Preventing *E. coli* in Egg-laying Chickens with SRP Vaccine

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Layers Facing New Health Challenges

- Restrictions on the use of antibiotics in food animals
  - Eliminating preventive uses, antibiotics of medical importance
  - "NAE" retail marketing programs; even meds of no value to humans
- Increasing regulatory, retailing concerns about Salmonella
  - Shell eggs: additional serotypes beyond SE?
  - Hatching eggs: tightening of salmonella thresholds at slaughter
- Cage-free/aviary systems: impact on flock health
  - Associated with increased mortality vs. conventional housing
    - Coalition for Sustainable Egg Supply 3-year hen housing study
  - Increased exposure to manure, dust, carcasses, pathogens
  - Increased behavioral issues, aggression, injuries (fighting/flying)

Salmonella – Food Safety, not Flock Health

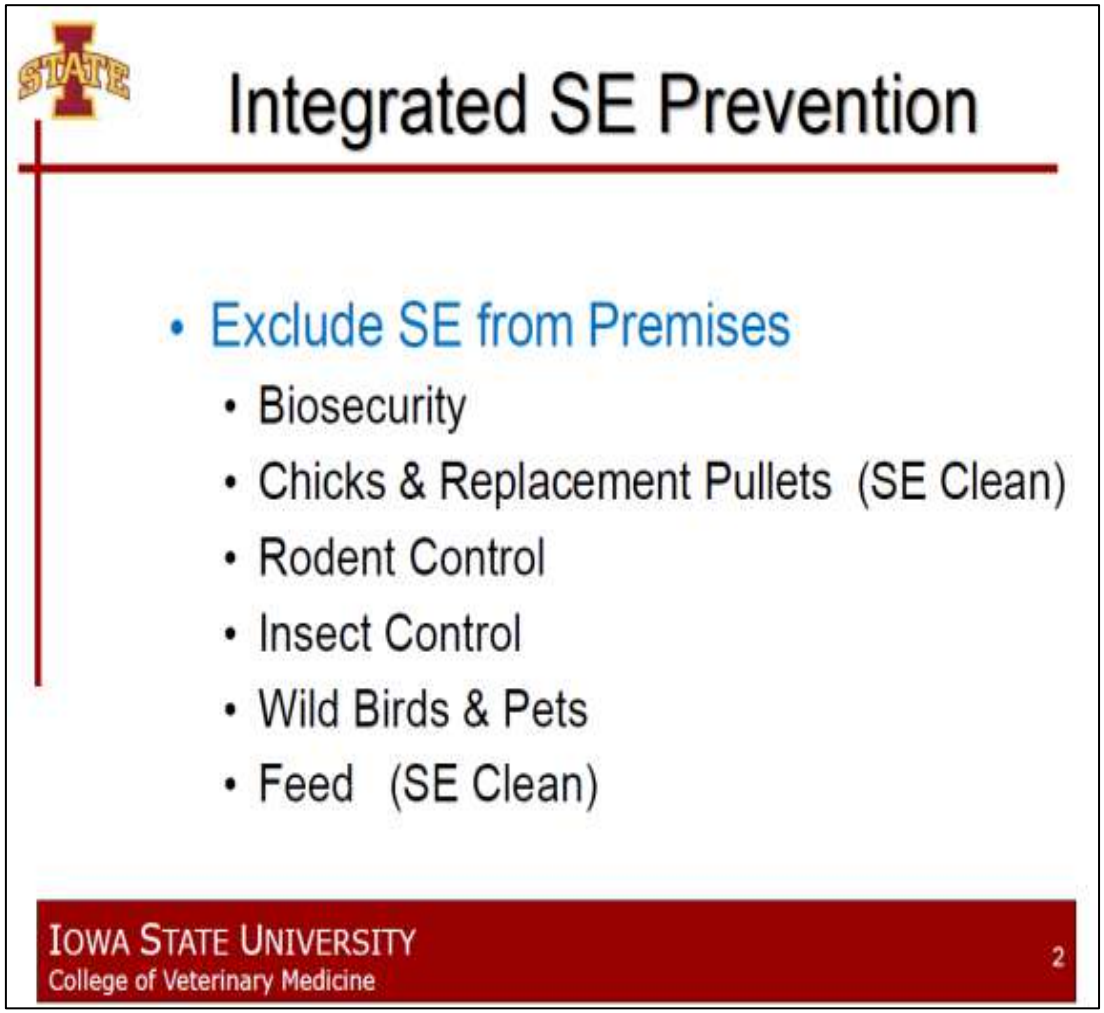
- S. Enteritidis has been well controlled by the layer industry; vaccination has been part of that success
- What is the status of S. Heidelberg, Oranienburg, etc?
  - Also Typhimurium, Newport, Senftenberg, Braenderup?
  - Coalition study: "Hens from all housing systems were shedding Salmonella spp. (89–100% of manure belt scraper swabs)"
- A couple problems with Salmonella vaccination:
  - Traditional killed vaccines (bacterins) are mostly serotype-specific
    - Similar to *E. coli* bacterin problem, too many strains
    - Need vaccines which target common surface proteins
  - Vaccine alone is not sufficient (see next slide)
    - Not just disease control, we expect **elimination** of organism

Integrated Farm Management to Prevent SE Contamination of Eggs

D.W. Trampel, DVM, PhD, DACVP

Iowa Egg Symposium 2013

In my experience:
Without a complete program, **vaccine alone is insufficient** to exclude Salmonella from premises



Why are Bacterins Serotype-Specific?

- The immunodominant molecule on the outer surface of Gram(-) bacteria is the polysaccharide (also called "somatic antigens" or "O-type")
- Inactivated bacterins stimulate host immunity to the polysaccharide; this is the reason we call it "serotype"
- A bacterin containing S. Enteritidis has no O-antigens in common with S. Kentucky, for example
- Same principle for *E. coli* bacterins

Name (Serotype)	Sero Group	Somatic O-type
Typhimurium	B	1,4,5,12
Heidelberg	B	[1],4,5,[12]
Agona	B	4,12
Mbandaka	C1	[1],6,7,[25]
Newport	C2	6,8
Hadar	C2	6,8
Kentucky	C3	(8),20
Enteritidis	D1	1,9,12
Pullorum	D1	1,9
Meleagridis	E1	3,10
Senftenberg	E4	1,3,19
Oranienburg	O	6,7,14

Characterization of E. coli Isolates from Peritonitis

Darrell W. Trampel, Yvonne Wannemuehler, and Lisa K. Nolan

"Based upon results of this pilot study, it may be necessary to use a different bacterin in each house on the farm to protect against *E. coli*-associated peritonitis in laying hens. It would be desirable to have vaccines that offer protection against homologous and heterologous *E. coli* challenge. Vaccines targeting proteins encoded by large APEC virulence plasmids might provide widespread protection against heterologous *E. coli* challenge because these plasmids are common among APEC."

A New Vaccine Technology: SRP®

- **Porins:** Protein pores in the cell wall which allow essential nutrients to enter the cell
- **Siderophore Receptors:** Specialized porin proteins which transport iron-siderophore complexes through the cell wall – *IRON is essential for most bacteria*
- **SRP® technology:** Siderophore Receptor and Porin proteins, extracted from the bacterial cell wall, used as vaccine antigens

SRP Vaccine Concept

Pasteurella

S. Enteritidis

E. coli

The immune system remembers what it has seen; for Gram negative bacteria, it mostly remembers the outer surface – polysaccharide or “serotype”

SRP Vaccine Concept – Find the Common Factor

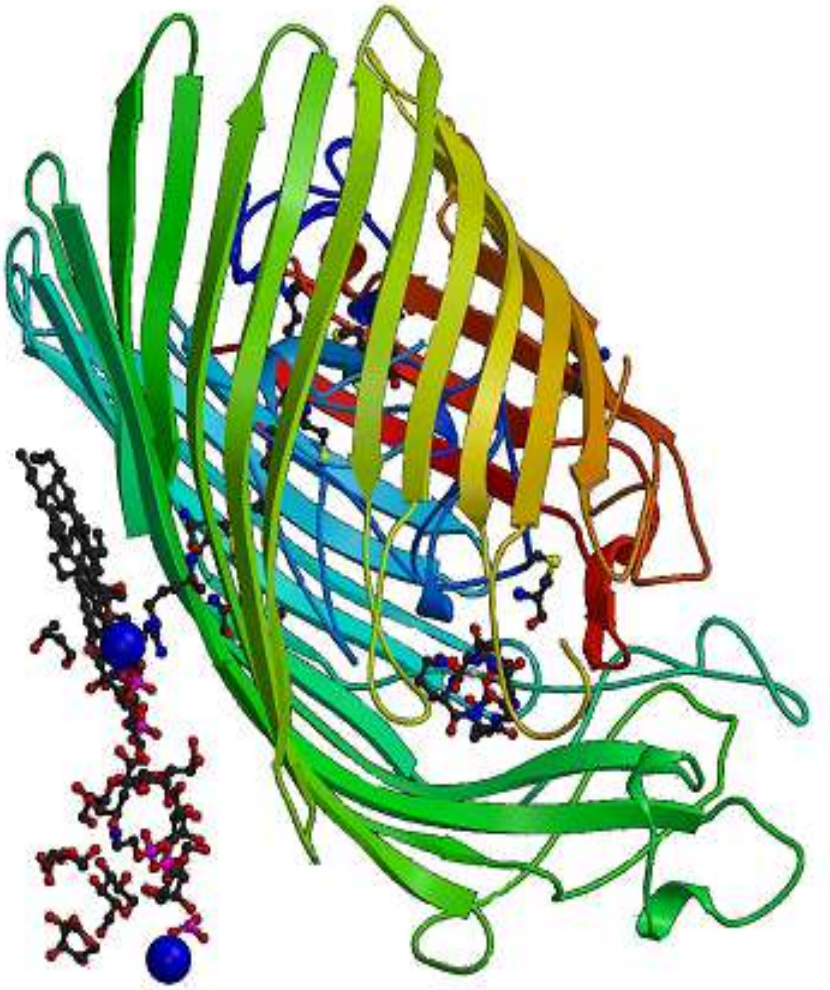
What do the balls have in common, on the exterior?

They all have air valves…
Which allow transport of much needed air into the football, basketball and volleyball.

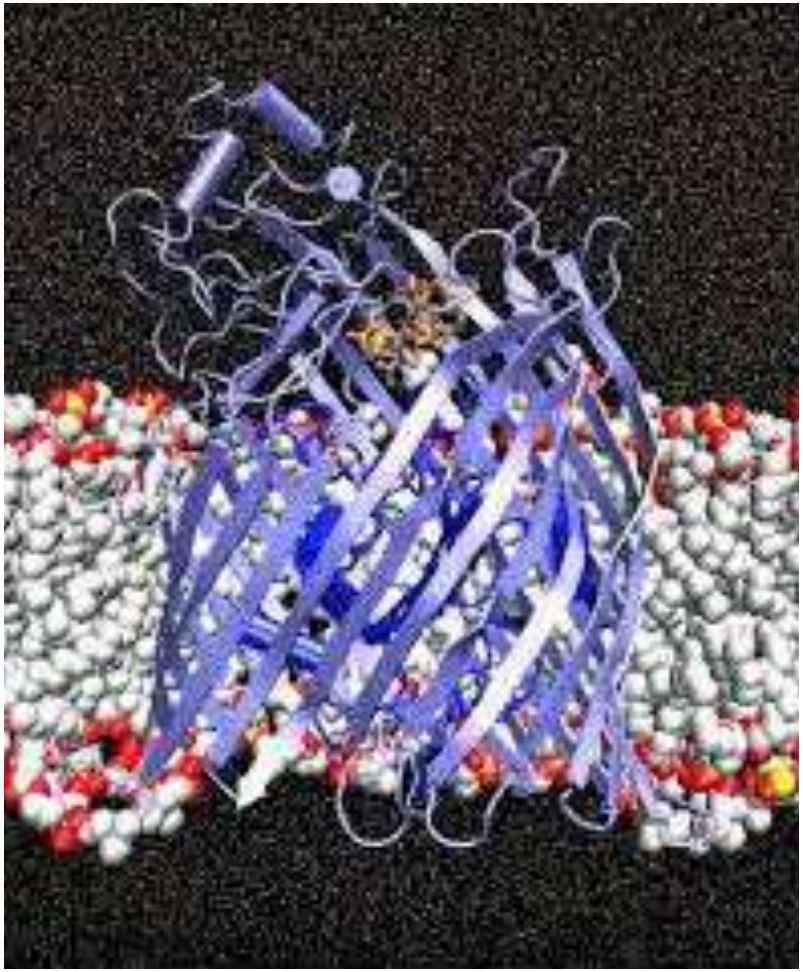
Siderophore Receptors: "match" certain siderophores, to acquire iron

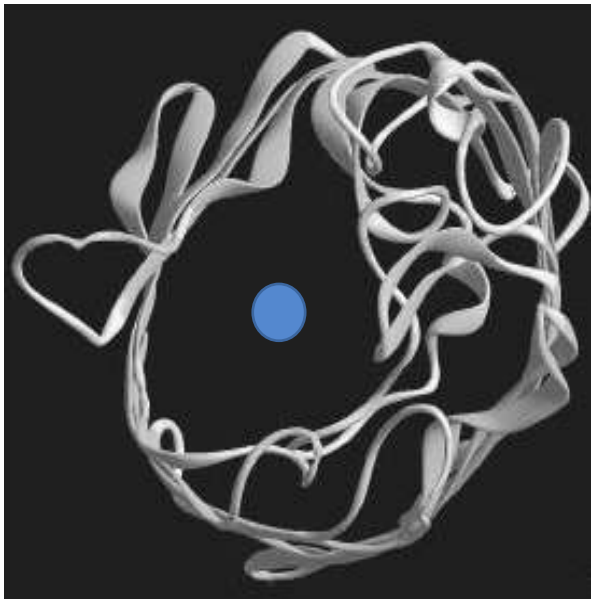
E. coli
Siderophore Receptor

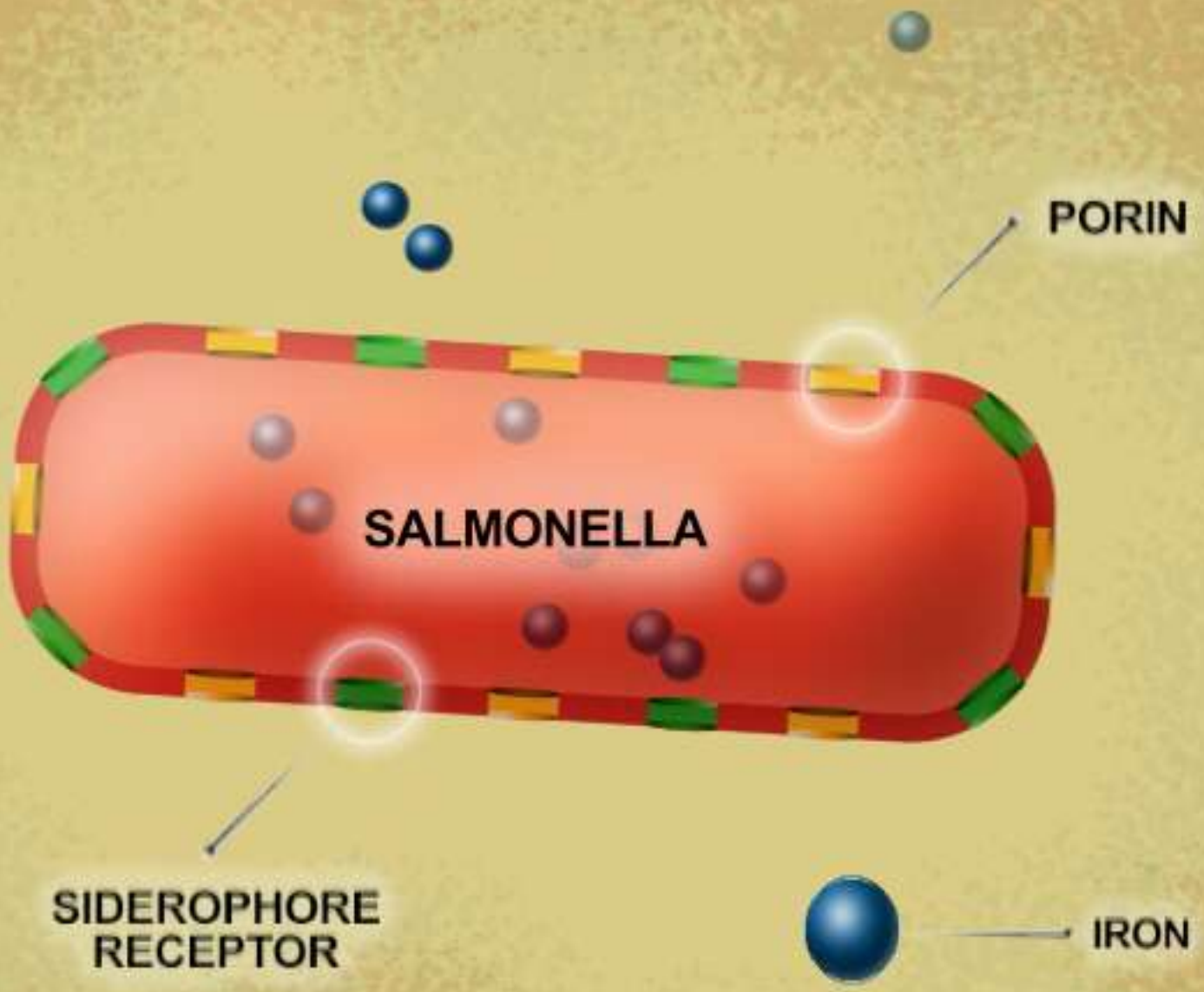
Enterochelin
(a siderophore) with bound iron

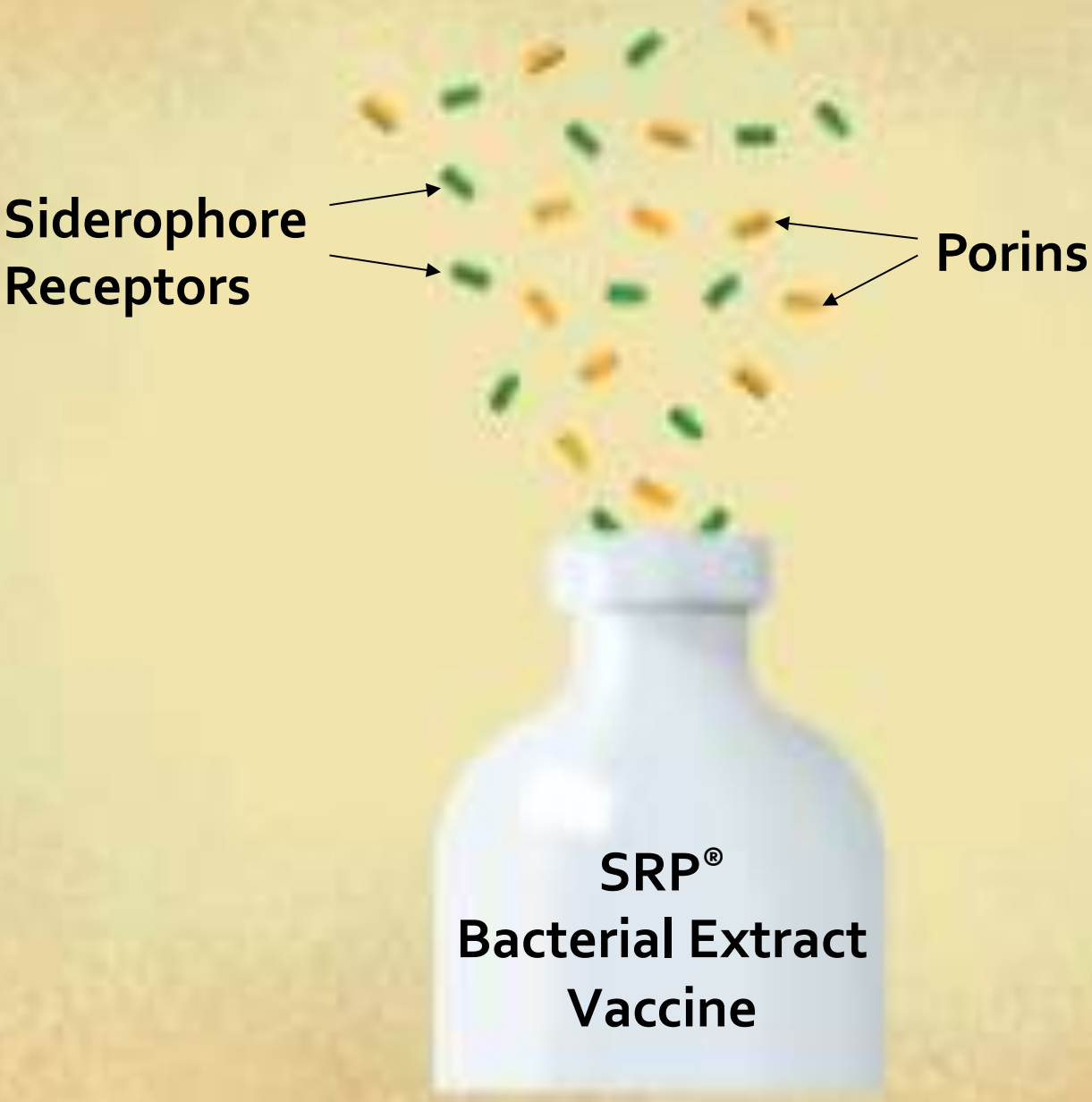


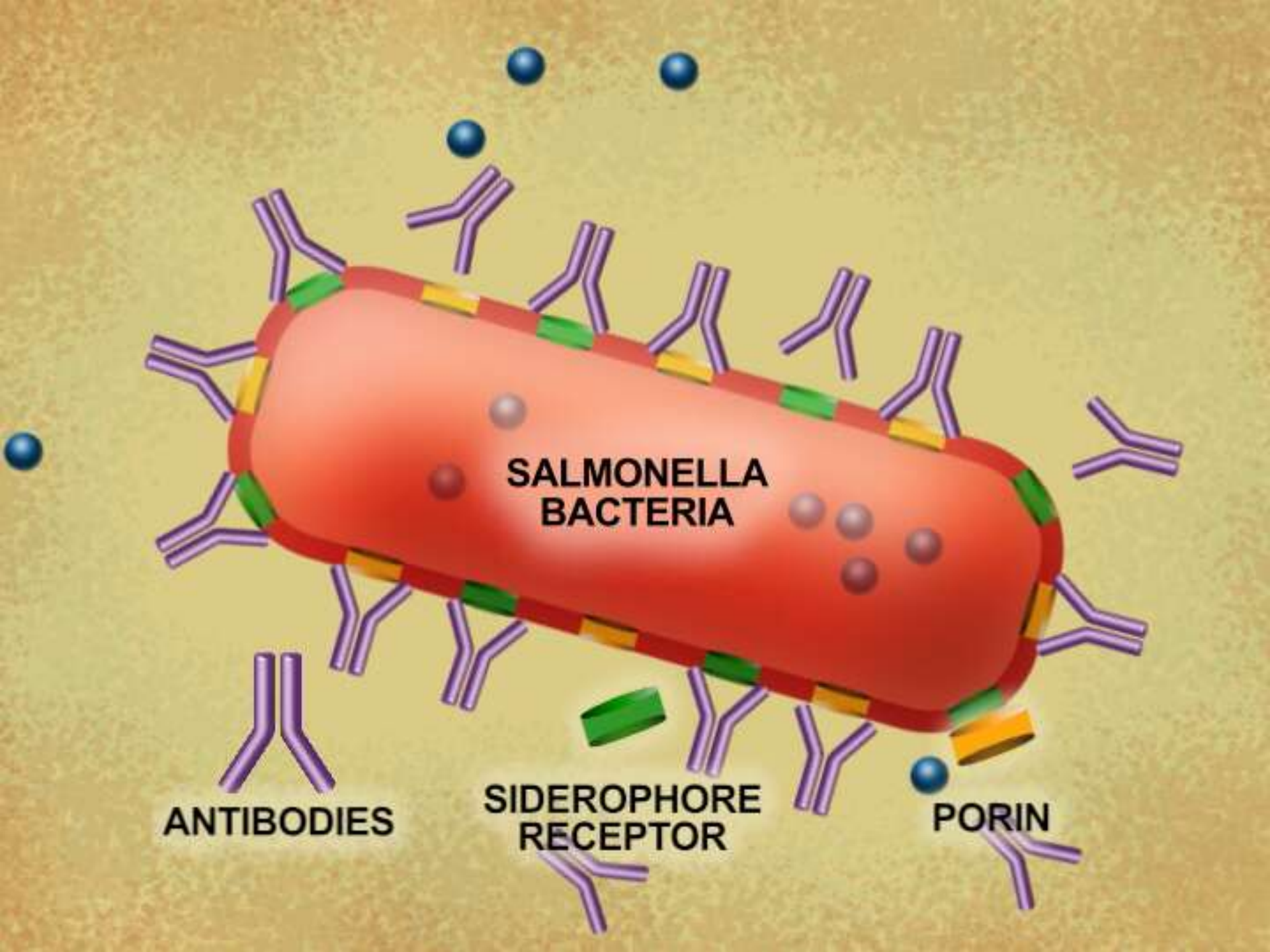
Orientation of Siderophore Receptors in Outer Cell Membrane






PORIN
SALMONELLA
SIDEROPHORE
RECEPTOR
IRON


Siderophore
Receptors
Porins
SRP®
Bacterial Extract
Vaccine


SALMONELLA
BACTERIA
ANTIBODIES
SIDEROPHORE
RECEPTOR
PORIN

Siderophore Receptor Proteins are "Conserved" Among Gram(-) Bacteria

SRP	Klebsiella	Enterobacter	E. coli	Salmonella
FepA	X	X	X	X
FecA	X	X	X	
CirA	X	X	X	X
FhuA	X		X	
ChuA	X		X	
IroN			X	X

SRP Vaccine Efficacy vs SE Challenge in Layers

- Clinical challenge model designed for USDA efficacy studies
- 100 SPF leghorn females, divided in 3 groups, commingled in floor pen
- Two doses of SRP vaccine or placebo at 10 and 18 weeks of age
- Challenged with SE at 22 weeks of age, terminated at 24 WOA

Oviduct & Ovary SE Isolation at 14 days Post Challenge			
	% Positive	Prevented Fraction	Log_{10} cfu/gm
Placebo	24%	-	0.45
Low dose SRP	3.7%	85%, p = 0.036	0.03
High dose SRP*	0.0%	100%, p = 0.014	0.0

* Results accepted by USDA for demonstration of efficacy

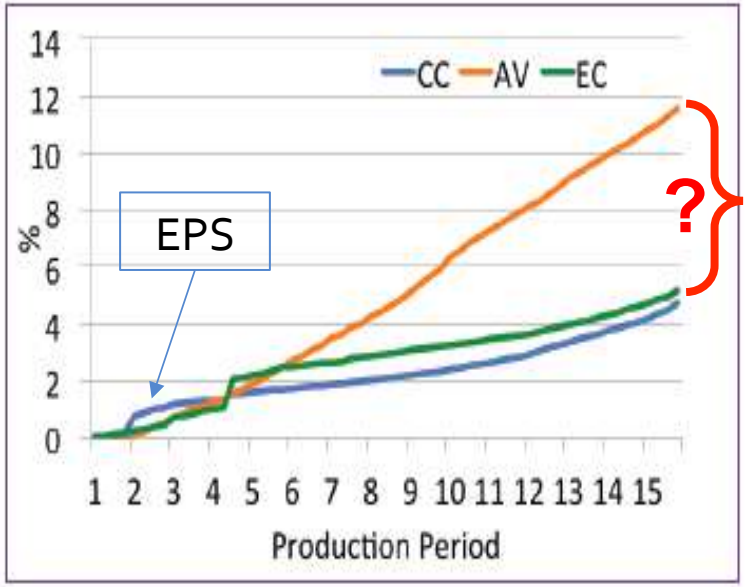
Laying Hen Housing Research Project

Quote from Summary Report:

"Hen mortality was much higher in the aviary system due to a variety of conditions, including hypocalcemia, egg yolk peritonitis, and [due] to behavioral issues, with hens either being excessively pecked, or picked out (vent). There was less mortality in the enriched colony due to behavioral issues, and the least in the conventional system. There was the most egg yolk peritonitis in the conventional cages, less in the aviary and the least in the enriched colony. It was also harder to detect dead birds in the aviary and enriched colonies than in conventional cages."

Cumulative mortality in flocks 1 and 2; Production Periods are four weeks, starting at 19 weeks of age.

A.



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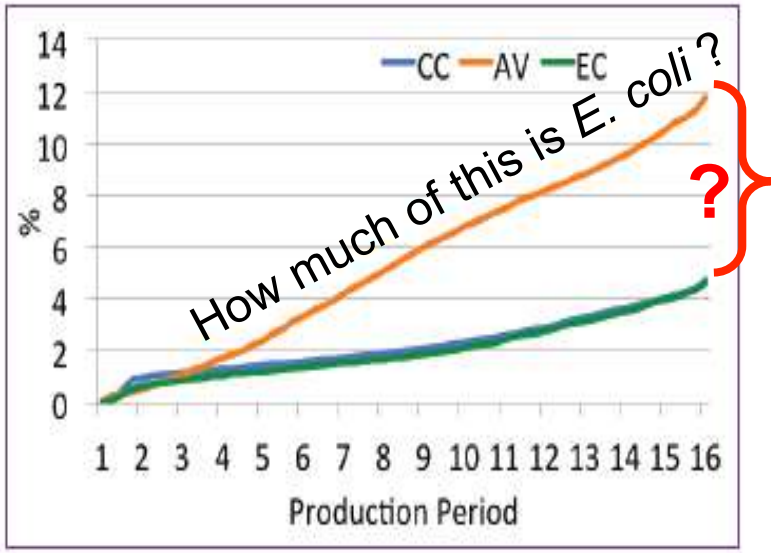
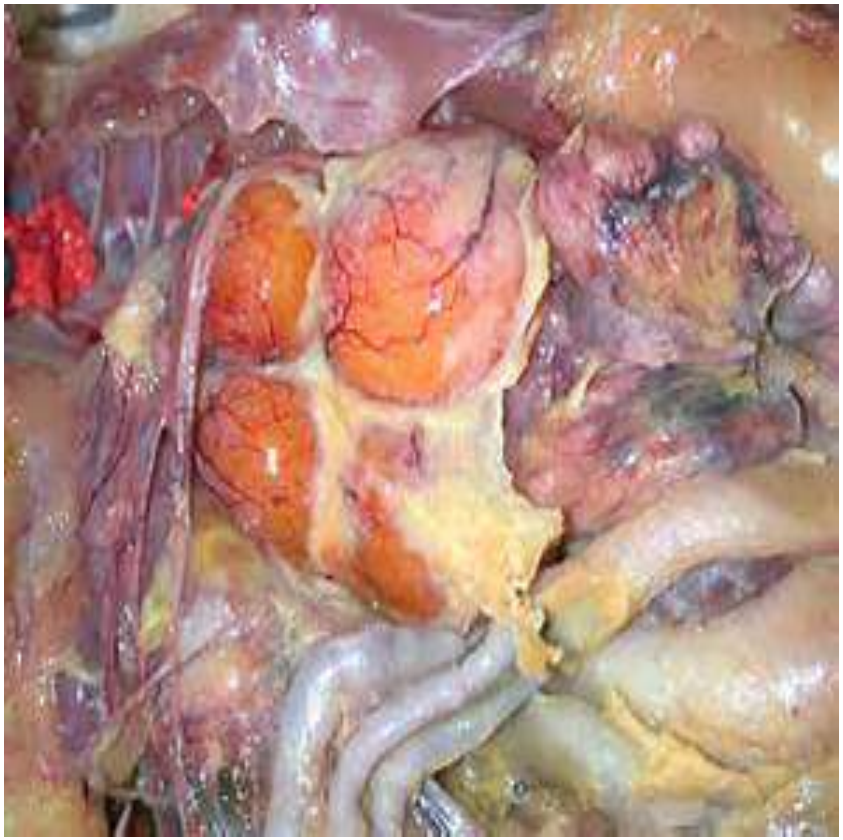


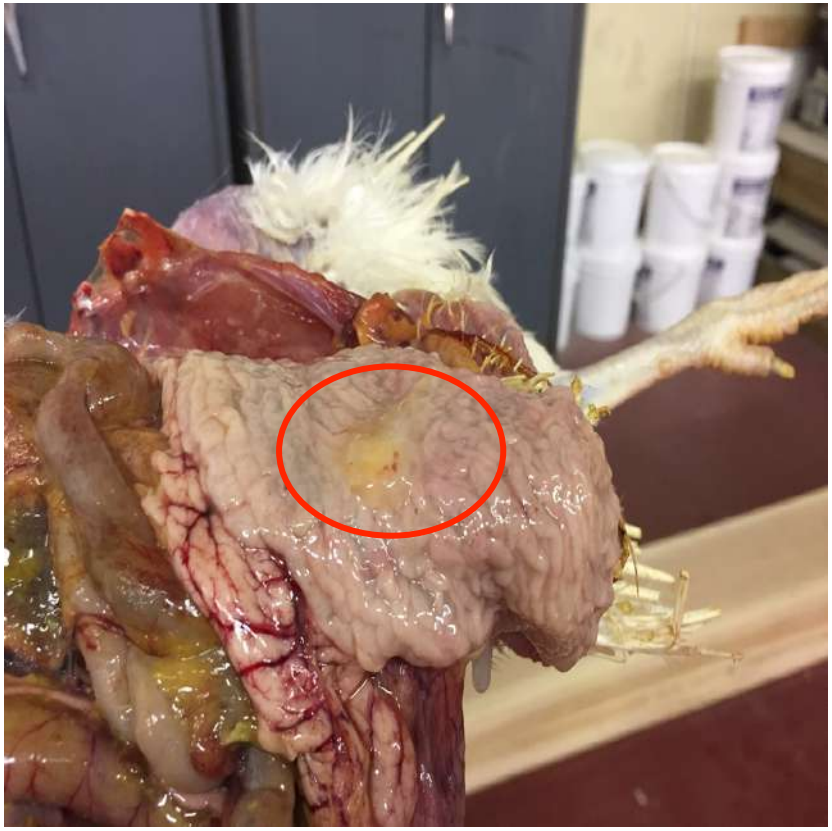
E. coli Peritonitis Syndrome (EPS)

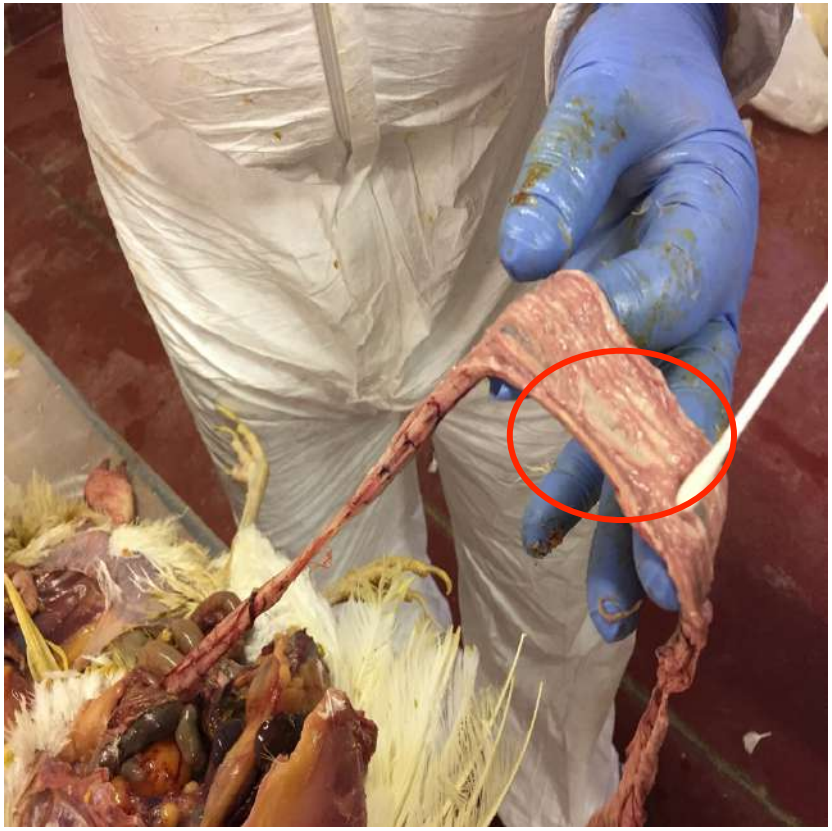
E. coli – APEC – Avian Pathogenic E. coli

- *E. coli* is normal in flora of poultry intestine and cloaca, but **not of oviduct**
- Can find *Gallibacterium anatis*, Pasteurella, Klebsiella
- EPS seems acute, usually in early lay
  - Internal layers, exudate in abdomen
- Coliform SPS seems more chronic:
  - Salpingitis/Periton./Salpingoperitonitis
  - Oophoritis and ovarian regression
- Infectious, but is it truly contagious?
- Affected hens in one house may all carry same strain of APEC (Trampel, Wannemuehler, Nolan 2007)
  - But, the adjacent house might have a totally different strain of APEC



Gallibacterium anatis





Commercial Field Trial: Autogenous E-S SRP

VACCINATION PROGRAM

KILLED MG 1 KILLED SE
2 KILLED SE/ECOLI IBD VECTOR

START 06/09/14
MODIFIED NEW ECOLI SE VACC

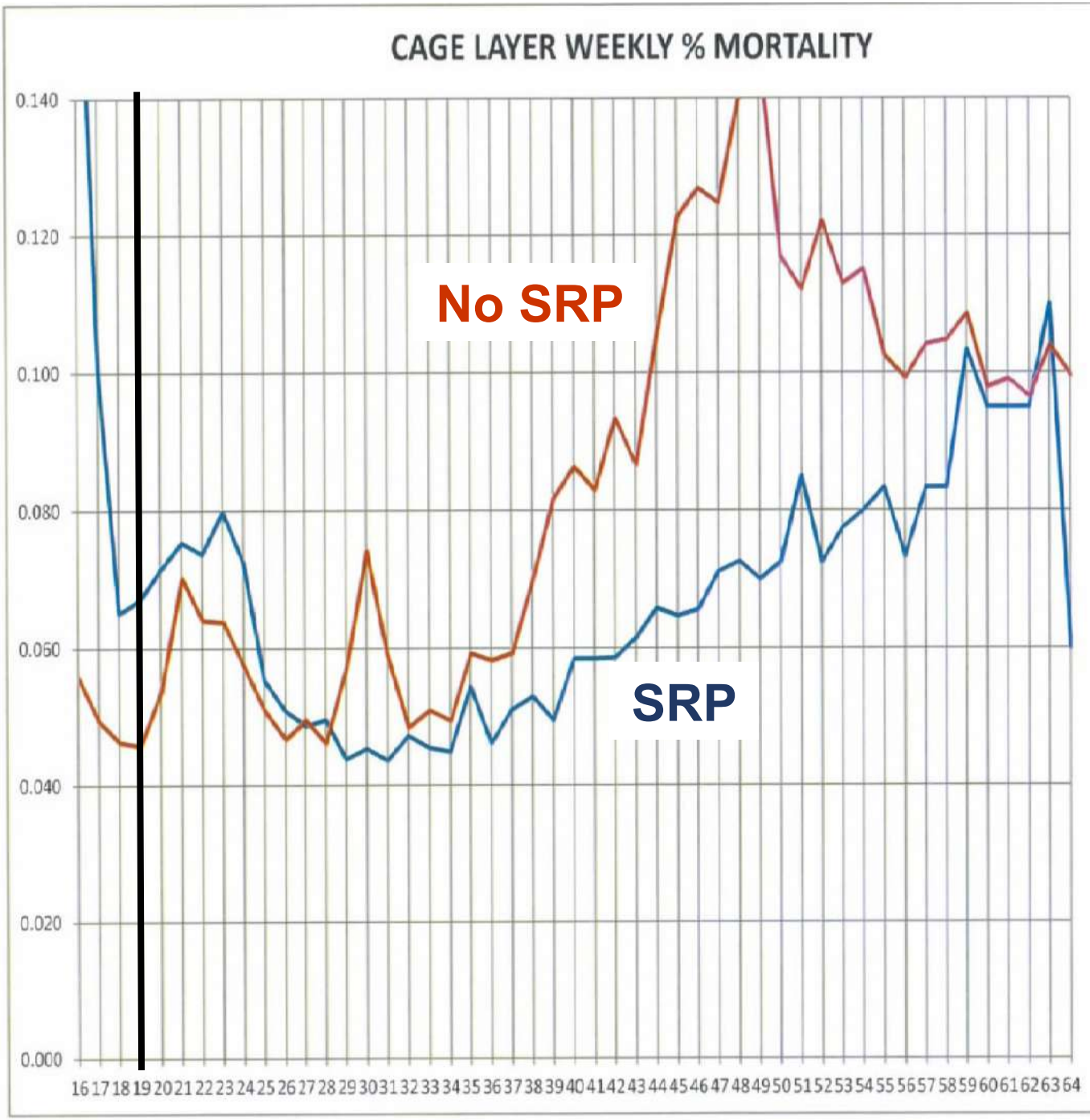
AGE	VACCINE	ROUTE	PRODUCT	MANUFACTURE
0 DAY	MAREKS	SUB-Q	CVI988 HVT/IBD	MERK SELECT
0 DAY	ST	COURSE SPRAY	MEGAN VAC	LOHMANN
18 DAY	NDV-IBV	COURSE SPRAY	B1-MASS / ARK	MERK
18 DAY	ST	COURSE SPRAY	MEGAN VAC	LOHMANN
6 WKS	NDV-IBV	COURSE SPRAY	ND-IB SOHOL	LOHMANN
6 WKS	ST	COURSE SPRAY	MEGAN EGG	LOHMANN
8 WKS	ILT	WATER X 3	LARYNGOVAC	SELECT
10 WKS	FOWL POX	WW	PIGEON POX	SELECT
10 WKS	FOWL POX - AE	WW	PT BLEN	SELECT
10 WKS	NDV-IBV / MG	SUB-Q oil	AVIPRO 304 .5	LOHMANN
10 WKS	SE / E-COLI	SUB-Q oil	AUTOGENOUS SRP .25	PHIBRO
12 WKS	ILT	WATER X 3	LARYNGOVAC	SELECT
14 WKS	SE	IM Inguinal Fold	MBL SE4-C oil	LOHMAN
14 WKS	SE / E-COLI	IM	AUTOGENOUS SRP oil	PHIBRO
15 WKS	NDV-IBV	FINE SPRAY	ND-IB SOHOL	LOHMANN

10-week and 16-week oil vaccinations combined using THAMA-VET 222 syringe

Cages

Total cumulative mortality during lay cycle approx. 4% without SRP, 2% with SRP

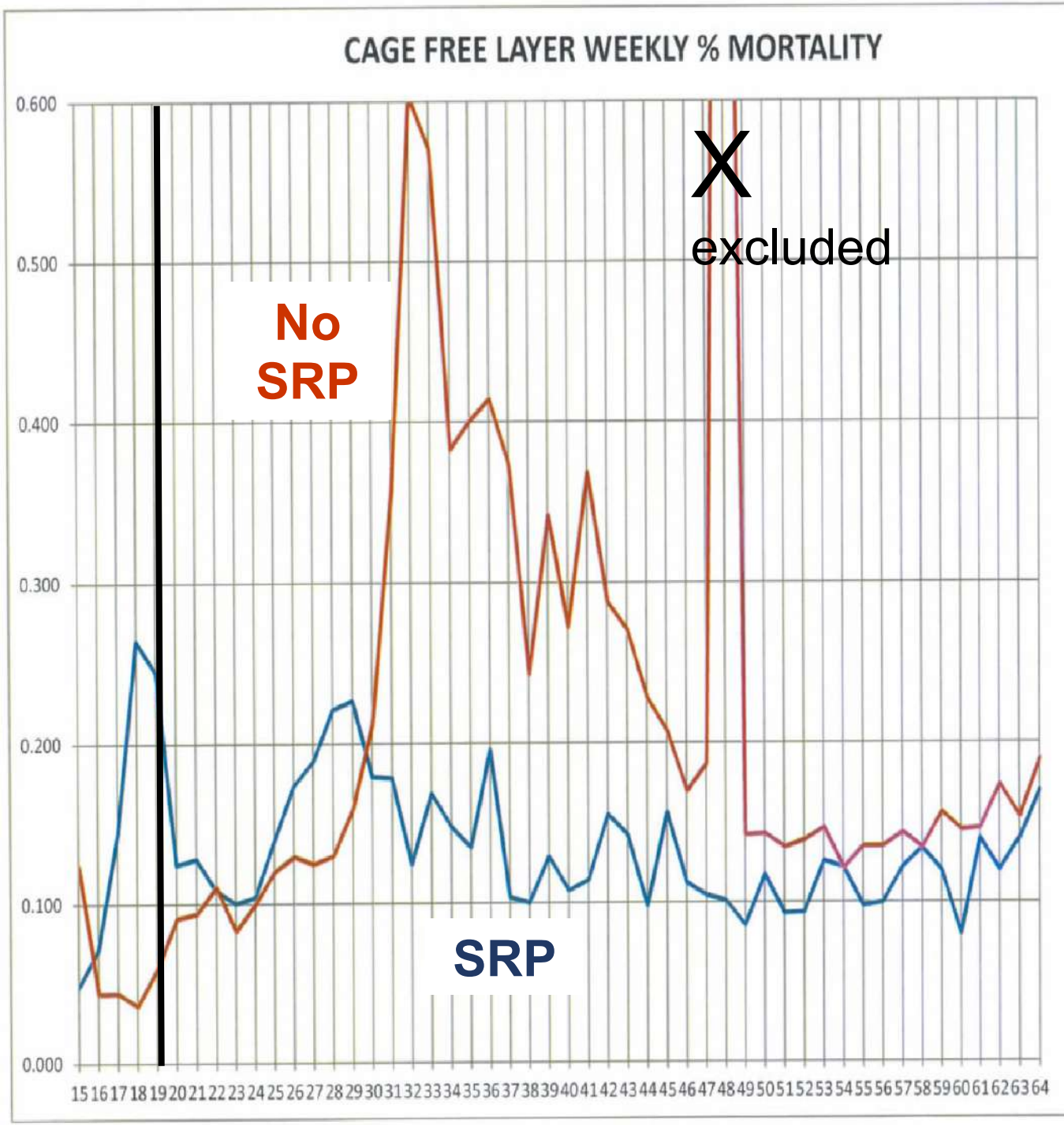
Early mortality attributed to "hemorrhagic hepatopathy" syndrome



Cage-free

Total cumulative mortality during lay cycle approx. 8% without SRP, 4% with SRP

(48-wk mortality episode was not associated with disease or vacc.)



Thank you!

vaxxinova