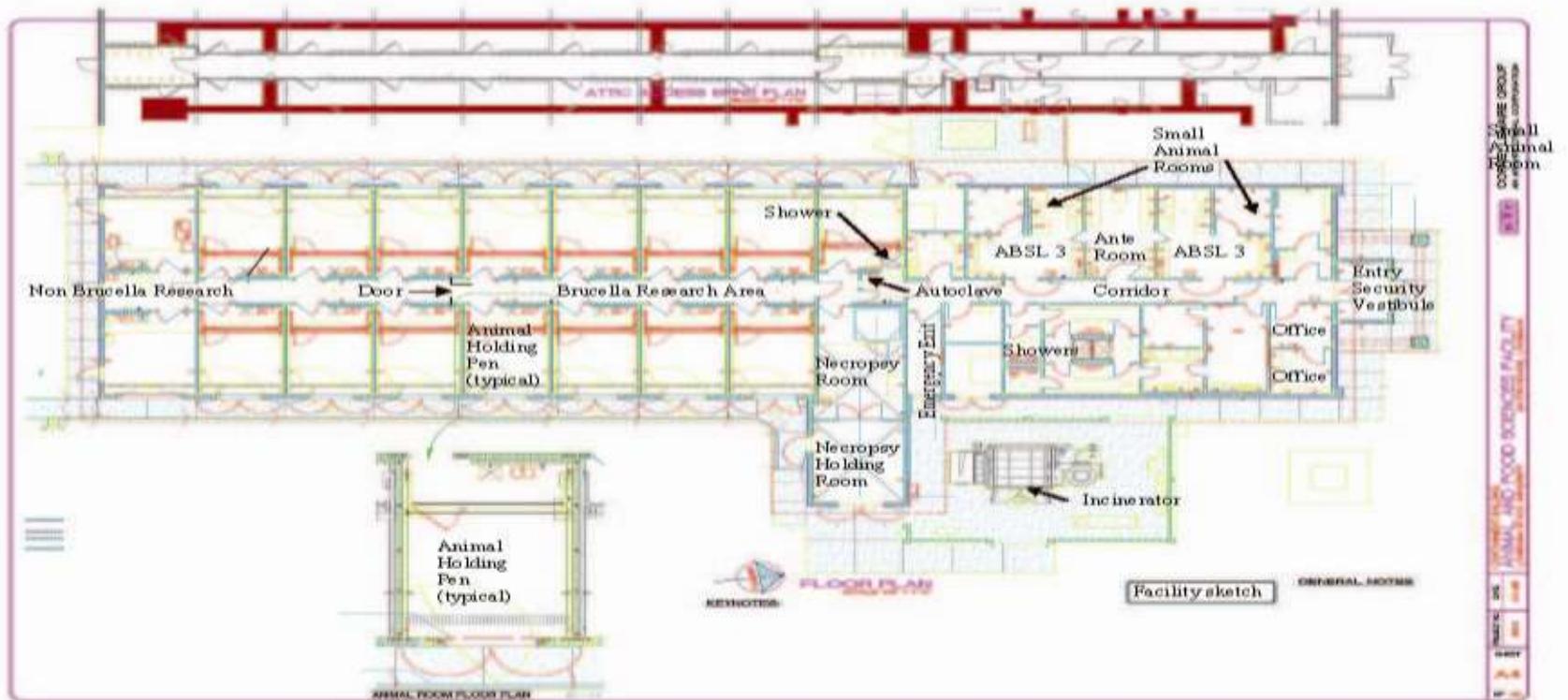


Western Blots – Procedure, Results and Interpretations

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Inside view of floor plan

Two - BSL 3 lab w/ABSL 3 small animal rooms; 15 large animal rooms w/ Hepa filters, sinks, boot wash, sealed with negative pressure



Animal holding room

Hydraulic gate/squeeze system, sealed floors, air handling,
sinks/boot wash

Western Blot Analysis or Immunoblots

- Electrophoretic separation of proteins
 - SDS page gel - size
- Transfer to nitrocellulose
 - Horizontal transfer - membrane
- Antibody assay
 - Test sample
 - Secondary antibody conjugate
 - Color development
 - **Bands only appear where test sample antibodies bound to the antigen**

Brucella vs Yersinia

- *Brucella abortus* vs *Yersinia enterocolitica* serotype 0:9
 - Both Gram negative bacteria
 - Both have outer membrane
 - Both have Lipopolysaccharide (LPS)
 - LPS composed of
 - Lipid A
 - Core
 - O-polysaccharide (OPS)

Brucella vs Yersinia

■ PROBLEM

– *Brucella abortus* OPS is identical to *Y. enterocolitica* 0:9 OPS

– SO

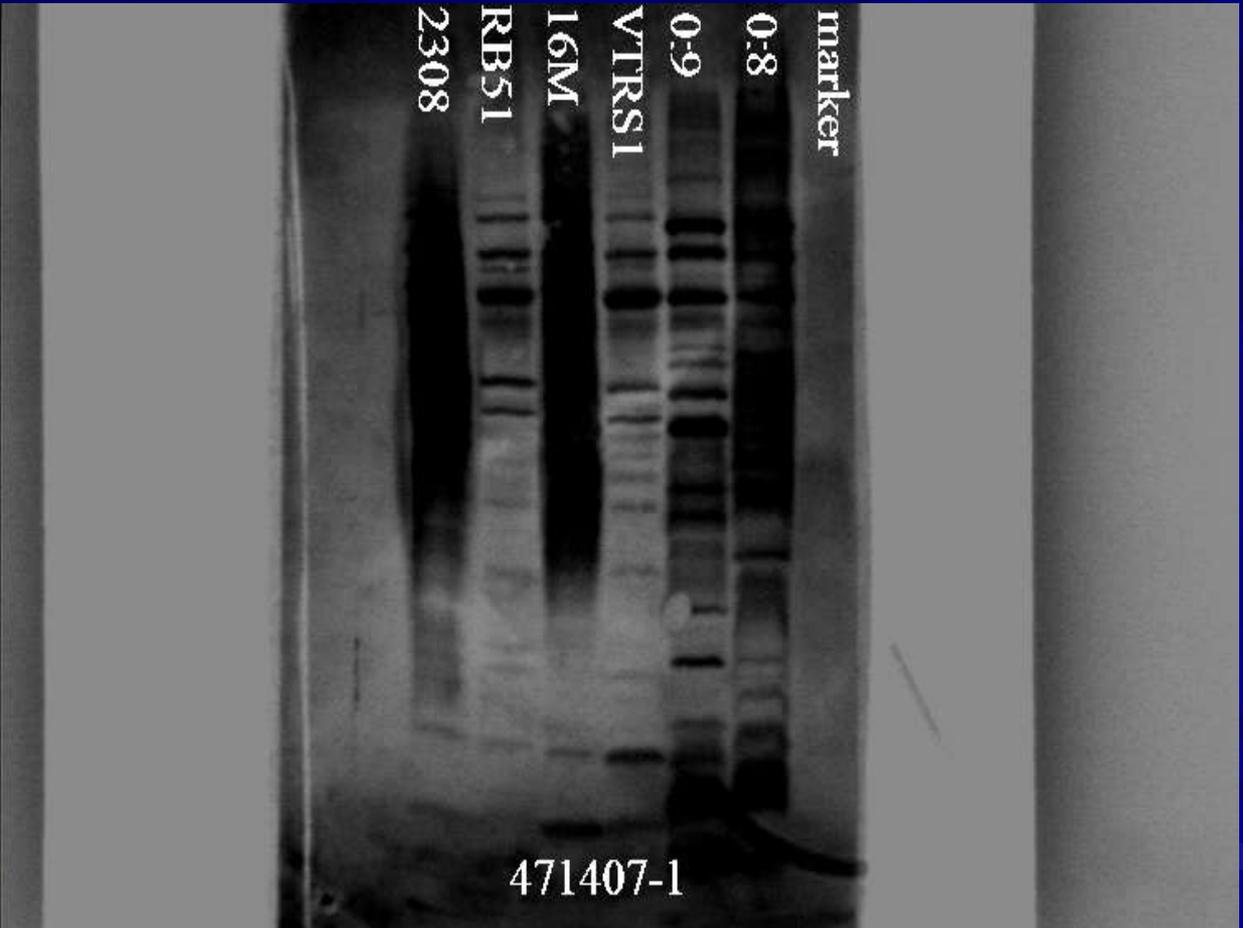
- Animals make antibodies against OPS of *Yersinia* and they cause cross reaction on *Brucella* tests.
- An animal infected with *Yersinia* may have a positive reaction on brucellosis tests

Yersinia enterocolitica 0:9

- Normal Flora
- Gut pathogen – might isolate from feces
- In old or young animal might get loose stool
- Asymptomatic in most cattle
- Easily transmitted
- Not problem in most animals
- Titters wax and wane – negative in 2-3 months

Theory

- Western blots can be used to differentiate
be Brucella and Yersinia infections
 - Protein banding and OPS smears
- Problems
 - Not a simple assay
 - Expensive
 - Difficult to interpret – one reader
 - What if sample does not fit the criteria all of
the time = ELK



Based on information:
Brucella field strain or recent S19
 vaccination

	Smooth <i>Brucella</i>	Rough <i>Brucella</i>	<i>Yersinia</i> 0:9	<i>Yersinia</i> 0:8
Protein	Bands	Bands	----	-----
Lipid A	Bands	Bands	----	----
Core	Bands	Bands	----	-----
OPS	Bands/ smear	----	Bands/ smear	----

RB51 vaccinate

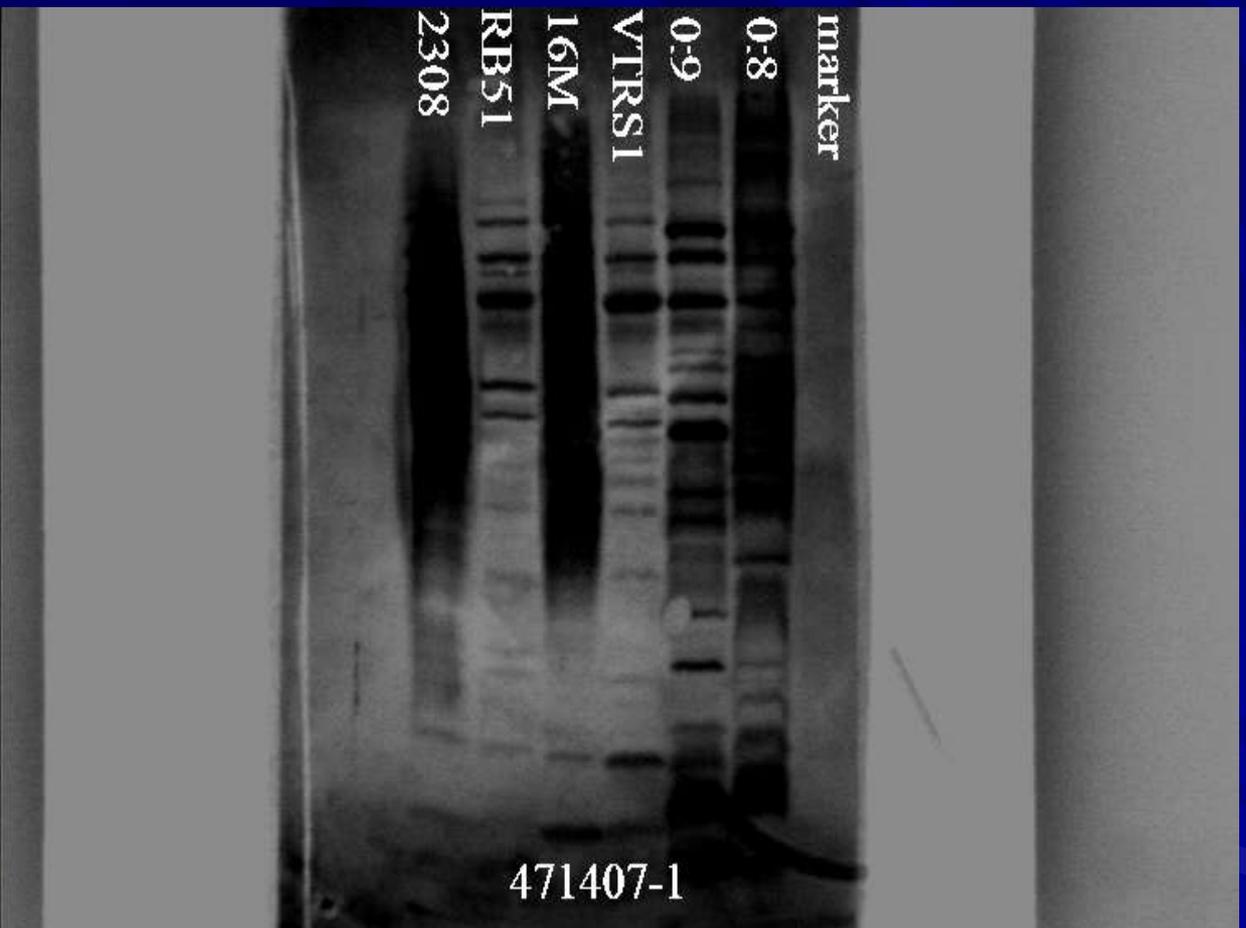
	Smooth <i>Brucella</i>	Rough <i>Brucella</i>	<i>Yersinia</i> 0:9	<i>Yersinia</i> 0:8
Protein	Bands	Bands	----	-----
Lipid A	Bands	Bands	----	----
Core	Bands	Bands	----	-----
OPS	----	----	----	----

Yersinia 0:9 infected animal

	Smooth <i>Brucella</i>	Rough <i>Brucella</i>	<i>Yersinia</i> 0:9	<i>Yersinia</i> 0:8
Protein	----	----	Bands	Bands
Lipid A	----	----	Bands	Bands
Core	----	----	Bands	bands
OPS	Bands/ smear	----	Bands/ smear	----

Brucella vaccinate + *Yersinia* 0:9

	Smooth <i>Brucella</i>	Rough <i>Brucella</i>	<i>Yersinia</i> 0:9	<i>Yersinia</i> 0:8
Protein	Faint	Faint	Strong	Strong
Lipid A	Faint	Faint	Faint	Faint
Core	Faint	----	Strong	-----
OPS	Strong	----	Strong	----



Other cross reacting organisms

- *Other Brucellae*
- *E.coli*
- *Vibro*
- *Salmonella*
- *Pseudomonas*
- *Francisella*
- *Pasturella*

Other cross reacting organisms

- *Brucella*'s close cousins the nitrogen fixing organisms in the soil
 - *Agrobacterium sp.*
 - *Rhizobium sp.*

Reporting

■ Yersinia

- unique banding patterns

■ Brucella infected

- high titer distinct banding

■ Brucella exposed

- cleared infection – still have some banding
- recent infection – lower titer some banding
- vaccinated animal

Reporting

- Background reactions
 - very few protein bands
 - some bands unique to the brucellae
 - bacteria frequently share proteins esp if they are related like brucellae and nitrogen fixers
 - Can not call it negative because criteria is more than 3 bands is a reaction

Conclusions

- No, it is not an official test; but it is a tool to aid the epidemiologist
- Antibody reactions are the immune response to an antigen
 - Antibody positive it has seen an antigen
 - Does NOT mean infection or disease
 - Vaccination or exposure to organism or a cross reacting antigen

QUESTIONS?

