

Food Safety Microbiology: some research priorities for the future

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(formerly known as The National Food Centre)

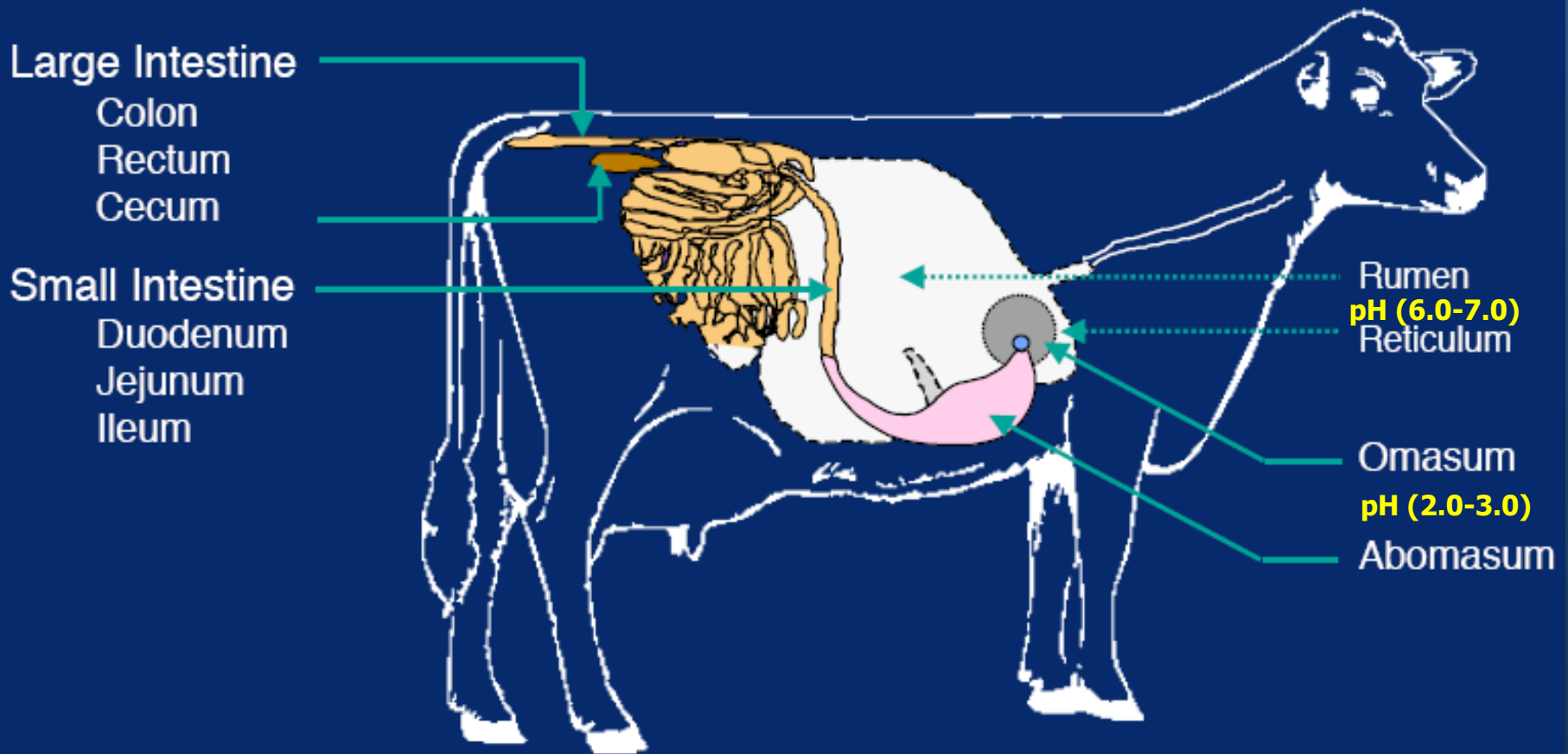
Future Research Priorities

1. Isolation & detection of pathogens
2. Pathogen identification
3. Control strategies for food pathogens
4. Techniques for the study of organisms in communities
5. Advances in risk assessment techniques
6. Mechanisms of survival & virulence ✓

Mechanisms of Survival

- In nature all bacteria are in stationary phase
- Bacteria adapt to a wide range of hostile environments
- Stresses include heat, cold, osmolarity (a_w) & acidity
- These stresses occur in animals, food & humans
- In this presentation acid adaptation will be discussed

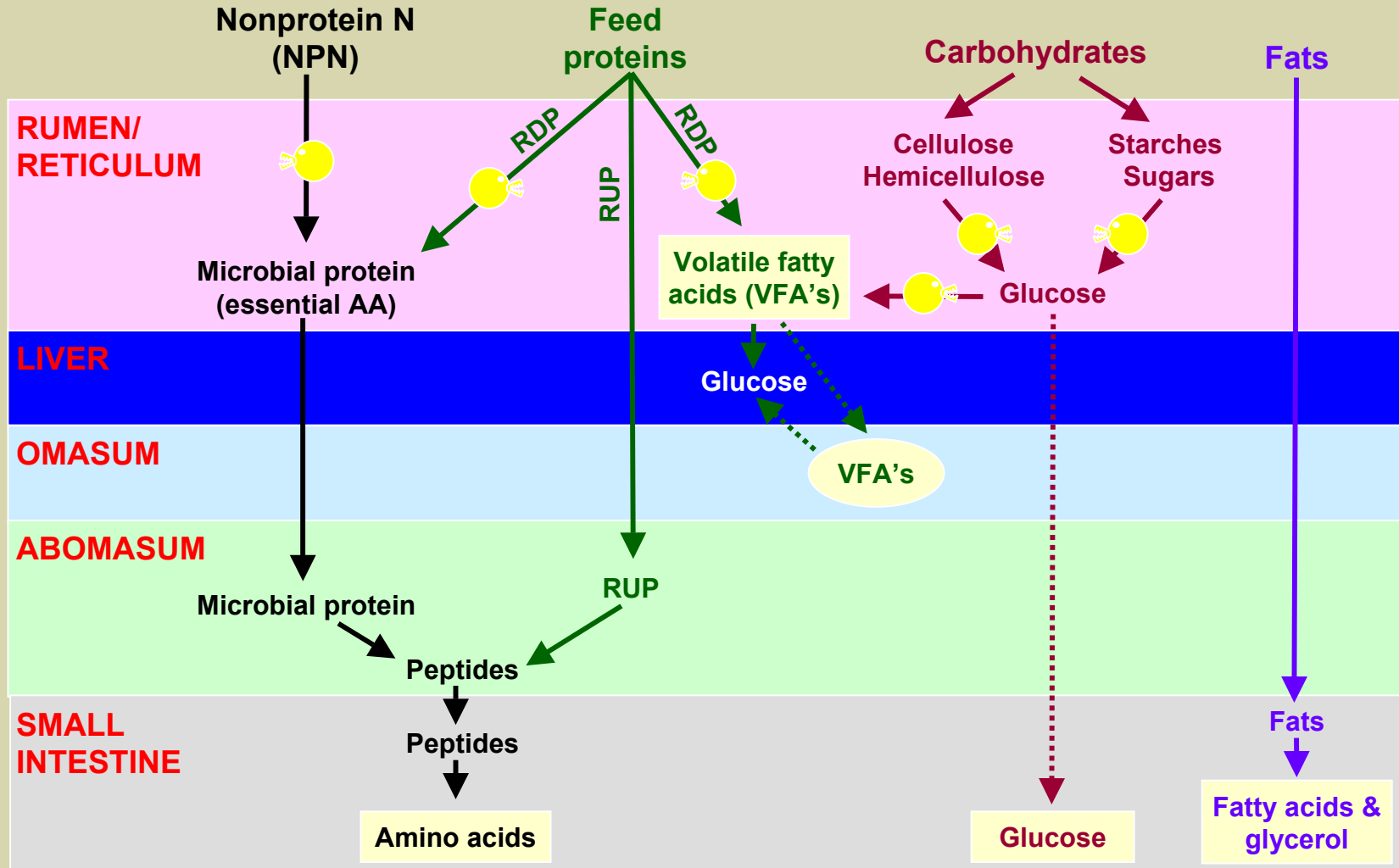
Bovine GI tract





Bovine GI tract

- The rumen has a pH of 5.5-7.0 depending on volatile fatty acid (VFA) production from feeds
- The main VFA's are propionic, acetic and butyric acids
- These produce a mild acidic environment (pH 4.0-5.0) in the rumen
- In the abomasum or stomach a harsh acidic environment (pH 2.0-3.0) is produced by HCL
- Acid from the abomasum is neutralised in the small intestines with an alkaline secretion (pH 7.0-8.0)

Volatile fatty acid production in the rumen



 = microbial action; RDP = rumen degraded protein; RUP = rumen undegraded protein;  = main site of absorption  = some absorption

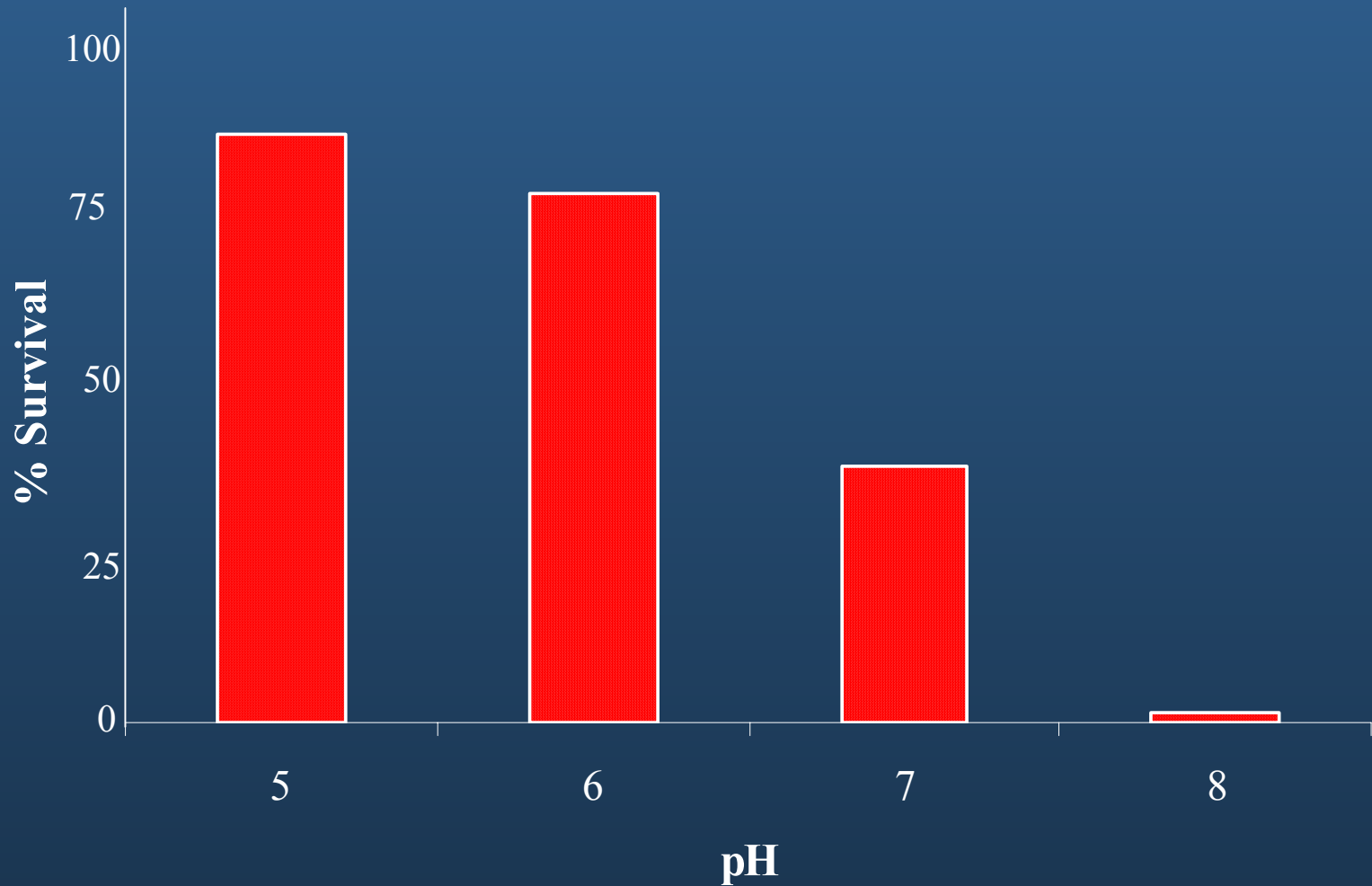
Pathogen survival in the GI tract

- Domestic livestock are primary habitats for *Escherichia*, *Salmonella*, *Shigella* & *Campylobacter*
- These organisms survive passage through the entire GI tract
- In the rumen pathogens are exposed to a mild acid stress (pH 4.0-5.0) in the presence of VFA's
- In the abomasum cells are exposed to severe acid stress (pH 2.0-3.0) in the presence of HCL

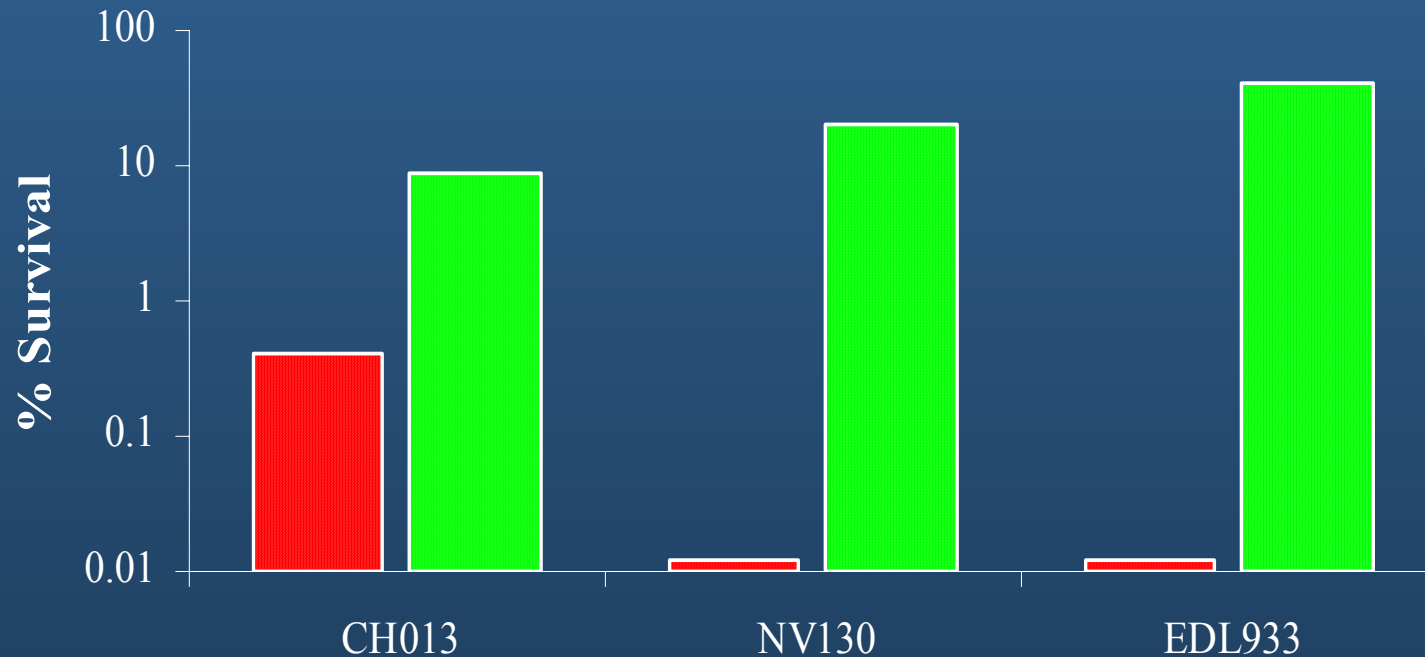
Rumen acid adaptation

- Volatile fatty acids (VFA's) in the rumen adapt cells to survive a subsequent severe acid challenge (pH 2.0-3.0)
- A low rumen pH enhances the acid tolerance effect
- Acid tolerance is enhanced in the anaerobic rumen

Acid adaptation in propionic acid of *S. Typhimurium* at different pHs & survival (%) after acid challenge at pH 3.0



E. coli O157:H7 acid adaptation in the rumen & survival (%) in the abomasum at pH 2.5



Rumen		
Feed	Hay	Grain
VFA's (%) Propionate	18	28
Acetate	73	62
pH	6.9	6.1

Survival in the abomasum

- Cells entering the abomasum were acid resistant (AR)
- *E. coli* O157:H7 survives acid challenge at pH 2.5
- Feeding regime had a major effect on survival
- At a lower pH survival is improved
- Strain variation was shown to occur
- These experiments were carried out in sterile fluids

Influence of feeding regimes on acid adaptation & resistance in *E. coli* O157:H7

- Cattle fed grain had more acid resistant *E. coli* than animals fed hay (Diez-Gonzalez & Russell, 1998)
- Cattle fed grain or hay had equal numbers of acid resistant *E. coli* (Hovde *et al.*, 1999)

Acid adaptation in the live animal

- Acid adaptation has not been shown to be associated with a particular system in the live animal
- It is likely that acid adaptation results from an overlapping between different systems

Acid adaptation and resistance in foods & humans

- During food processing enteric pathogens may become acid adapted
- This ensures survival in the human stomach
- Stresses induced in foods are also found in humans

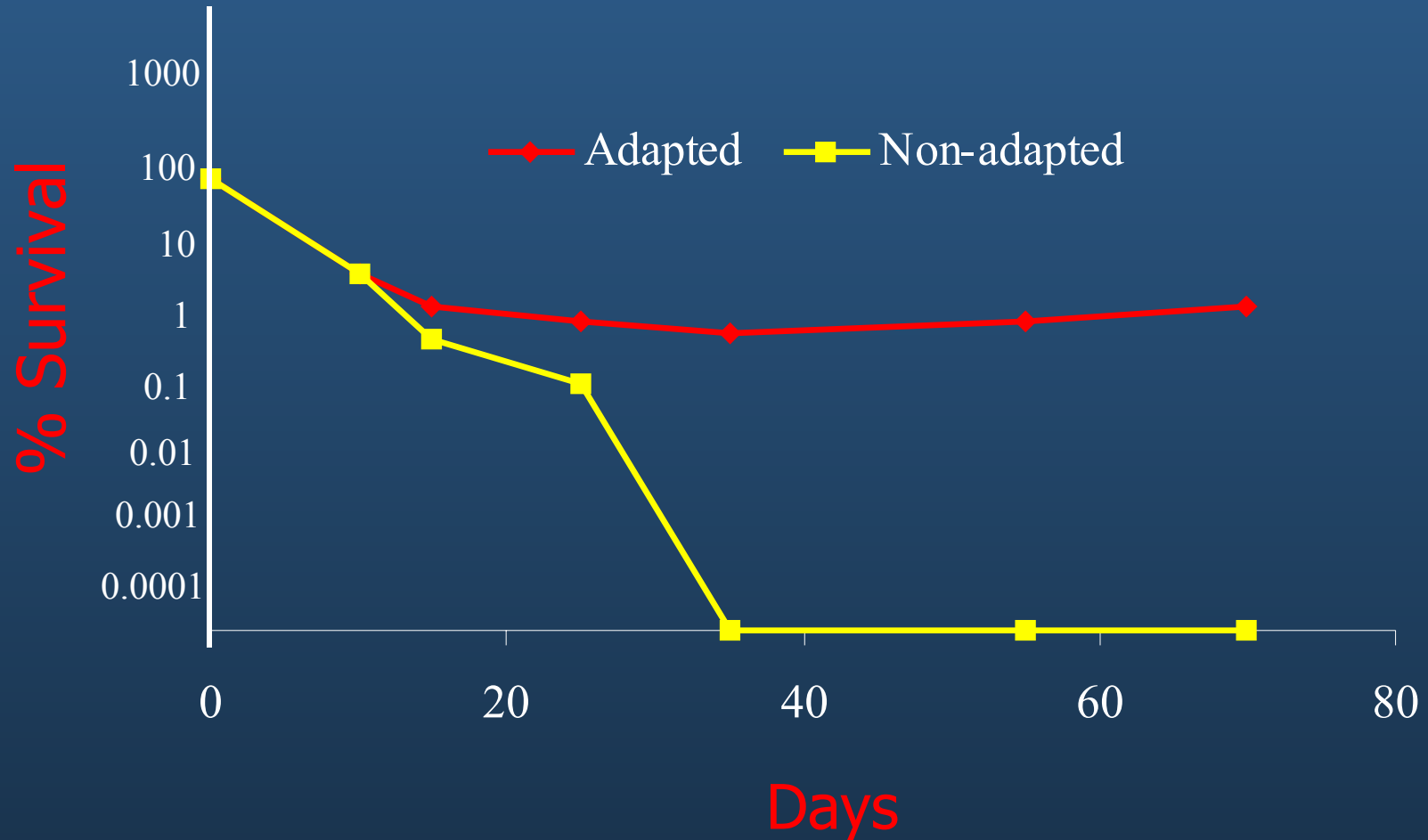
Stresses which occur in foods & in humans

Foods/process	Stress	Human body
Mayonnaise, fermented foods pepperoni, salami	Acid	Stomach/small intestine colon/phagosomes
Cooking/processing	Heat	Temperature upshift- intracellular environment
Fish, brines, marinades (salt solutions)	Osmolarity	Stomach
<i>Sous vide</i> - VP of meat	Anaerobiosis	Phagosomes

Survival on foods and in humans

- Acid resistance develops throughout the animal intestinal tract in the rumen, abomasum & intestines
- Acid resistant pathogens in faeces may contaminate carcasses during slaughter
- Resistance may persist in adapted cells during cold storage for periods >70 days
- Cells from animals or foods are acid adapted for persistence in the human stomach

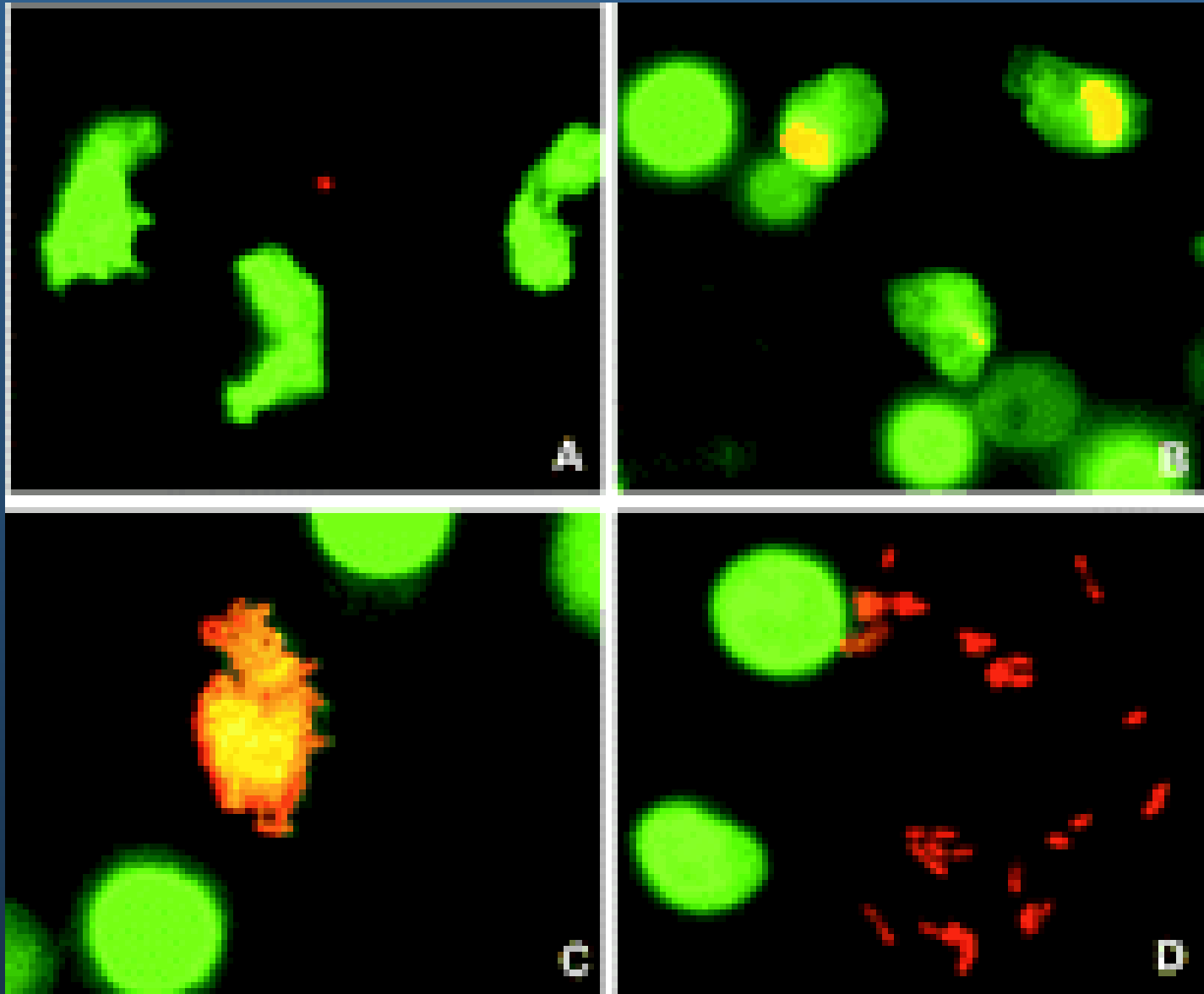
The influence of acid adaption on survival of *Salmonella* in cheese



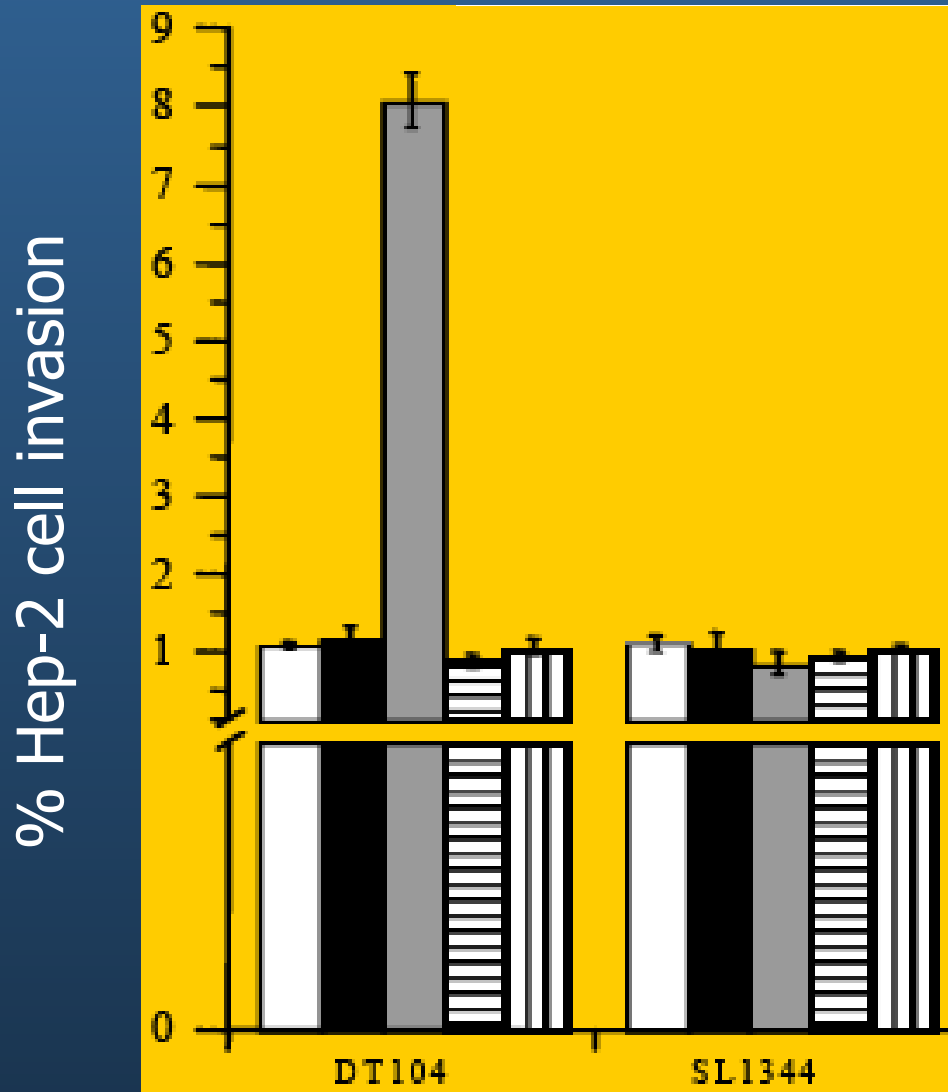
Pathogen survival & replication in protozoa

- The rumen has very large numbers of protozoa 10^6 ml⁻¹
- Protozoa are present in large numbers in water & soil
- Enteric pathogens may use protozoa as a means of survival, replication & increasing virulence in these environments

Pathogen replication in *Acanthamoeba*



Virulence enhancement in rumen protozoa



Salmonella strains

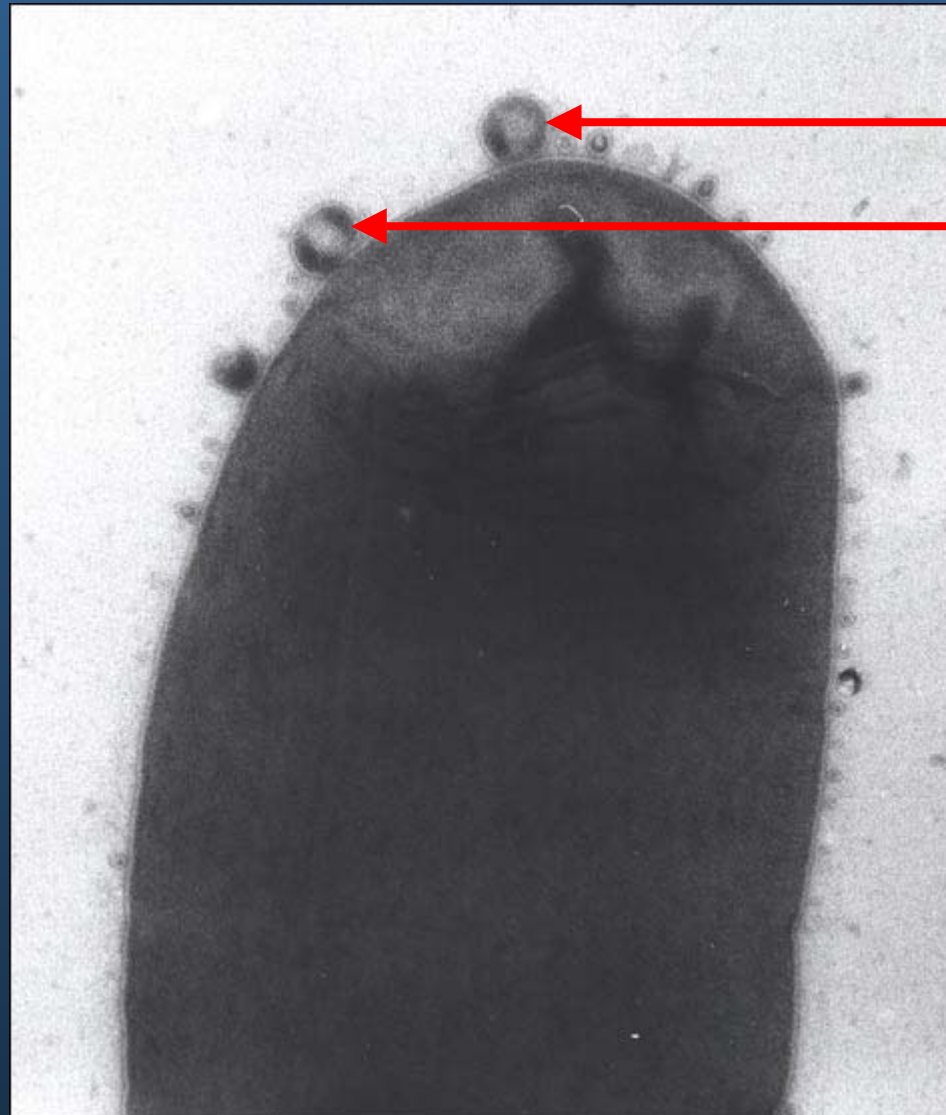
Pathogen survival in protozoa

- Enteric pathogens may survive & replicate in protozoa, mainly *Amoeba*
- Pathogens within protozoa may show enhanced virulence
- Increased virulence is related to the presence of the SGI1 resistance integron
- This may be a significant pathogen survival mechanism in water, the rumen & in soil

Alternative pathogen survival mechanisms - predator cytotoxicity

- Gram negative organisms produce vesicles on their surfaces
- Vesicles from *E. coli* O157:H7 contain shiga toxins
- When engulfed by *Acanthamoeba* the toxin kills the organism
- A possible survival mechanism in soil?

E. coli O157:H7 producing vesicles containing shiga toxins



Vesicles

Conclusions

- Research is required to determine how pathogens survive in complex communities such as the GI tract
- Controlling acid adaptation of enteric pathogens in the rumen would offer a unique opportunity for control since unadapted cells would not survive the pH of the abomasum
- Control of enteric pathogens in the live animal will depend on the development of methods & a detailed understanding of pathogen acid adaptation & resistance
- The significance of pathogen survival, replication & virulence enhancement in protozoa requires further investigation