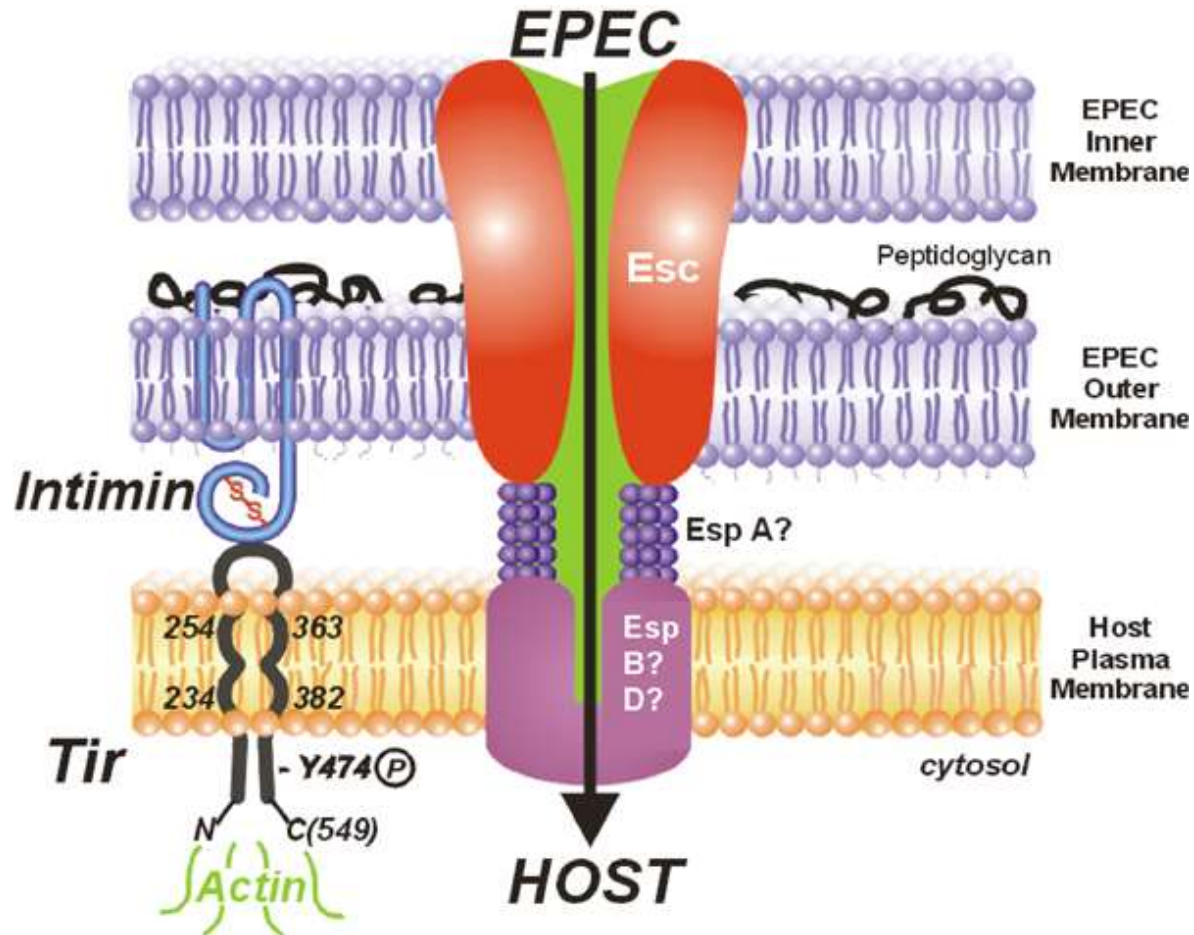


Type III Secretion System



Pedestal Formation

Cytoskeletal Proteins Recruited

Tip: active in pedestal formation

Tir, N-WASP, α -actinin, Arp2/3, gelsolin, talin, VASP, Nck, CD44

Length: structural components

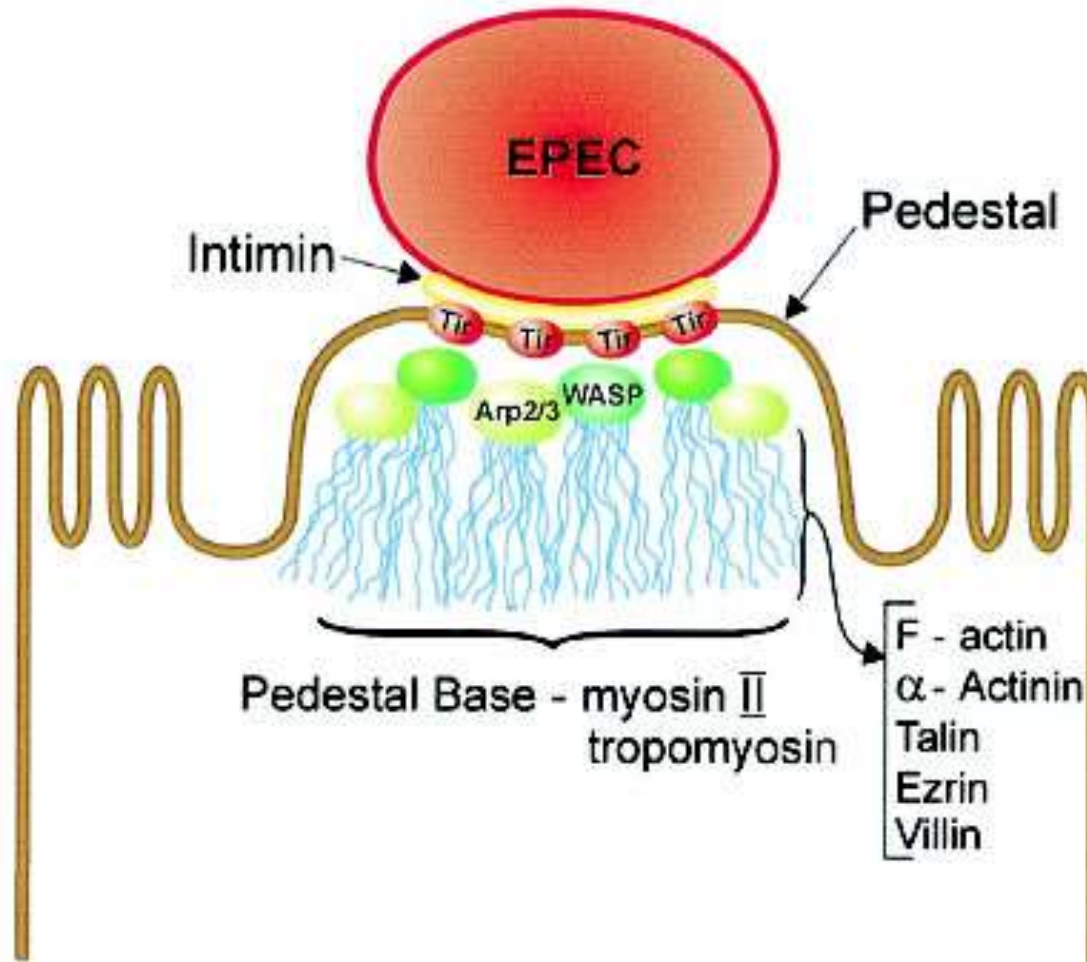
α -actinin, Arp2/3, talin, ezrin, calpactin, cortactin, Shc, CrkII, Grb2, vinculin, zyxin, LPP, gelsolin, paxillin, cofilin, gelsolin

Base: motor proteins?

tropomyosin, myosin II light chain



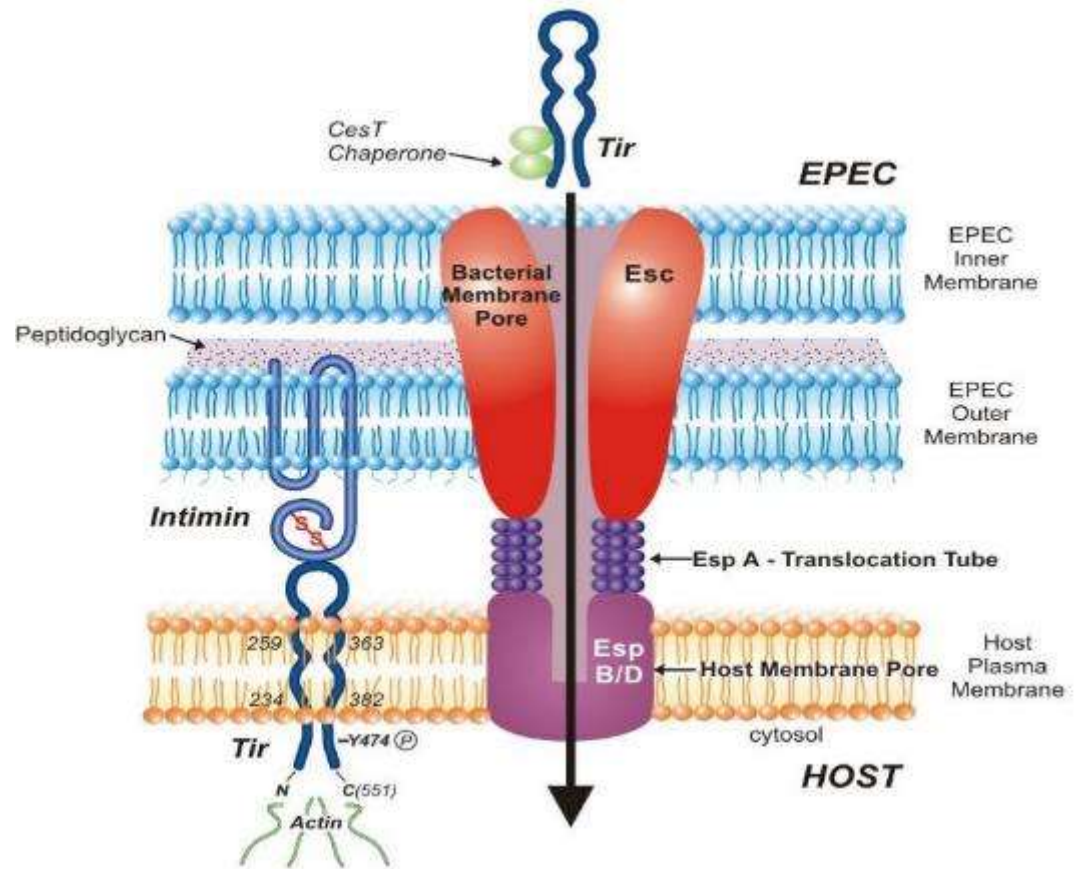
Pedestal Formation



- Vallance & Finlay. 2000. Proc. Natl. Acad. Sci. 97:8802

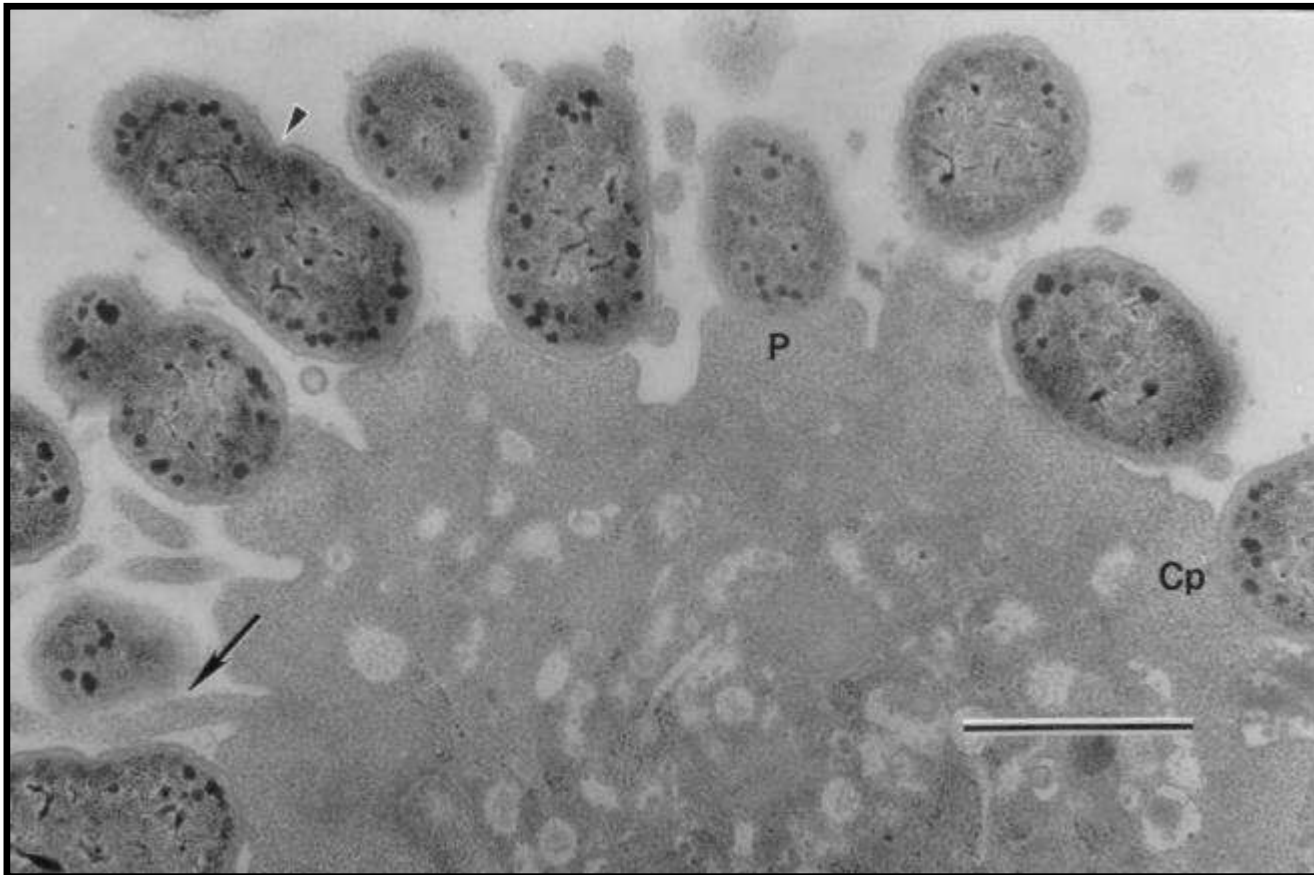
Translocon and Effector Proteins

- **Translocon**
 - EspA (tube)
 - EspB and EspD (pore)
- **6 known LEE effectors**
 - Tir (mediates adherence)
 - Others affect cytoskeleton
- **Translocon PLUS effectors are needed for vaccine**



Pedestal Formation

Rectum — EHEC O5:NM Inoculated Neonatal Gnotobiotic Calf



Moxley & Francis Infect. Immun. 53:339-346.

Attaching & Effacing Lesions

Rectal and Colonic Mucosal Epithelium



Baehler & Moxley. FEMS Microbiol. Lett 185:239-242.

Shiga Toxin

- Following the establishment of attaching and effacement lesions, *E. coli* O157:H7 release shiga toxin (Stx) into the host cell
- Two types of shiga toxin were originally recognized – Stx1 and Stx2
 - Both molecules have an $\alpha_1\beta_5$ structure
 - Both possess the same mechanism of action
 - 55% amino acid sequence identity between the α subunits of Stx1 & Stx2
- The Stx toxins bind to globotriaosylceramide (Gb3) receptors in endothelial cells and induce cell death by inhibiting translation
- The α subunit possesses enzymatic activity that enables the toxin to cleave a specific adenine base from the 28 S rRNA and thereby prevent protein synthesis
- The cluster of β subunits of the Stx bind to specific glycolipid receptors on the surface of cells, permitting internalization of the toxin molecule

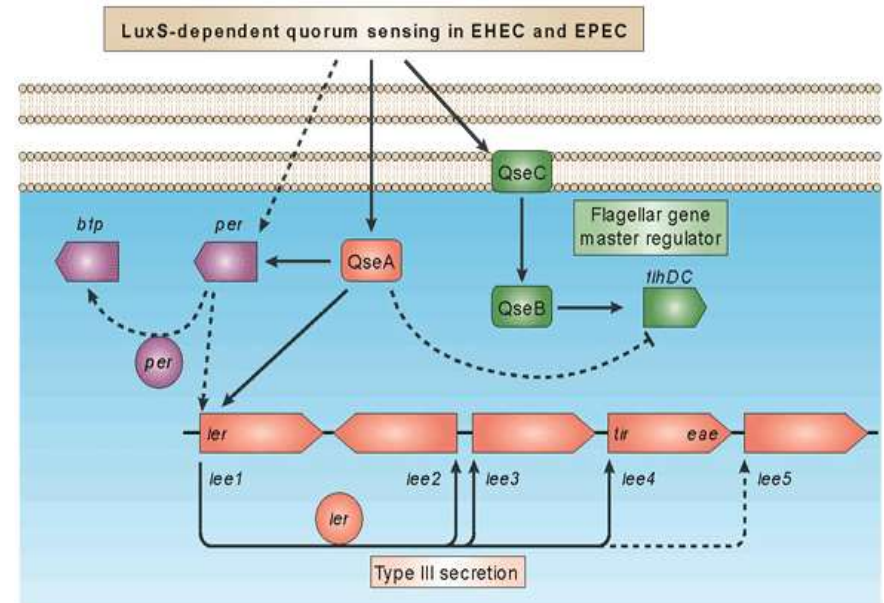
E. coli O157:H7 Regulation of Gene Expression

- Regulation of LEE genes involves several non-LEE-encoded and LEE-encoded regulators

- Non-LEE-encoded regulators:

- H-NS (repressor)
- IHF (activator)

- EHEC use quorum sensing, via QseA (quorum-sensing *E. coli* regulator A), to regulate the expression of LEE genes required for colonization



Nature Reviews Microbiology 3, 383-396 (May 2005)

- LEE encoded regulators:

- Ler (H-NS-like transcriptional regulator Ler (LEE-encoded regulator))
- GrlA (global regulator of LEE activator)
- Ler is necessary for the expression of grlA and that Ler and GrlA induce each other's expression partly through counteracting H-NS-mediated repression

Hazard Analysis & Critical Control Point (HACCP) System

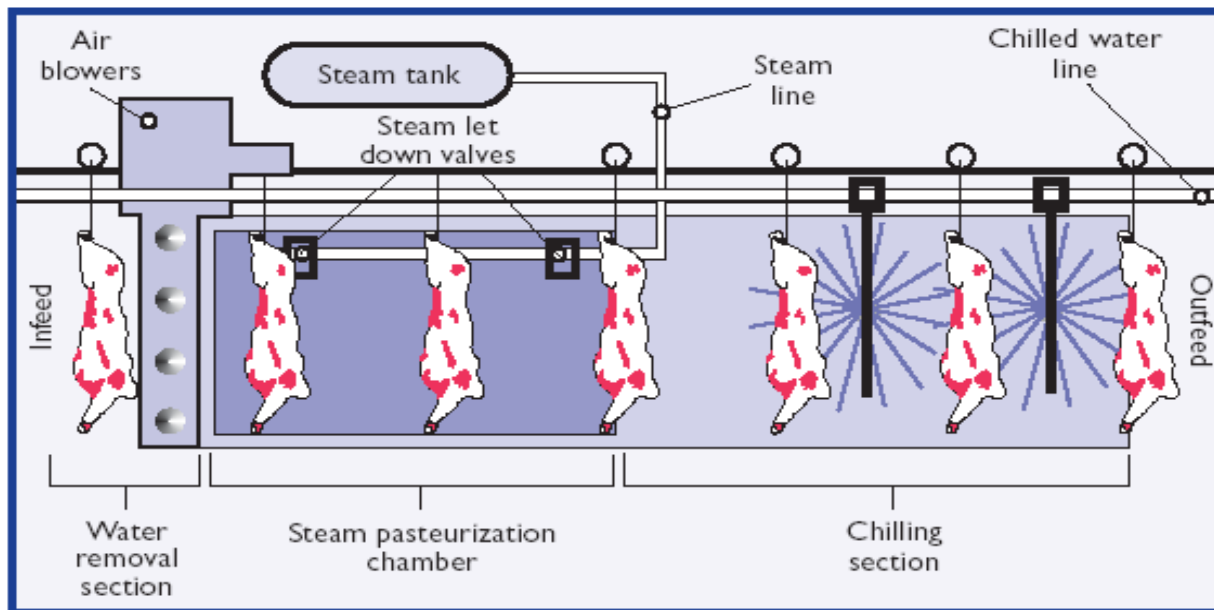
- USDA mandated HACCP in all meat and poultry processing plants in January 1999
- FDA mandated HACCP for seafood in 1996 and juice in 2002. Now HACCP is standard in all food processing:
 - Analyze hazards
 - Identify critical control points
 - Establish preventive measures with critical limits for each control point
 - Establish procedures to monitor the critical control points
 - Establish corrective actions to be taken when monitoring shows that a critical limit has not been met
 - Establish procedures to verify that the system is working properly
 - Establish effective record keeping to document the HACCP system

HACCP Induced O157 Control Measures

- Hide washes
- Line upgrades
- Carcass washes, steam pasteurization, lactic acid wash
- **Test and hold**

Figure C-3

Beef Steam Pasteurization System — Static Chamber Unit



Source: Frigoscandia Equipment

E. coli O157:H7 in the Feedlot

- Live cattle populations are an important reservoir of *E. coli* O157:H7
- *E. coli* O157:H7 is constantly recirculated within the environment

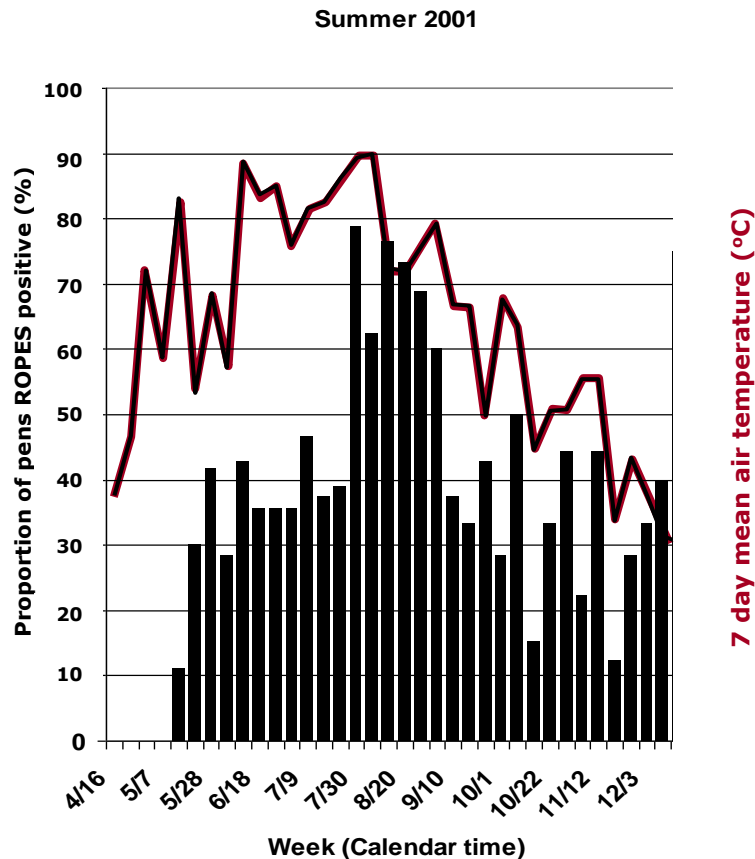


- Smith et al. J Food Prot. 2001, 64 (12) 1899-1903.
- Khaitsa et al. J Food Prot 2003, 66 (11) 1972-1977.
- Smith et al. Foodborne Pathogens and Disease. 2005, Vol 2(1): 50-60

Prevalence = Magnitude of exposure x Duration of infection

E. coli O157:H7 in the Feedlot

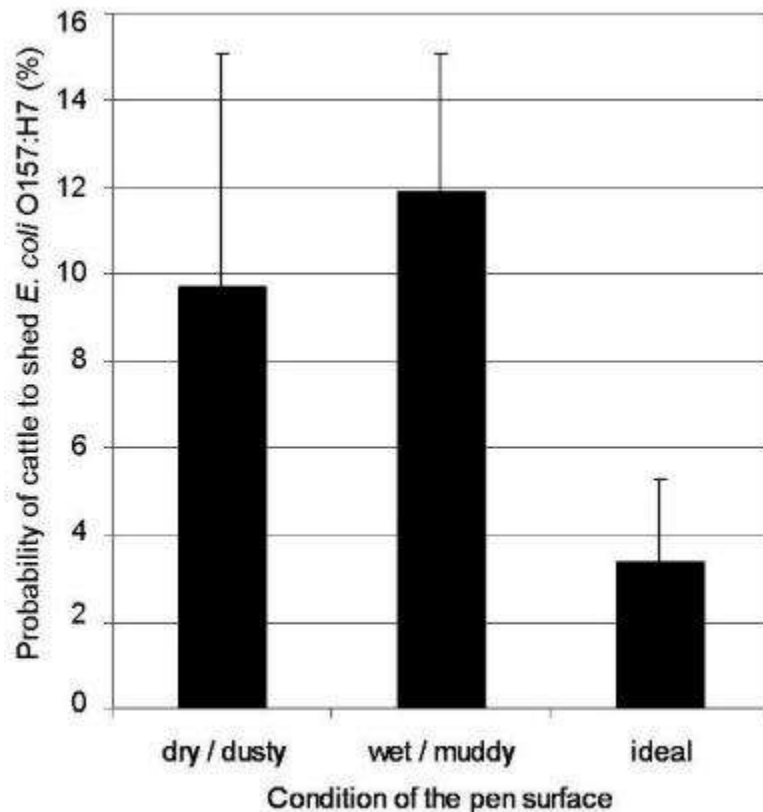
The natural ecology of *E. coli* affects the probability for cattle to shed *E. coli* O157:H7



E. coli prevalence is greatest during the hot summer months

E. coli O157:H7 in the Feedlot

The natural ecology of *E. coli* affects the probability for cattle to shed *E. coli* O157:H7



E. coli prevalence is highest during:

- dry / dusty
- wet / muddy

Ideal is **neither** dry/dusty nor wet/muddy

O157:H7 Feedlot Prevalence

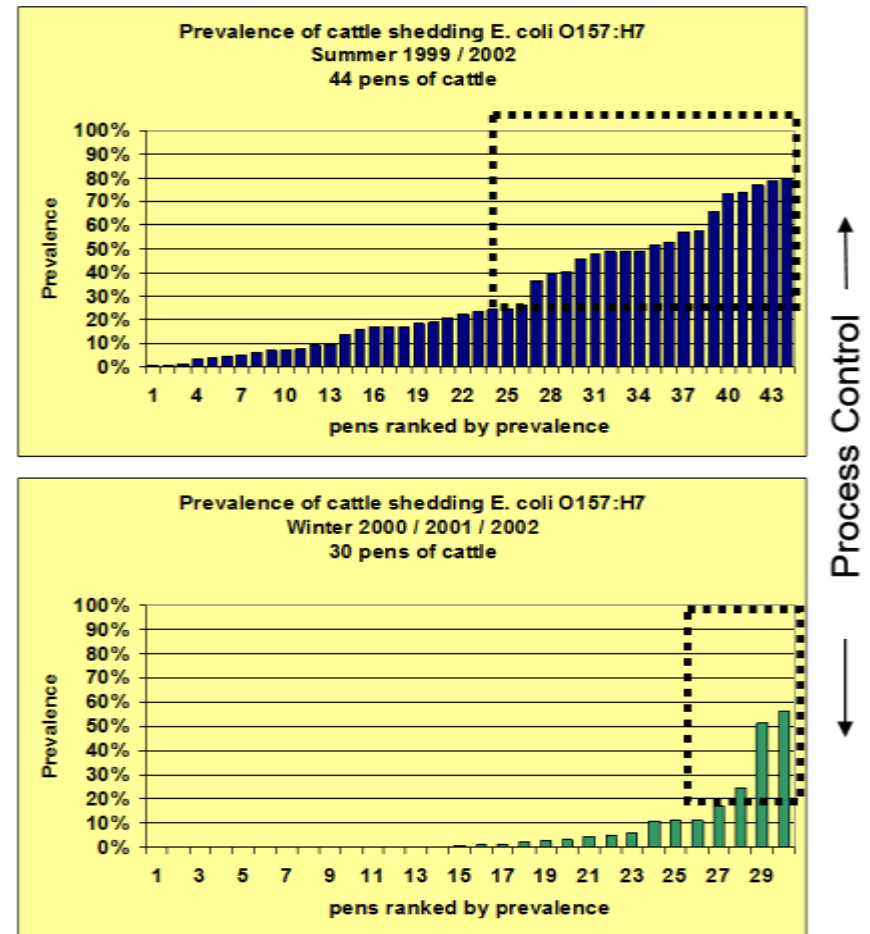
Significant difference by season:

Summer *E. coli* O157:H7

- 4,952 cattle, 44 pens
 - 30% of cattle culture positive
 - 100% of the pens positive
 - Variable prevalence (1-80%)

Winter *E. coli* O157:H7

- 2,941 cattle, 30 pens
 - 6.1% of cattle culture positive
 - 53% of the pens positive
 - Variable prevalence (0-56%)



• Smith DR, et al. 2001. J Food Prot 64 (12) 1899-1903

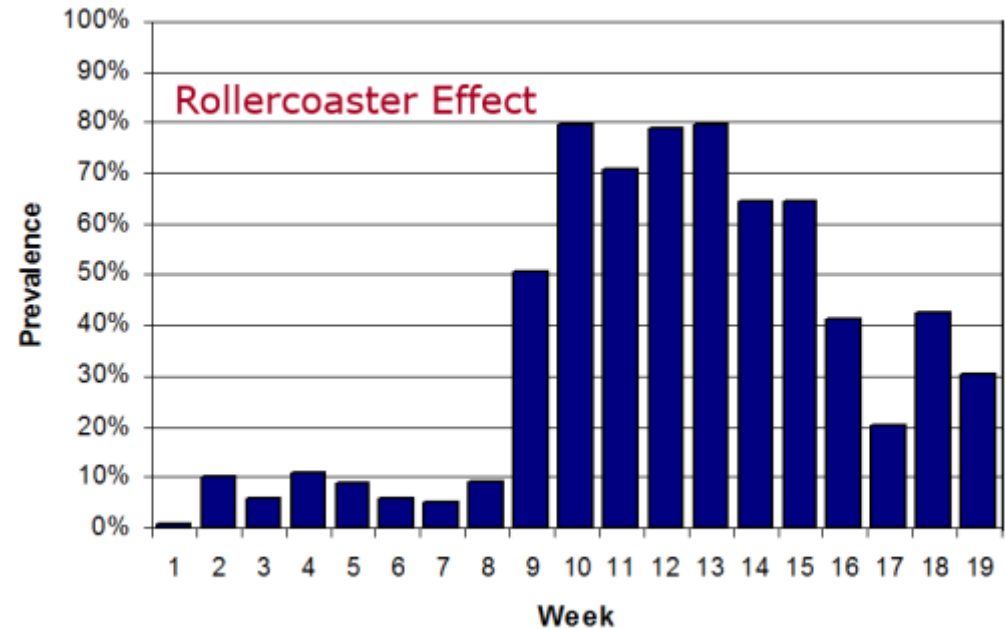
In the summer a high number of pens have a high prevalence

O157:H7 Feedlot Prevalence

Prevalence over time:

Summer *E. coli* O157:H7

- Natural exposure to *E. coli* O157
- Feces from 100 steers (10 pens of 10) cultured each week
- *E. coli* O157:H7 recovered every week and at least once from every animal



Prevalence varies by TIME and PLACE

Conclusions:

- ***E. coli* O157:H7 is present in all cattle populations**

O157:H7 Reduction Through Vaccination

- ❖ *E. coli* contamination of beef products, water supplies etc. could be controlled with mass vaccination of cattle and elimination of organisms from its major source
- ❖ Scientists around the world believe that best way to control contamination of beef products is to reduce or eliminate *E. coli* from the gut of animals in the pre-slaughter period

O157:H7 Reduction Through Vaccination

- ❖ *Vaccination would decrease bacteria counts in the environment by reducing replication of bacteria in the gut of cattle, therefore decreasing the risk of human contamination*
- ❖ *Gradual decrease of the bacterial load in the environment could minimize the risk to humans, and over time the incidence of disease*

O157:H7 Reduction Through Vaccination

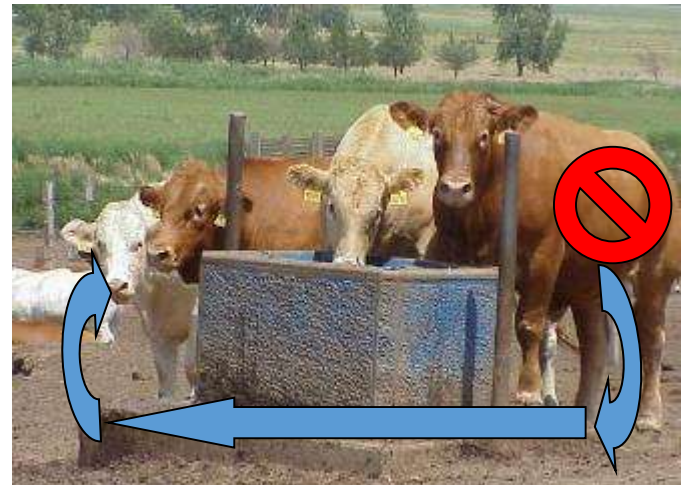
Potential Vaccine Candidates

- ❖ *Based on recent evidence, Tir, EspA, EspB and EspD are protective proteins against E. coli O157:H7 disease*
- ❖ *Antibodies elicited against Adhesin and Tir can block colonization, block infection and prevent disease*

Evaluating *E. coli* O157:H7

Scientists agree that a reduction in any of the following parameters will have a positive impact on *E. coli* O157:H7 associated food safety:

- Duration of bacterial shed
- Magnitude of shed
- Colorectal colonization
- Hide contamination
- Pen-level prevalence (ROPES)



- Smith et al. J Food Prot. 2001, 64 (12) 1899-1903.
- Khaita et al. J Food Prot 2003, 66 (11) 1972-1977.
- Smith et al. Foodborne Pathogens and Disease. 2005, Vol 2(1): 50-60

Evaluating *E. coli* O157:H7

E. coli O157:H7 can be quantified in the feedlot by:

Prevalence ROPES



Colonization Terminal Rectal Mucosa (TRM)



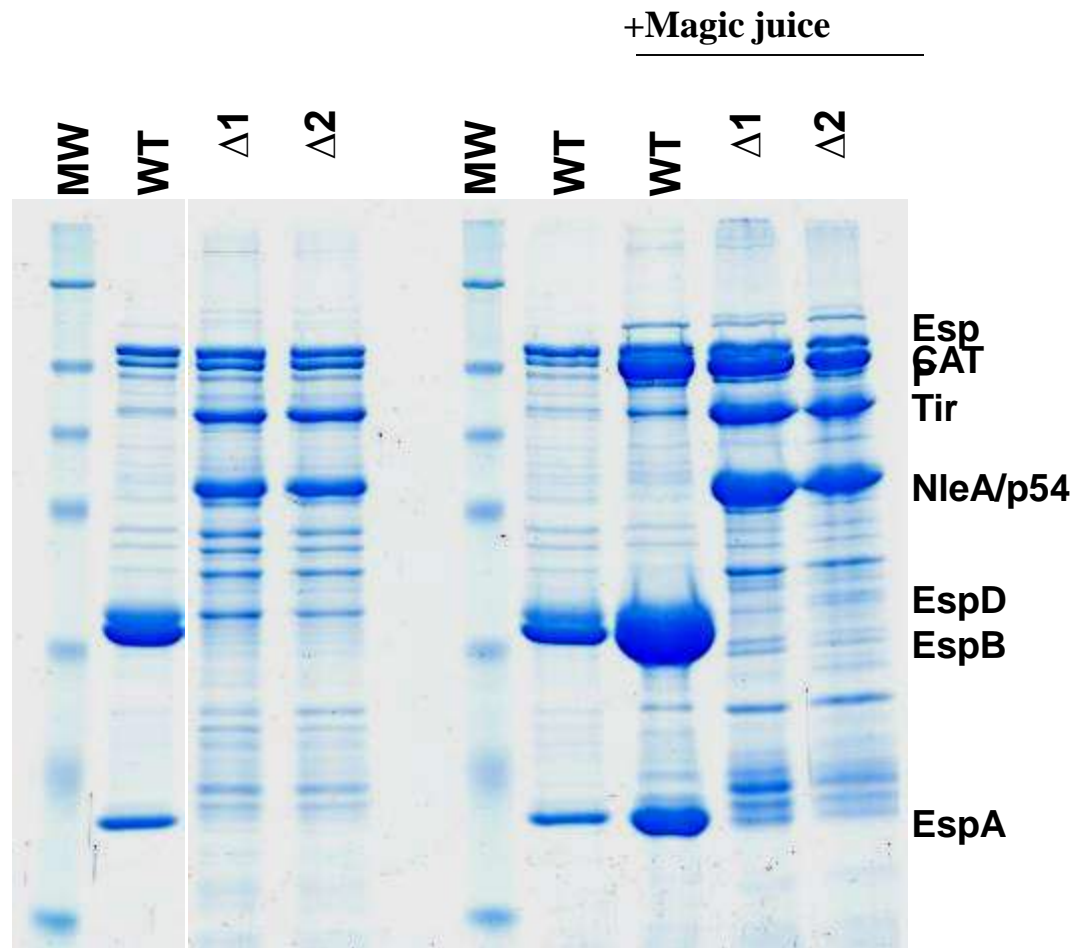
Fecal Shedding

- Immunomagnetic Separation (IMS)
- Direct Fecal Sampling



- Irwin et al. 2002. Bov Practitioner 36 (1) 5-9.
- Smith et al. 2004. Epid Infect 132:297-302.
- Naylor et al. 2003. Infect. Immun. 71:1505-1512.

UBC identified a 2 member hierarchical switch for type III secretion of translocators, plus a way to oversecrete effectors in EHEC



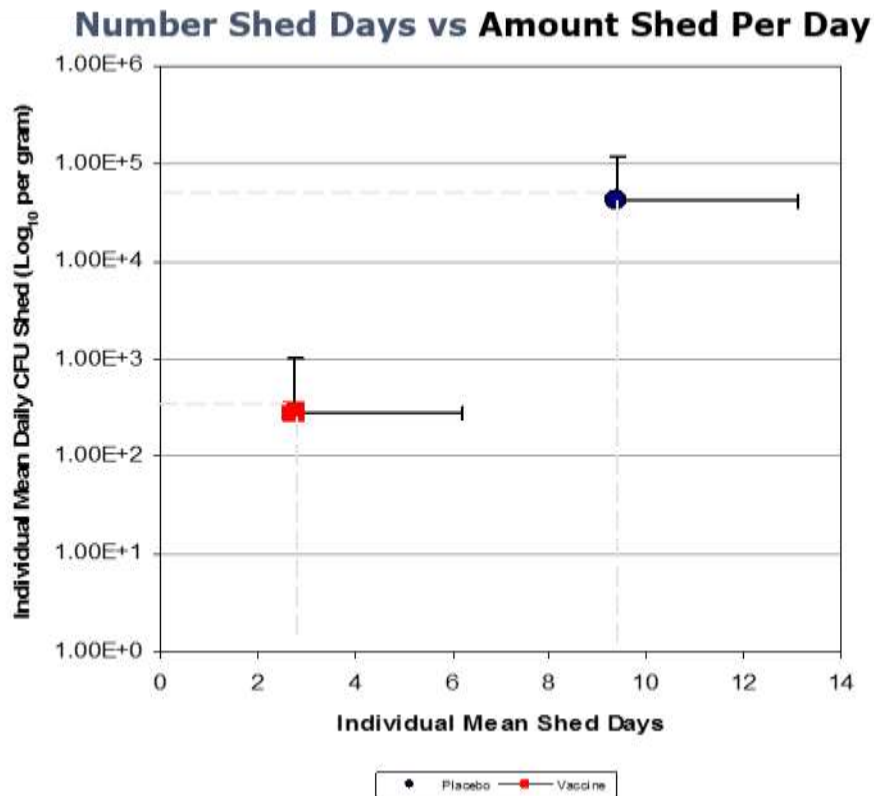
EHEC stain was induced in DMEM. Shown are secreted proteins analyzed in 12% SDS-PAGE.

E. coli O157 Vaccine Studies

Effect of Vaccination on Magnitude and Duration

Challenge Trial (Vaccine and Infectious Disease Organization)

- 6 month-old calves
- 8 vaccinates, 8 placebo controls
- 14 days of observation
- Challenged with *E. coli* O157:H7 by oral-gastric intubation at 14 days



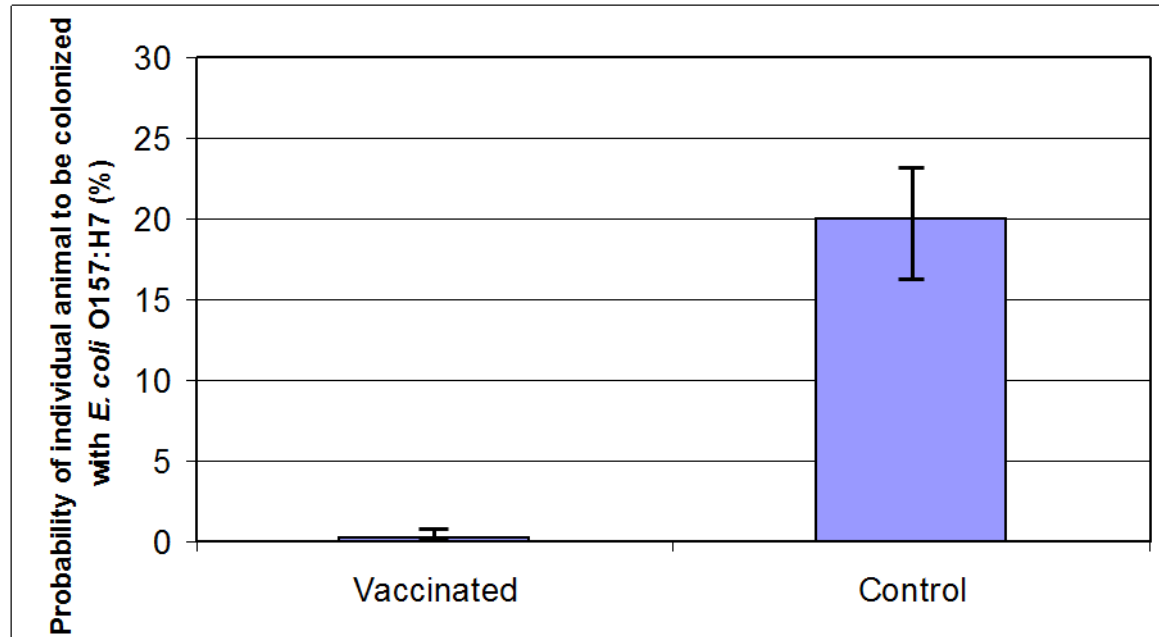
- The vaccine resulted in a **2.28 log₁₀ reduction** in the magnitude of shedding in vaccinated animals compared to control animals (over 99% reduction)
- **63.9% efficacy** in the vaccine's ability to reduce the number of days *E. coli* O157:H7 was shed in the feces

E. coli O157 Vaccine Studies

Effect of Vaccination on Colorectal Colonization

Field Trial 2004a (University of Nebraska-Lincoln Feedlot)

- 3 doses (0, 21, 42 days)
- 144 vaccinates + 144 placebo controls (288 total)
- 5 sample periods, (14, 28, 42, 56 days post-vaccination)



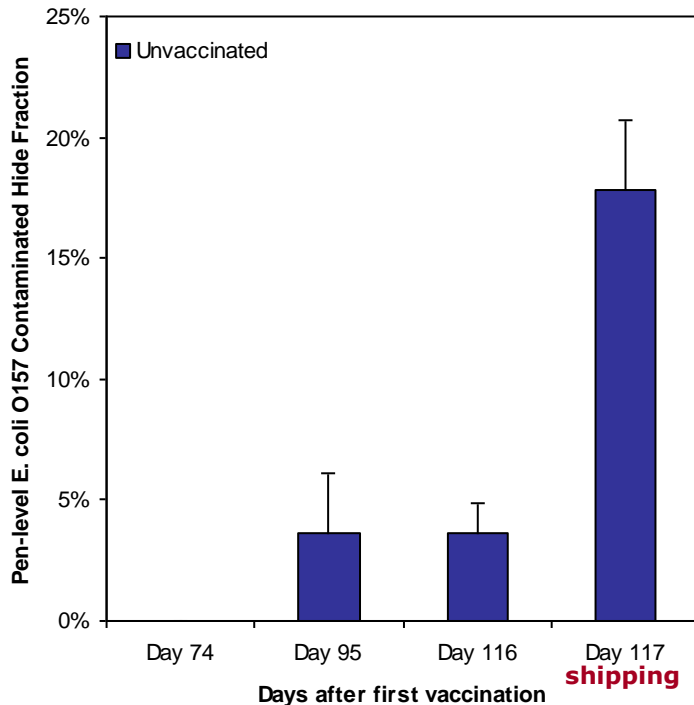
Vaccinated animals were less likely to be colonized by *E. coli* O157:H7 in the mucosa of the terminal rectum (OR=0.014, $p < 0.0001$), resulting in a **vaccine efficacy of 98.3%**.

E. coli O157 Vaccine Studies

Effect of Vaccination on Hide Contamination

Field Trial 2005 (University of Nebraska-Lincoln Feedlot)

- University of Nebraska-Lincoln Feedlot
- 2 doses (arrival, reprocessing)
- 7 pens vaccinated and 7 pens non-vaccinated, 8 animals per pen (504 total)
- 3 sample periods, 3 weeks apart
- Hides sampled pre- and post-shipping



The probability for unvaccinated cattle to have *E. coli* O157:H7 on their hides differed significantly by test period ($p < 0.0001$)



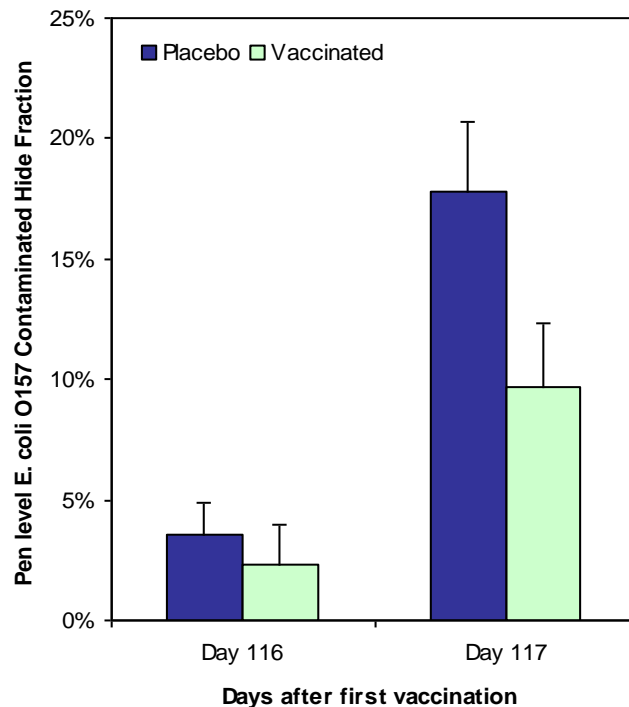
Shipping causes a dramatic increase in shedding

E. coli O157 Vaccine Studies

Effect of Vaccination on Hide Contamination

Field Trial 2005 (University of Nebraska-Lincoln Feedlot)

- University of Nebraska-Lincoln Feedlot
- 2 doses (arrival, reprocessing)
- 7 pens vaccinated and 7 pens non-vaccinated, 8 animals per pen (504 total)
- 3 sample periods, 3 weeks apart
- Hides sampled pre- and post-shipping



Vaccinated cattle were less likely to have contaminated hides in BOTH the feedlot and the abattoir, resulting in a **vaccine efficacy of 53.8%**.

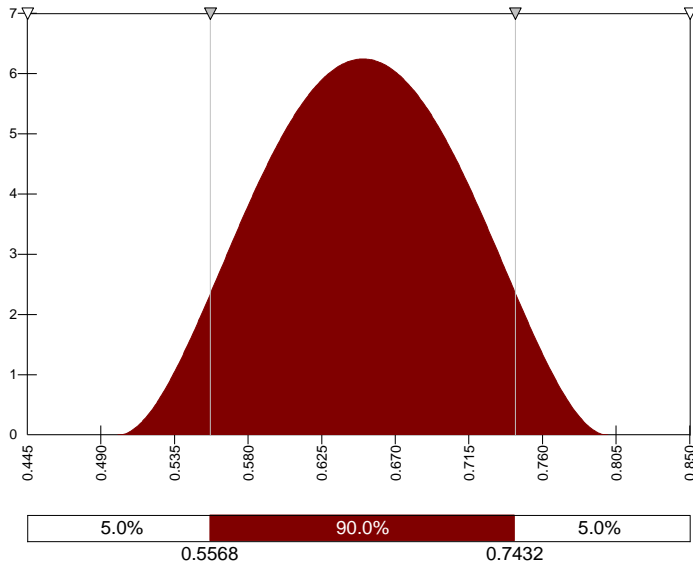
E. coli O157 Vaccine Studies

In conclusion, vaccination results in:

- Reduction in duration of shed - 63.9% efficacy
- Reduction in magnitude of shed - 2.28 log₁₀ reduction
- Reduced colorectal colonization - 98.3% efficacy
- Reduced hide contamination - 53.8% efficacy
- Reduced pen level prevalence (ROPES) - OR=0.59 (p=0.004)

So What?

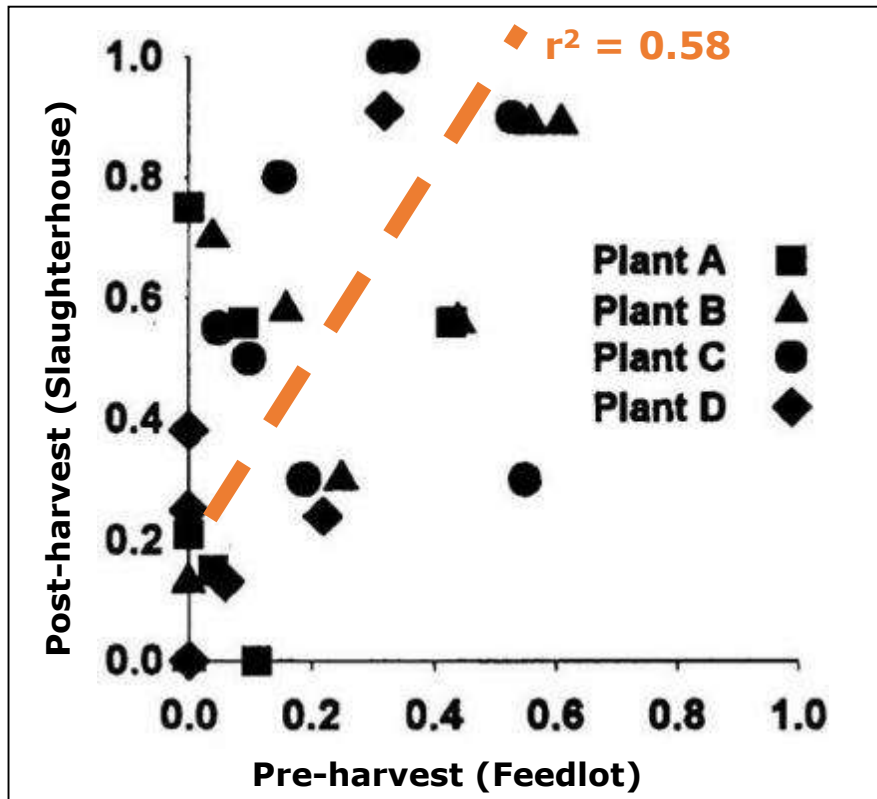
What might be the effect of vaccinating cattle against *Escherichia coli* O157:H7?



A modeling approach

Modeling: Effect of Vaccination

Carcass contamination and prevalence of O157:H7 in feces of cattle



Feedlot prevalence is correlated to carcass contamination

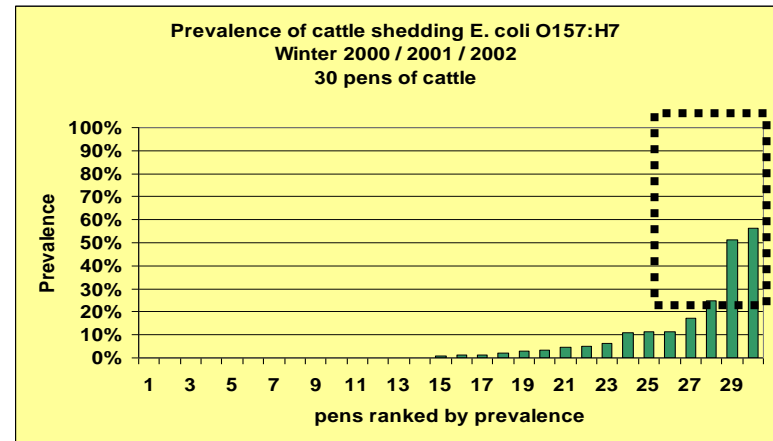
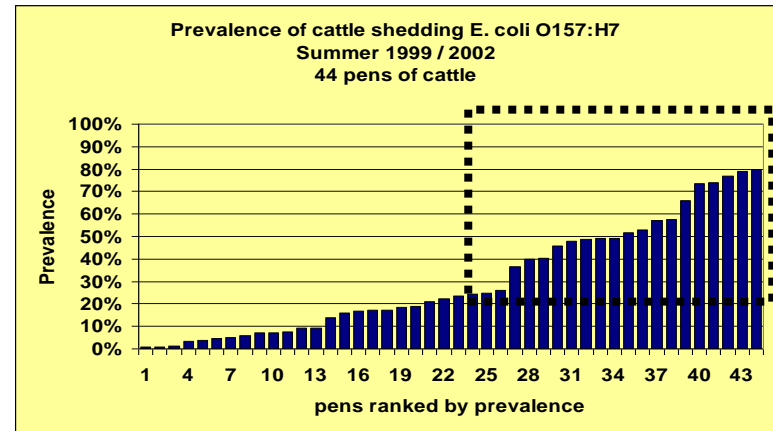
Fig. 1. Spearman rank correlation of EHEC O157 prevalence in all fecal and hide samples (preharvest) versus prevalence of carcasses positive on any sample (postharvest), by lot. Spearman rank correlation coefficient (r_s) = 0.58 (95% confidence interval 0.27–0.78), $P = 0.001$, $n = 29$.

Modeling: Effect of Vaccination

Seasonal Prevalence in Commercial Feedlots

Q: How might vaccination affect O157:H7 shedding compared to what we've observed in winter of summer?

A: Turn Summer into Winter

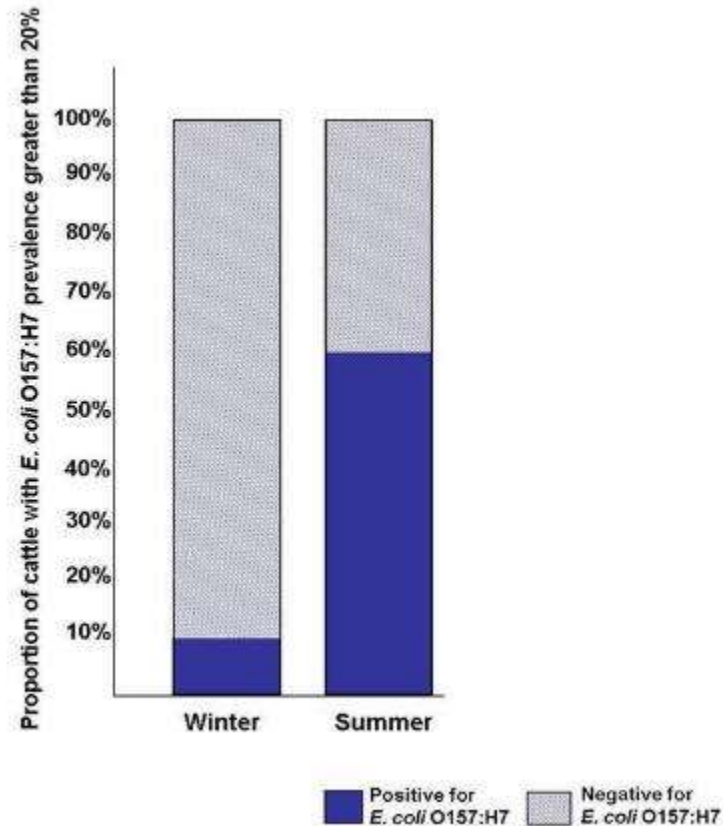


- Smith et al. 2001. J Food Prot 64 (12) 1899-1903.

Modeling: Effect of Vaccination

How would O157:H7 shedding of vaccinates compare to non-vaccinates in winter or summer?

- Stochastic model of the prevalence of O157:H7 in live cattle
- Simulation:
 - Unvaccinated summer
 - Vaccinated summer (2 or 3 doses)
 - Winter
- 5,000 pen simulations
- 500,000+ cattle



Summer = 60% of cattle have more than 20% prevalence

Winter = only 10% of cattle have more than 20% prevalence

Modeling: Effect of Vaccination

How would O157:H7 shedding of vaccinates compare to non-vaccinates in winter or summer?

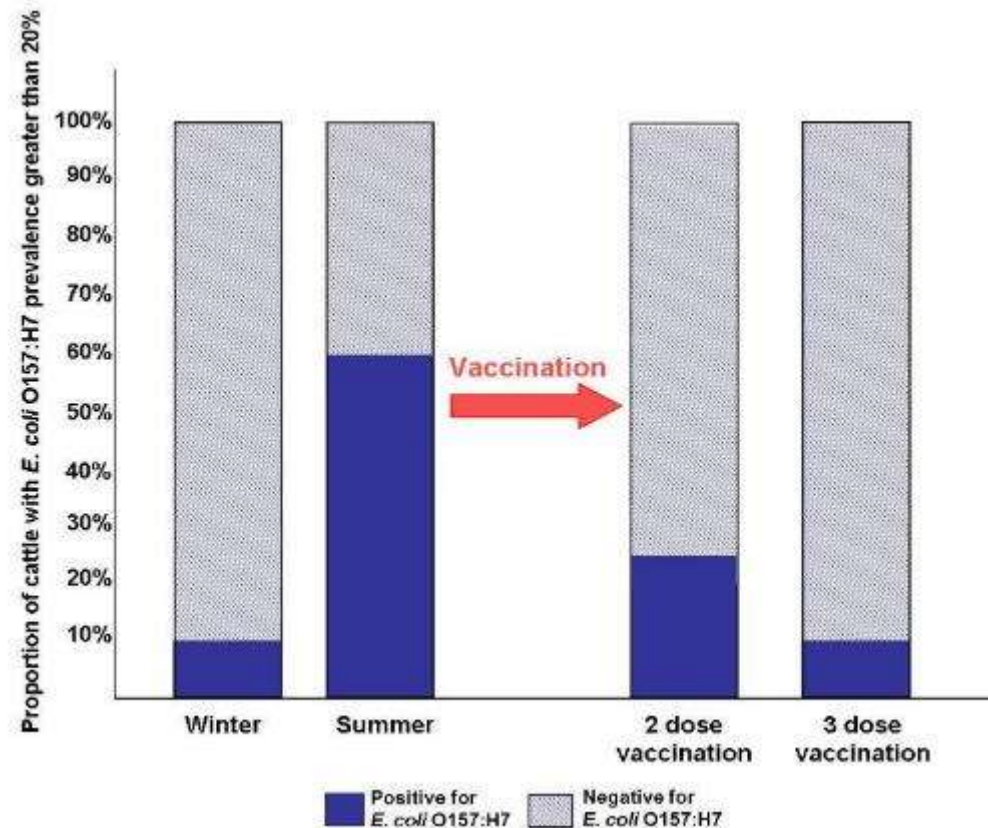
Summer = 60% of cattle have more than 20% prevalence

Winter = only 10% of cattle have more than 20% prevalence

With Vaccination:

2 dose = only 25% of cattle have more than 20% prevalence

3 dose = only 10% of cattle have more than 20% prevalence



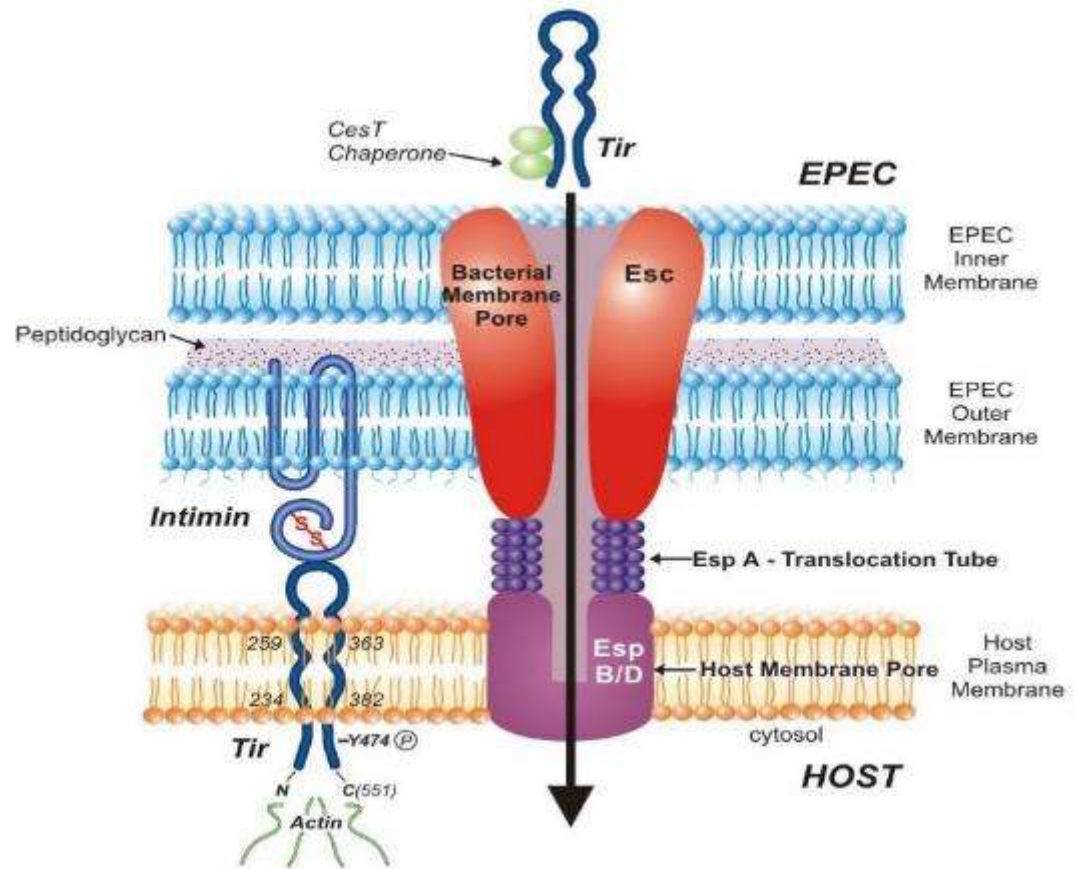
Modeling: Effect of Vaccination

Conclusion:

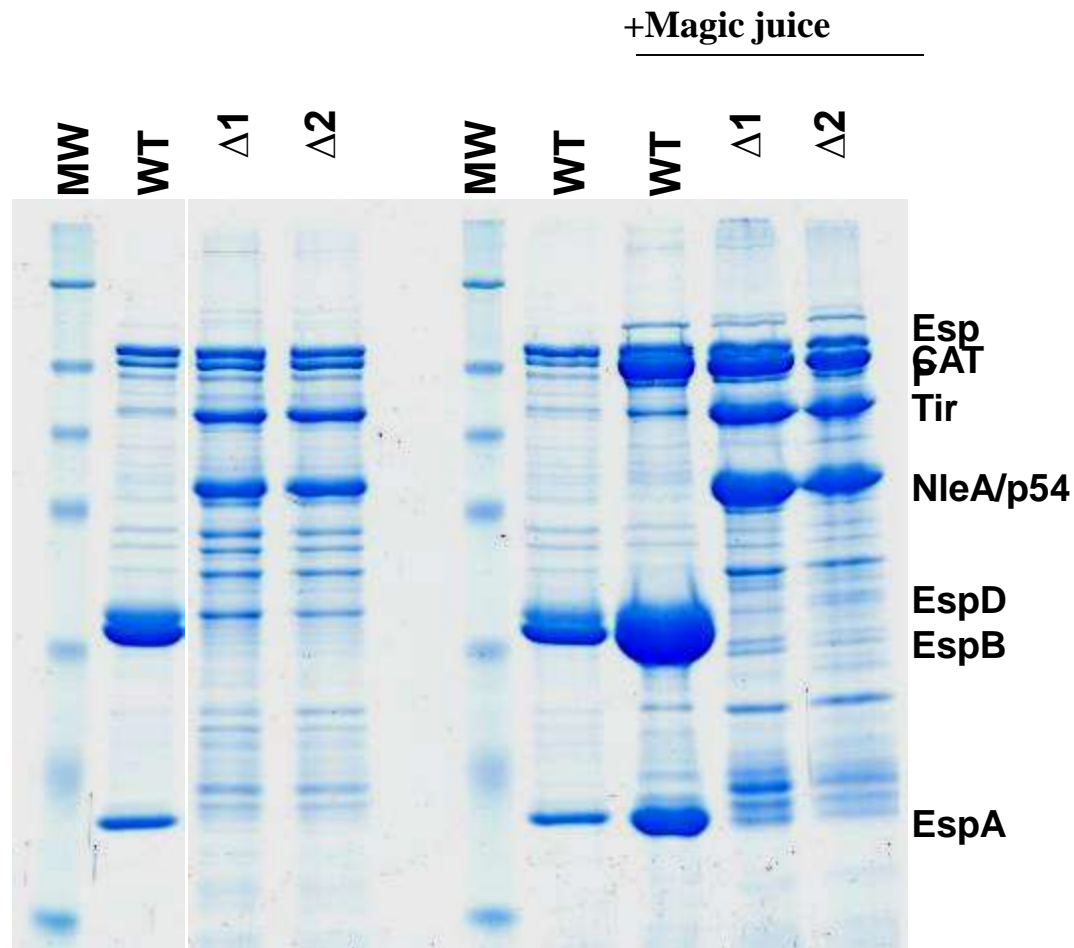
- ❖ *The model suggests that winter shedding levels can be approximated by vaccinating summer-fed cattle*
- ❖ *Establishes proof of concept for vaccination as a useful pre-harvest intervention against E. coli O157:H7*

Translocon and Effector Proteins

- Translocon
 - EspA (tube)
 - EspB and EspD (pore)
- 6 known LEE effectors
 - Tir (mediates adherence)
 - Others affect cytoskeleton
- Translocon PLUS effectors are needed for vaccine



UBC identified a 2 member hierarchical switch for type III secretion of translocators, plus a way to oversecrete effectors in EHEC



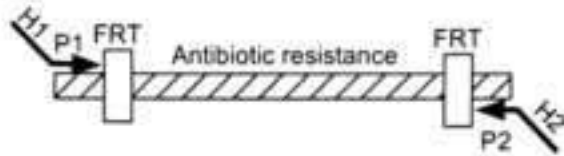
EHEC stain was induced in DMEM. Shown are secreted proteins analyzed in 12% SDS-PAGE.

Proposed plan

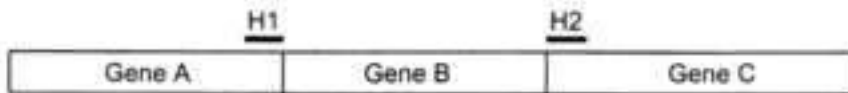
- 1. Construction of *switch* mutants**
 - ***switch1* and *switch2* mutants show higher levels of effector secretion**
 - **WT strain predominantly secretes translocators**
- 2. Overexpression of Magic Juice on WT and Switch mutant strains**
- 3. Construction of Shiga-toxin negative strains in sequenced (EDL933) strain**
 - **Mutations of both Shiga toxin genes in both strains**
- 4. Evaluate the possibility of a vaccine against non-O157 shiga toxin-producing *E. coli* (STEC) using same technology**

Lambda-red recombination

Step 1. PCR amplify FRT-flanked resistance gene



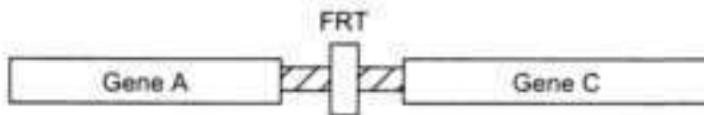
Step 2. Transform strain expressing λ Red recombinase



Step 3. Select antibiotic-resistant transformants

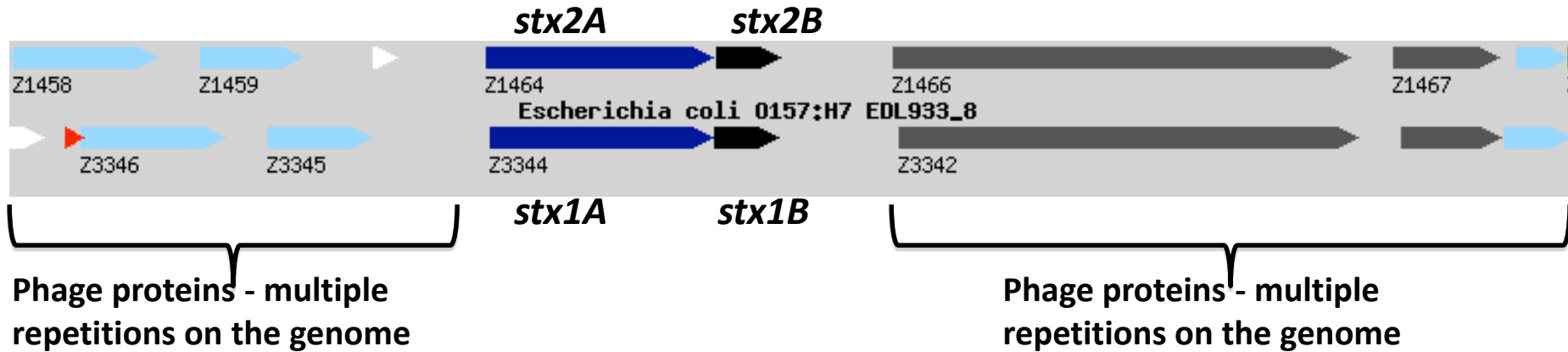


Step 4. Eliminate resistance cassette using a FLP expression plasmid



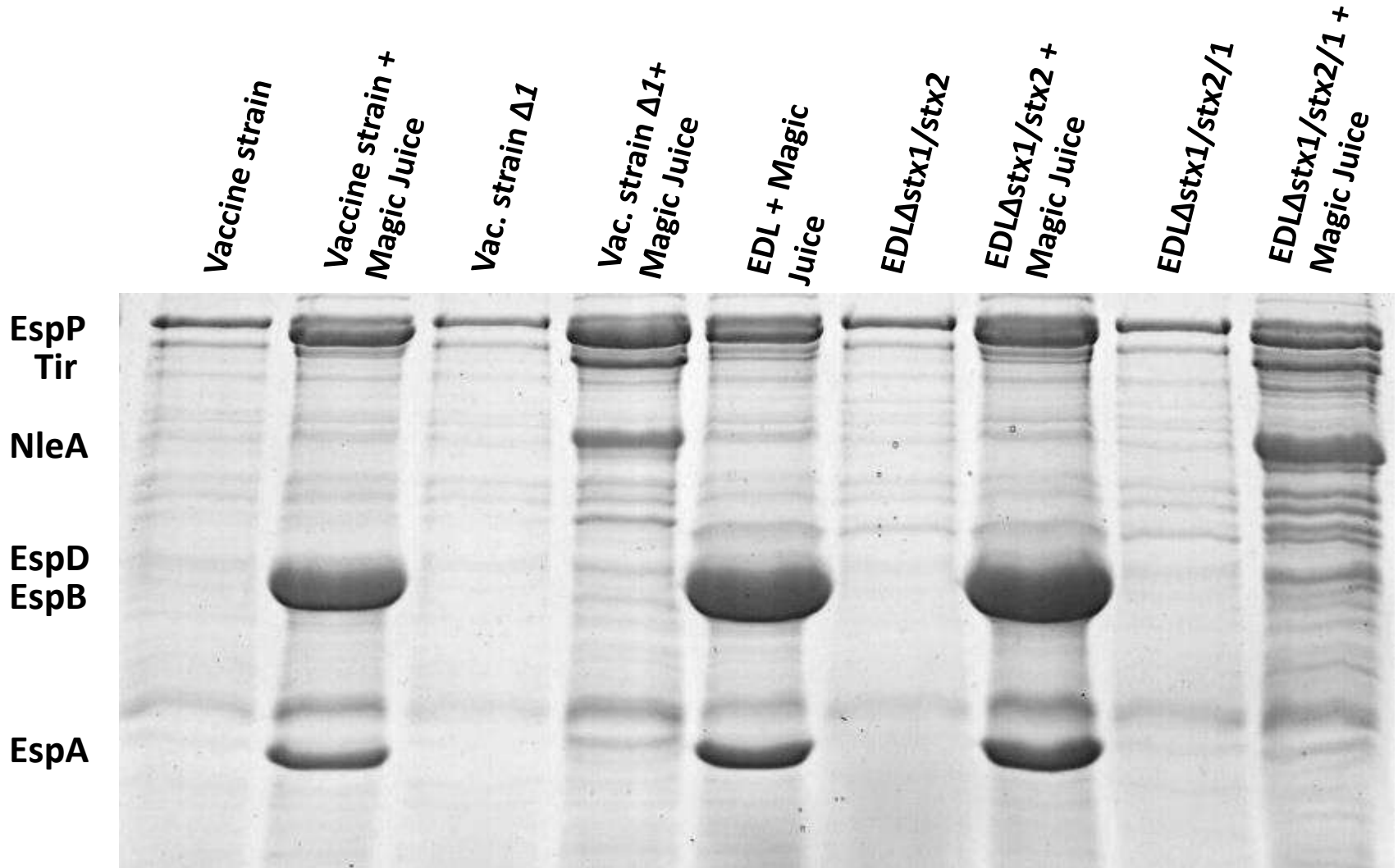
- pKD13
 - Kanamycin cassette as a template to disrupt gene
 - Non-polar deletions
- pKM208
 - Recombination system from lambda phage, IPTG-inducible
 - Temperature sensitive origin of replication

Construction of *stx* mutants in EDL933



- Lambda red recombination with kanamycin cassette in both directions into *stx*

Strains constructed



Second Generation Vaccine Scale-up

- Two strains:
 - “E2” oversecretes translocon (EspA, EspB, EspD)
 - “T2” oversecretes effectors (Tir, NleA,...)
- Both strains have both Shiga Toxins deleted cleanly
- Harvest supernatants from both strains and combine for vaccine
 - (or combine strains and make supernatant)

Antimicrobial Resistance (AMR)

Multi resistant organisms (MRO)

- AMR or MRO are increasing concern for human and animal health and does not recognize geographic or human animal borders
- MRSA (Methicilin-Resistant Staphylococcus aureus)
- VRE (Vancomycin-Resistant Enterococci)
- CRE (Carbapenem-Resistant Enterobacteriaceae)
- ESBL (Extended spectrum beta Lactamase producing organisms)

Points to be considered in Serbia

- ❖ Pre harvest interventions to reduce pathogens in live animals hold potential to reduce food borne pathogen dissemination on farms , in the environment and entering food chain
- ❖ Traditional barriers between interdisciplinary scientists, policy makers and Government officials in animal health, human health and environmental field should be overcome and harmonized for the benefit of OH and overall prosperity of the country “if barriers exist”
- ❖ It is absolutely necessary to form committee, commission with CEO that will have well defined mandate.

Points to be considered in Serbia

- ❖ When guidelines, directives and policies are not well defined shifting of responsibilities between the Institutions and Ministries would be reasonable to expect
- ❖ There is need to increase research on Zoonoses, food safety, antimicrobial resistance and environmental health and to improve the understanding of the one health concept

Points to be considered in Serbia

- It is obvious that in now days access to guidelines, directives, organization charts, polices and recommendations of developed countries could be obtained very easily
- Our polices, guidelines, directives and laws are very similar or almost identical with developed countries
- Implementation and Endorsement of those legislations should be imperative

Points to be considered in Serbia

- ❖ Constant education of all involved in OH *is must*
- ❖ Allocate \$ for the research and diagnostic laboratories
- ❖ Select the most knowledgeable people for the job
- ❖ Promote team work
- ❖ Do what is best for your country!!!
- ❖ Vision for OH approach is required at the Government level!!!

Dragan Rogan's *Escherichia coli* Publications

- 2009 Moxley RA, Smith DR, Lubbe MK, Erickson GE, Klopfenstein TJ, Rogan DR. **ESCHERICHIA COLI O157:H7 VACCINE DOSE-EFFECT IN FEEDLOT CATTLE.** Foodborne Pathogens and Disease. 2009. 6(7)[Ahead of print]
- 2009 Misyurina O, Asper DJ, Deng W, Finlay BB, Rogan D, Potter AA. **THE ROLE OF Tir, EspA AND NleB IN THE COLONIZATION OF CATTLE BY SHIGA TOXIN-PRODUCING ESCHERICHIA COLI O26:H11.** Journal of Infection and Immunity [SUBMITTED]
- 2009 Potter AA, Asper D, Rogan D, Finlay B. **VACCINATION OF CATTLE WITH STEC TYPE III SECRETED PROTEINS: IMMUNE RESPONSES AND CROSS-SEROTYPE REACTIVITY.** 7th International symposium on Shiga Toxin (Verocytotoxin) – Producing *Escherichia coli* Infections. 10th – 13th of May 2009. Buenos Aires, Argentina. Proceedings pp 24.
- 2008 Babiuk S, Asper DJ, Rogan D, Mutwiri GK, Potter AA. (2008). **SUBCUTANEOUS AND INTRANASAL IMMUNIZATION WITH TYPE III SECRETED PROTEINS CAN PREVENT COLONIZATION AND SHEDDING OF ESCHERICHIA COLI O157:H7 IN MICE.** Microb Pathog. 2008 Mar 26.
- 2007 Peterson RE, Klopfenstein TJ, Moxley RA, Erickson GE, Hinkley S, Bretschneider G, Berberov EM, Rogan D, Smith DR. **EFFECT OF A VACCINE PRODUCT CONTAINING TYPE III SECRETED PROTEINS ON THE PROBABILITY OF ESCHERICHIA COLI O157:H7 FECAL SHEDDING AND MUCOSAL COLONIZATION IN FEEDLOT CATTLE.** J Food Prot. 2007 Nov;70(11):2568-77.
- 2007 Peterson RE, Klopfenstein TJ, Moxley RA, Erickson GE, Hinkley S, Rogan D, Smith DR. **EFFICACY OF DOSE REGIMEN AND OBSERVATION OF HERD IMMUNITY FROM A VACCINE AGAINST ESCHERICHIA COLI O157:H7 FOR FEEDLOT CATTLE.** J Food Prot. 2007 Nov;70(11):2561-7.
- 2007 Asper DJ, Sekirov I, Finlay BB, Rogan D, Potter AA. **CROSS REACTIVITY OF ENTEROHEMORRHAGIC ESCHERICHIA COLI O157:H7-SPECIFIC SERA WITH NON-O157 SEROTYPES.** Vaccine. 2007 Nov 28;25(49):8262-9. Epub 2007 Oct 12.

Dragan Rogan's *Escherichia coli* Publications - continued

- 2006 Rogan, D. **ENTEROHAEMORHAGIC *E. COLI* O175:H7 VACCINE REDUCES SHEDDING AND COLONIZATION OF BACTERIA IN VACCINATED ANIMALS.** Animal PHARM Vaccines – Scientific Advances and Regulatory Developments. Dec 5-6, 2006. Hamburg, Germany. Invited speaker.
- 2006 R.E. Peterson ,R. Smith, R.A. Moxley, , T. J. Klopfenstein, G. E. Erickson, D. Rogan S. Hinkley. (2006). **VACCINATING AGAINST TYPE III SECRETORY PROTEINS OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* REDUCED COLONIZATION OF *ESCHERICHIA COLI* O157:H7 AT THE TERMINAL RECTUM IIN THE BOVINE HOST.**
- 2006 R. Smith, R.A. Moxley, R.E. Peterson, T. J. Klopfenstein, G. E. Erickson, S. Hinkley, G. Bretschneider, E.M. Berberov, D. Rogan (2006). **EFFECT OF A TYPE III SECRETED PROTEIN VACCINE ON *ESCHERICHIA COLI* O15:H7 FECAL SHEDDING AND RECTAL COLONIZATION OF FEEDLOT CATTLE.**
- 2006 D. R. Smith, R.A. Moxley, R.E. Peterson, T. J. Klopfenstein, G. E. Erickson, S. Hinkley, D. Rogan (2006). **EFFECT OF DOSAGE NUMBER OF AN *ESCHERICHIA COLI* O157:H7 TYPE III SECRETED PROTEIN VACCINE ON FECAL SHEDDING AND HEARD IMMUNITY IN FEEDLOT CATTLE.**
- 2005 Rogan, D., Strauss,C.A. (2005). **USE OF BIOTECHNOLOGY FOR THE PRODUCTIONOF PHARMACEUTICAL PRODUCTS.** IV International Symposium of Animal Reproduction, June 24-26, Cordoba, Argentina. Key note paper.
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