

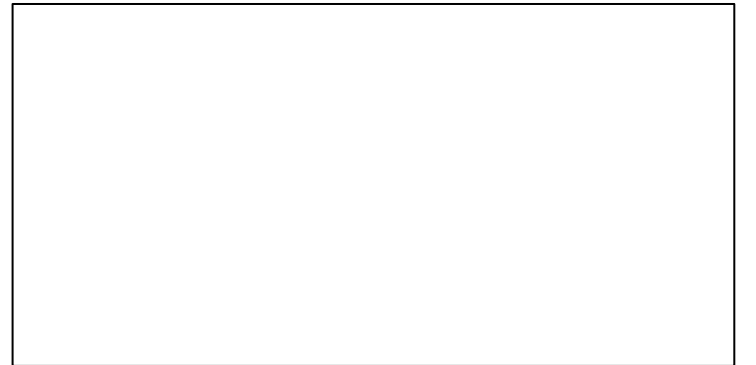
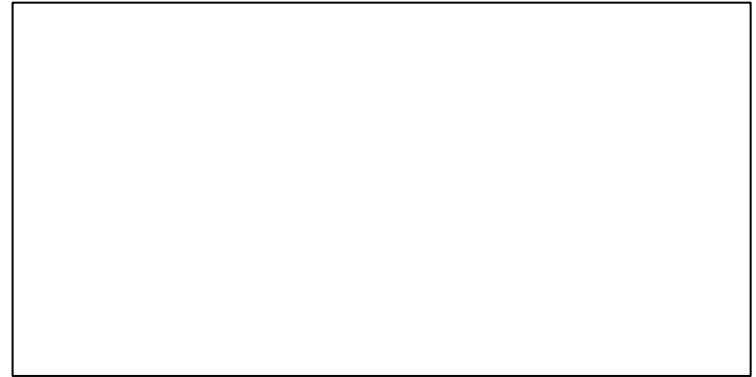
Molecular Epidemiology – Examples from the world of Milk quality and Mastitis

Ruth N. Zadoks, DVM, PhD

Quality Milk Production Services
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Molecular Tools

- Routine methods:
grow live bacteria
- Molecular methods:
detect bacterial DNA
 - dead/alive
 - milk/manure/bedding
 - species/strains/genes
 - many techniques



Milk quality and Mastitis

- Rapid detection of food borne pathogens
- Antimicrobial resistance mechanisms
- Source tracing of spoilage organisms
- Host adaptation of pathogen strains
- Strain specific epidemiology
- Species identification
- Pathogen evolution

Mastitis Paradigm

CONTAGIOUS

- *Strep. agalactiae*
- *Staph. aureus*
- Mycoplasma

ENVIRONMENTAL

- Coliforms
 - *E. coli*
 - *Klebsiella*
- “Strep. species”
 - *Strep. uberis*
 - *Strep. dysgalactiae*
 - *Enterococci*

Streptococcus agalactiae

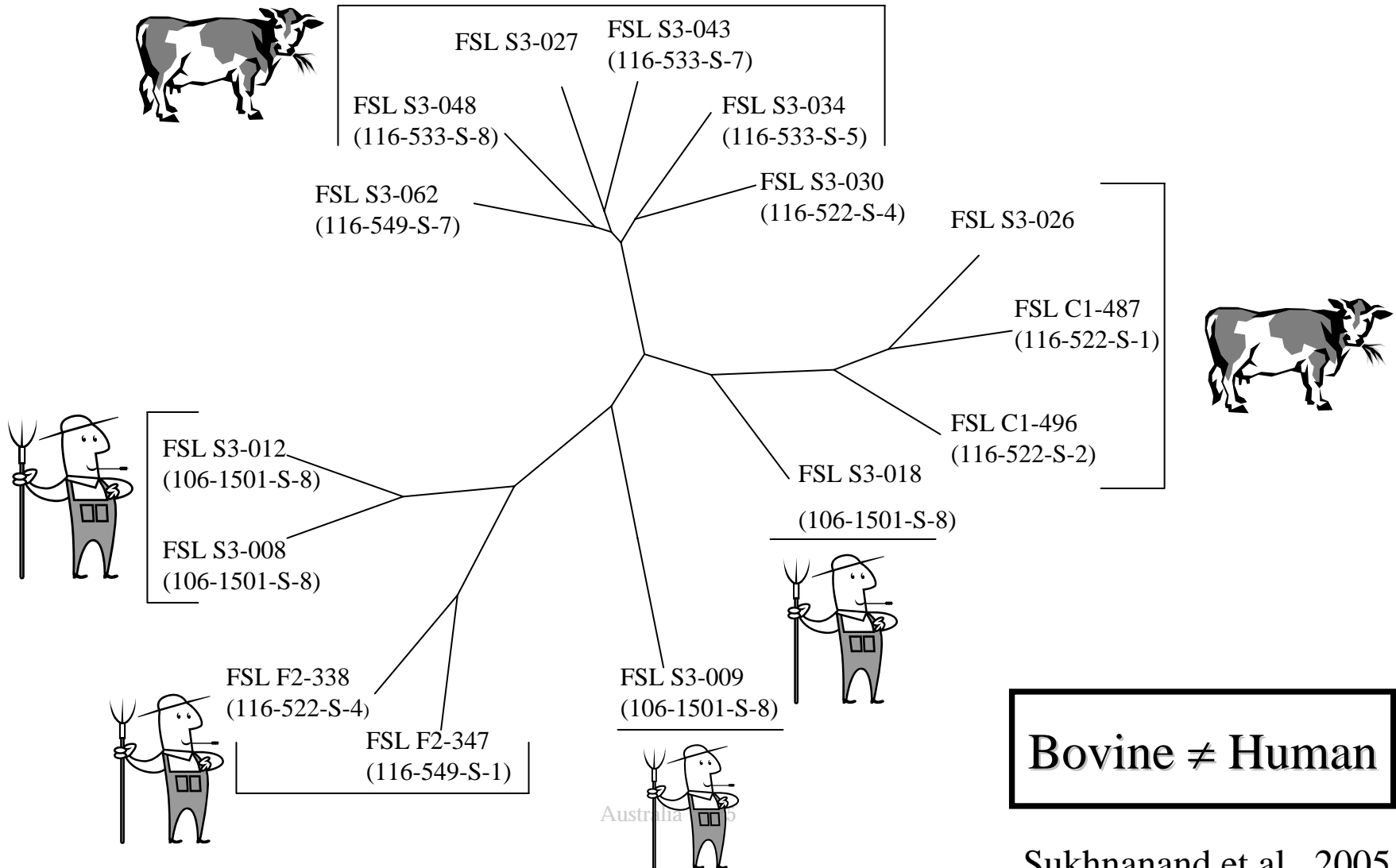
- Cows

- “*Strep. agalactiae*”
- high SCC (cow, tank)
- no clinical mastitis
- rapid spread via milking machine
- introduction with purchased animals
- good response to treatment

- People

- “group B streptococcus”
- severe disease, death, permanent damage
- mother-to-child and sexual transmission
- people, dogs and cats can be carriers
- treatment options depend on allergies

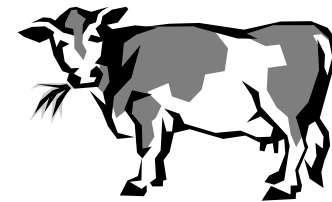
Not All *Strep. ag.* is Equal



Bovine ≠ Human

Sukhnanand et al., 2005

Case Study - *S. canis*



- Contagious or environmental?
- Source identification: cat with chronic sinusitis
- Host species barrier jump
- Rapid spread due to circumstances

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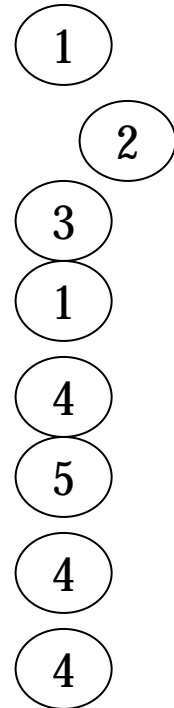
Tikofsky and Zadoks, 2005

Environmental sources of *S. uberis*

- Organic dairy
- Environmental samples
 - What they eat/drink
 - Where they lie down
 - Where they congregate
 - Seasonal extra's

S. uberis in the Environment

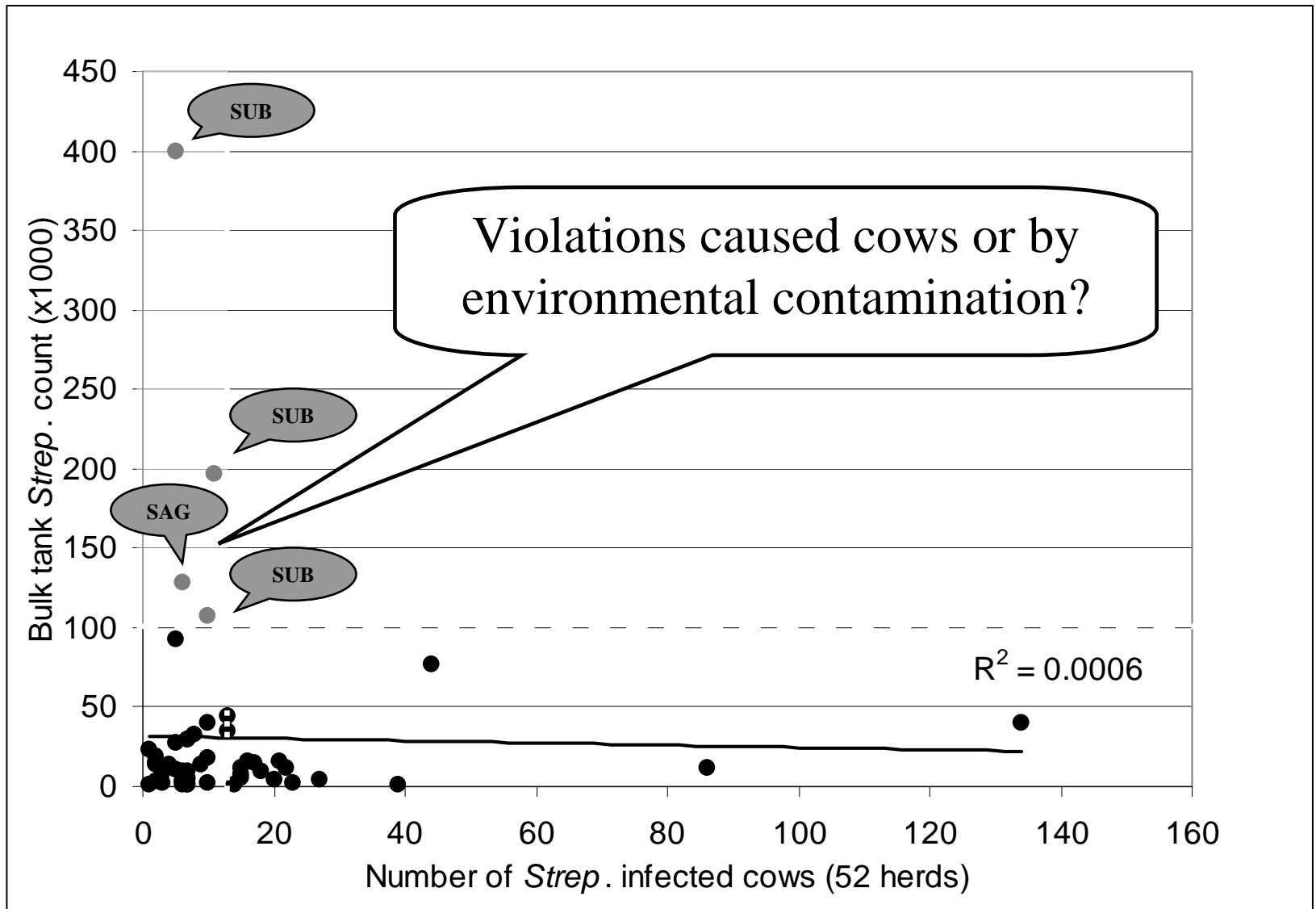
- 63% of env. samples
 - 89% of soil
 - 64% of grazing matter
 - 42% of water
 - hay, flies, leaves
 - bedding (clean, used)
- 23% of fecal samples



High Bacteria Counts in BTM

- Poor milking time hygiene
- Poor equipment cleaning
- Poor milk cooling
 - *Coliforms*
 - *Environmental streptococci*
- Cows with mastitis
 - *S. agalactiae*
 - *S. aureus*

Poor correlation between counts

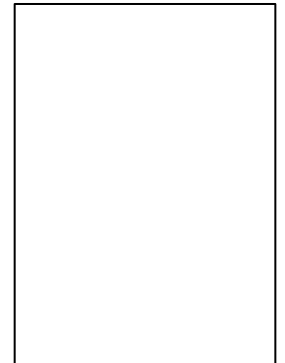


It's a Cow!

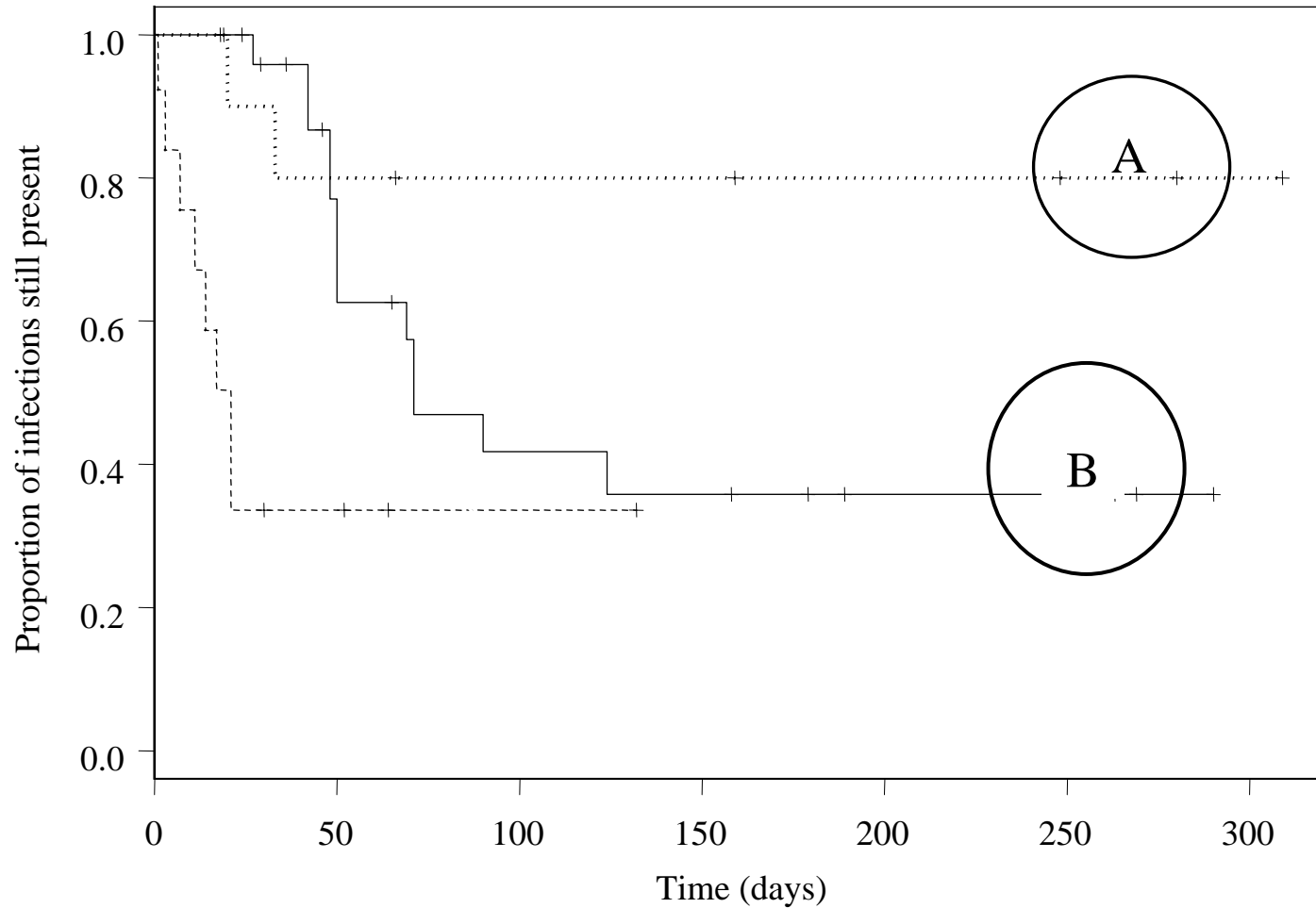
Farm	Source	Ribotype	RiboPrinter™ pattern
A	Bulk Tank	116-795-5	
	Bulk Tank	116-795-5	
	Bulk Tank	116-795-5	
	Cow 1	116-795-5	⇒
	Cow 2	116-793-3	
E	Bulk Tank	116-788-6	
	Bulk Tank	116-788-6	
	Bulk Tank	116-788-6	
	Cow	116-788-6	⇒

How Many Cows Does it Take?

- Saran, 1995 – shedding 10^9 cfu/ml
- Van Werven, 1997 – shedding $>10^9$ cfu/ml
- Schulte, 2004 – shedding 10^8 cfu/ml
- PMO limit – 100,000 = 10^5 cfu/ml
- Dilution by factor 10^3 – 10^4 would still result in violation of PMO limit
- That is 1 quarter per 250-2500 cows!



S. uberis – duration by strain



Some Strains are More Contagious Than Others

Streptococcus agalactiae (Jensen, 1982)

- bovine strains > human strains

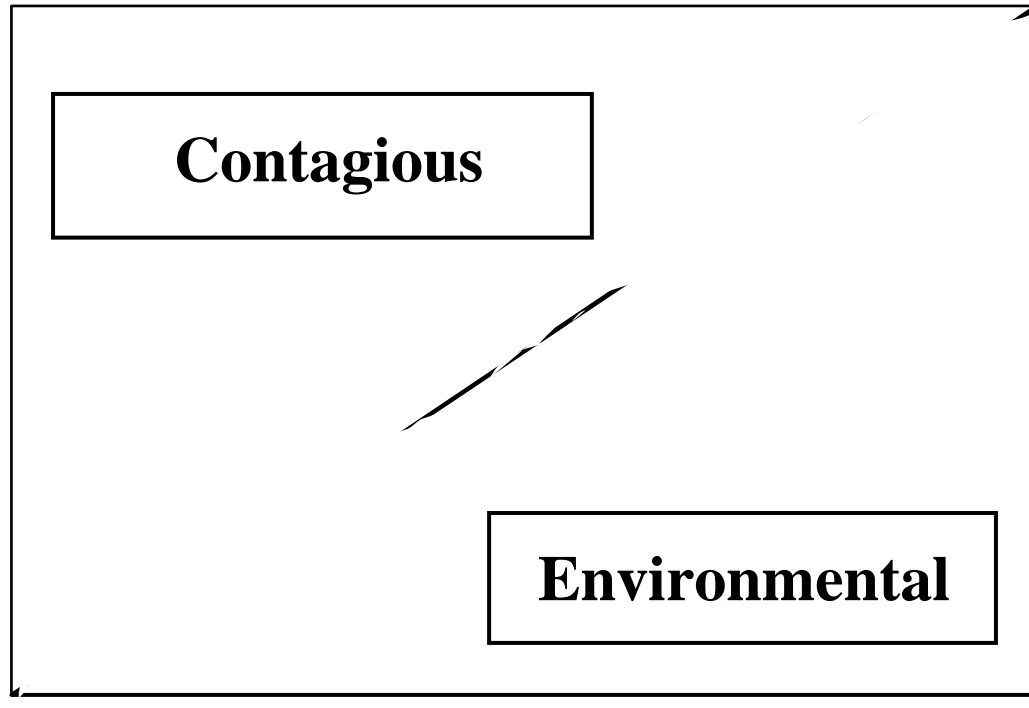
Staphylococcus aureus (Smith et al., 1998)

- novel strain > standard test strain

Streptococcus uberis (Zadoks et al., 2003)

- strain B > other strains

One Size Does Not Fit All



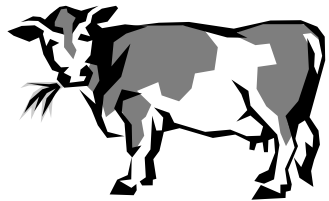
Garrison, Zadoks
(QMPS, FSL)



Comparative vs. Library Typing

- Comparative Methods
 - banding patterns
 - within study
- Library Methods
 - standardized
 - web based

MLST



Source

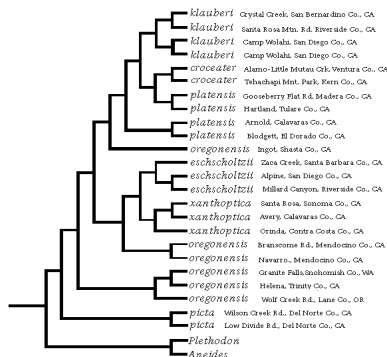
Isolated colonies

Amplified DNA



Phylogenetic analysis

Alignment

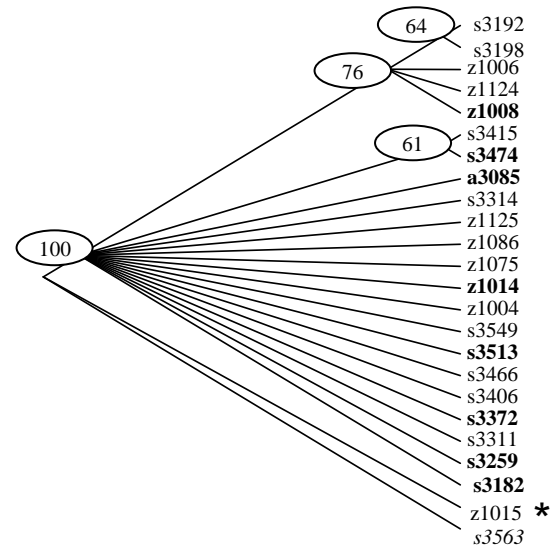
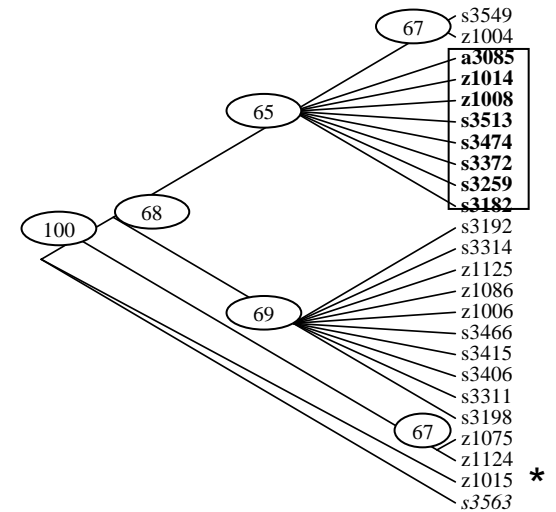


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Nucleotide sequence

MLST - *S. uberis*

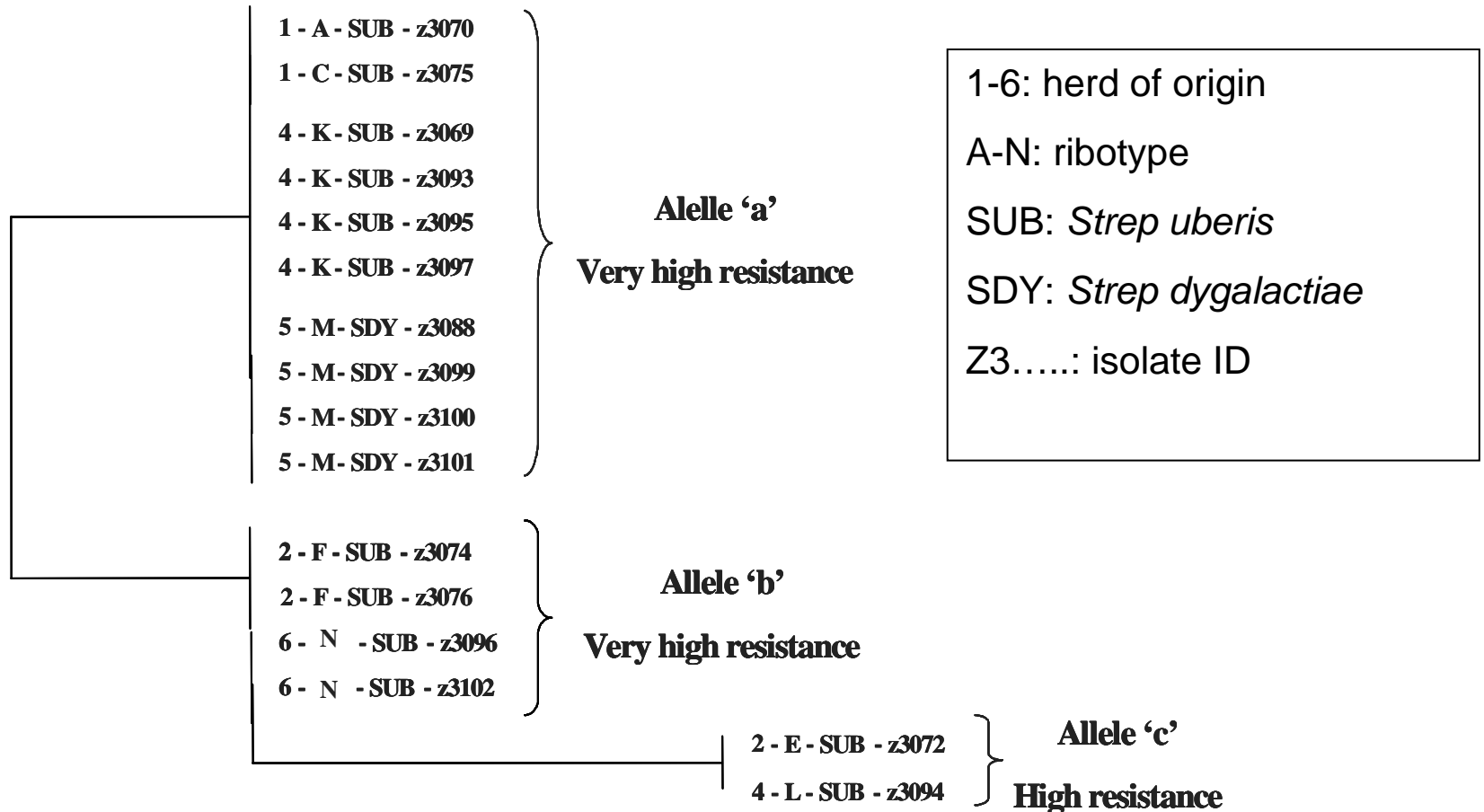
- Non-clonal population
- New subtaxon
- *pauA* negative isolates
 - diagnostics
 - vaccination
- Contagious strains?
- Strain specific management?



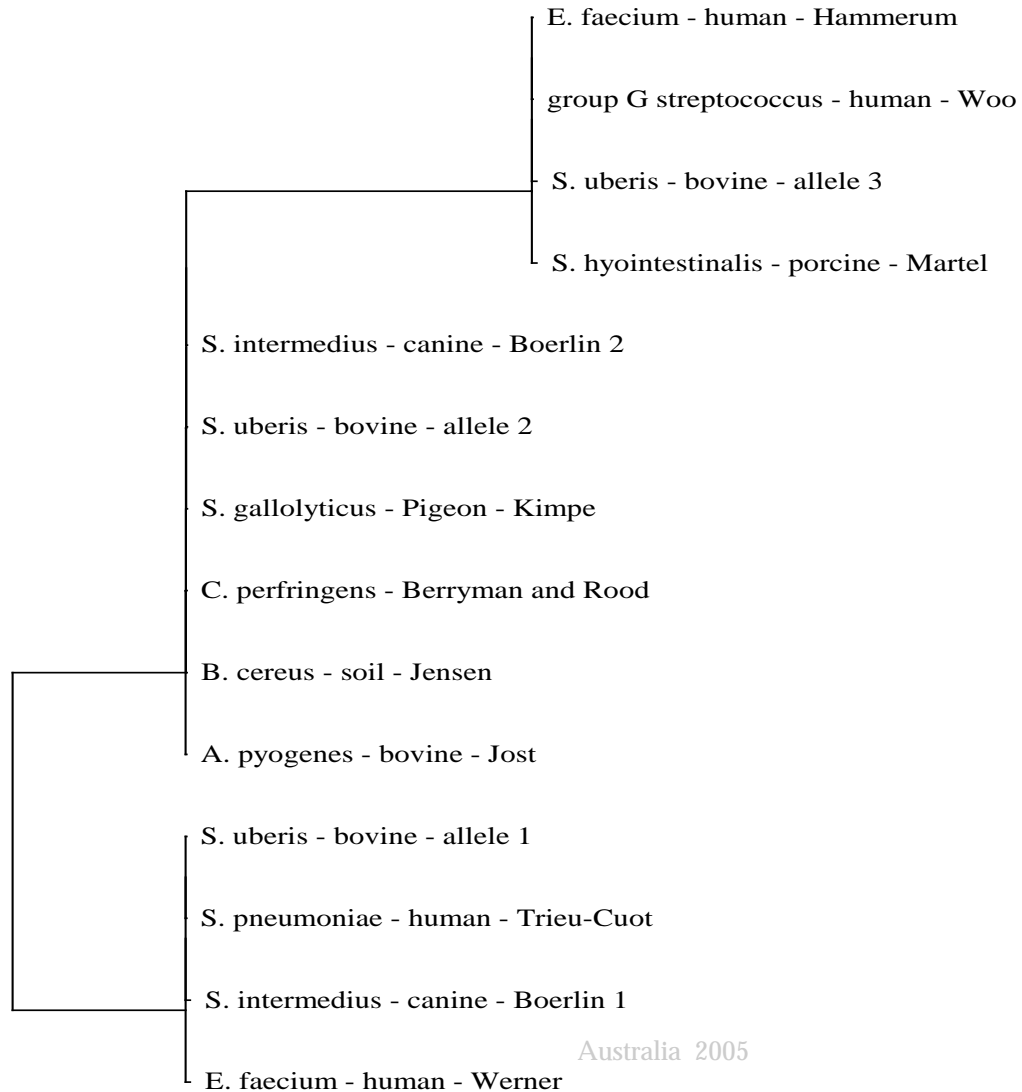
Case Study – *Antibiotic Resistance*

- “No increase in resistance due to mastitis Tx”
- Treatment of subclinical mastitis recommended
- Marcolides only registered antibiotics
- Macrolide resistance in Streptococci increased
- Prevalence of Streptococci increased
- Which resistance genes are present?
- Can resistance result from gene transfer?
 - Intraspecies or interspecies?
 - Between host and environment?

Species, Strains and Genes



Interspecies Resistance Gene Transfer



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One Size Does Not Fit All

- Not all drugs are equal
- Spread of resistance is more likely for some antibiotics than for others
- Strain typing provides deeper understanding of mastitis epidemiology

Questions?

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Australia 2005