BACTERIAL VACCINES IN CURRENT USE

Immunity

- **Active immunity** – develops as a result of infection (colonisation or disease) with a **microorganism**
- OR administration of **vaccine** prepared from live or inactivated organisms, antigenic fractions or detoxified exotoxins

Immunity

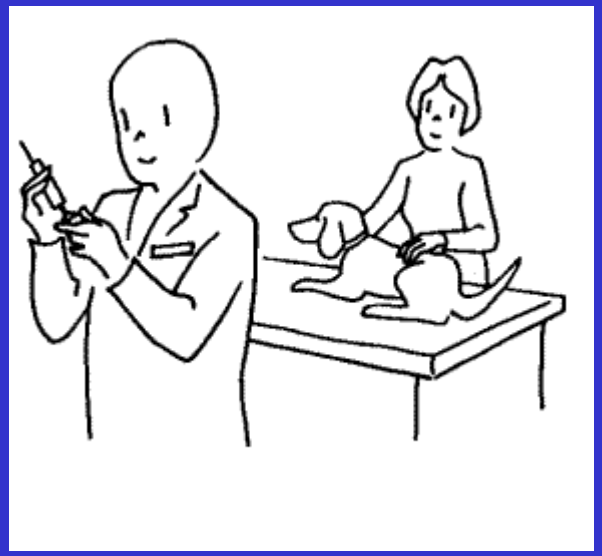
- **Passive immunity** – transfer of maternal antibodies to offspring or injection of antibodies
- Domestic Mammals – intestinal absorption of antibodies from **colostrum** within 1st few hours of life
- Birds – maternal antibody transferred to **yolk** where developing chick absorbs it
- No maternally derived passive immunity in fish

Vaccines

- Preparations of antigenic material
- Administered to induce active immunity in recipient animal against a specific organism
- Vaccines may be single component or mixed combined preparations
- Immune response usually specific for each agent although cross protection may occur

Reasons For Vaccination

- Antimicrobials mostly used for treatment
- May be used for preventing infection/disease
- Antimicrobials not always acceptable for prevention
- Vaccines prevent disease
- Cost effective
- Cheap
- Efficient prevention
- Profit for veterinarian



Current vaccines

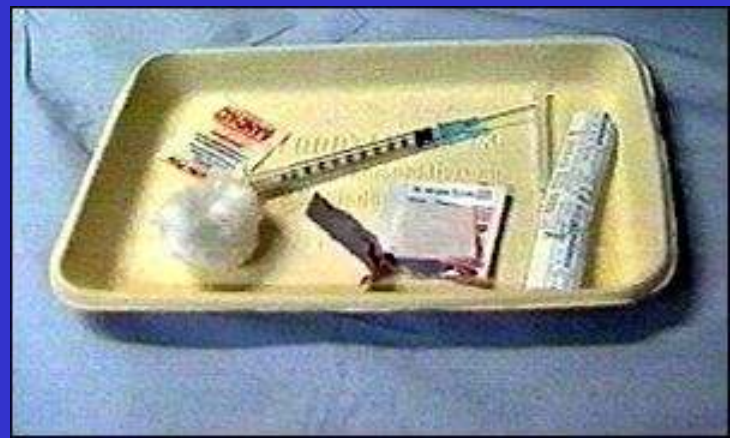
- Available for most classes of veterinary bacterial pathogen (Gram positives, Gram negatives, *Chlamydophila*, *Mycoplasma*)
- Mostly inactivated vaccines
- Live vaccines
- Single component
- Multi component
- Passive protection (antisera)

Emergency vaccines (autogenous)

- Prepared from microorganisms from an infected animal (eg, during a farm outbreak for control)
- **Intended for administration to flock or herd from which that animal belongs**
- Used when commercial vaccines not available
- Authorisation required by VMD

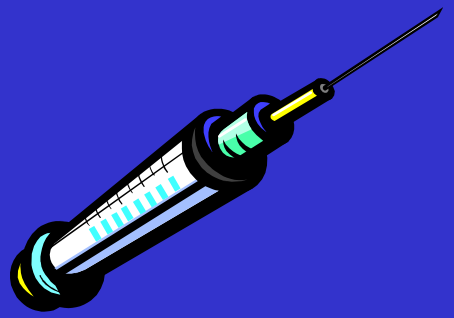
Inactivated Vaccines

- Most commonly used at present
- Known as BACTERINS
- Whole culture or bacterial cells enriched with pathogenic determinants, or parts of bacterial cell
- Formaldehyde inactivated
- Preserved with phenol
- Mixed with adjuvant



Antigens preserved in inactivated vaccines

- Fimbriae
- Capsules
- Outer membrane proteins
- Cell wall lipopolysaccharides
- Iron binding proteins and heat shock proteins
- Prototoxins and toxins/ toxoids
- Other secreted antigens



Production of Inactivated Vaccines

- Seed stock maintained at low passage to conserve pathogenic determinants
- Grow culture to produce optimum yield of pathogenic determinants
- Harvest cells or precipitate supernatant
- Inactivate toxins to toxoids
- Add adjuvants and preservative
- Pack for distribution
- Normally require more than one administration

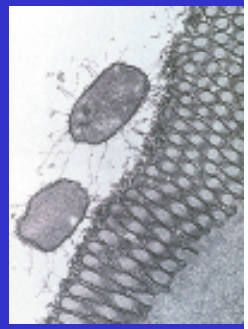
Inactivated (killed) vaccines

- Contain sufficient antigen to stimulate antibody production
- Generally require 2 doses in order to generate sufficient response for protection
- Inactivation may modify surface antigens
- Organisms are dead so wont replicate
- May be whole killed bacteria or bacterial components known as sub-units
- Given by injection
- Annual boosters

Whole Cell/Culture
Kavak L, Fort Dodge, Merthiolate-killed *Leptospira icterohaemorrhagiae* and *L. canicola* for dogs

Capsules
Suvaxyn APP, Fort dodge, killed whole cell vaccine with capsular antigens from serotypes 3,6, and 8 of *Actinobacillus pleuropneumoniae* for pigs

Cell membranes
Stellamune, Pfizer, *Mycoplasma hyopneumoniae* killed culture vaccine for enzootic pneumonia in pigs



Fimbriae
Porcilis Porcol 5, Intervet, K88ab, K88ac (F4), K99 (F5) 987P (F6) and LT Toxoid of *E. coli* against piglet diarrhoea

Crude culture supernatant
Blackleg vaccine BP (Vet), Schering Plough, Whole culture inactivated *Clostridium chauvoei* vaccine for cattle

Iron restricted proteins
Salenvac, Intervet, killed *Salmonella enteritidis* vaccine for poultry grown in iron restricted conditions

Use of Inactivated Vaccines

- Intramuscular parenteral administration
- Two or more injections
- Elicit IgM and IgG antibodies
- Poor local (mucosal) and cellular immunity
- Poor duration of immunity
- Passive immunity for offspring (vaccinate mum)

Toxoids

- Toxins obtained from bacteria (supernatant, secreted) and treated by heat or chemicals (formaldehyde)
- Destroys toxicity and capability of causing disease but retains immunogenicity
- Stimulate the formation of toxin neutralising antibodies
- Usually contain adjuvants

Toxins

Lambivac, Intervet, Lamb Dysentery, Struck, Pulpy kidney and tetanus vaccine for sheep, - TOXOIDS of *C. perfringens* Types B, C and D and *C. tetani* with alum and thiomersal preservative

Adjuvants

- Inactivated vaccines contain adjuvants that enhance the immune response to the vaccine
- Induce inflammation at the site of vaccination
- May cause local irritation and swelling at site of vaccination
- Adjuvants used are aluminium hydroxide, aluminium phosphate, alum, mineral oil such as liquid paraffin

Component Vaccines

- One or more protective antigens
- Toxoid, secreted antigen and/or structural proteins
- Increasingly used
- Require moderate amounts of antigen
- Administered with adjuvant

Live Bacterial Vaccines

- Not many live vaccines in use at present
- Many former vaccines have been withdrawn
- New engineered/vector vaccines not yet available

Live Vaccines

- Live microorganisms that have lost ability to cause disease by treatment with -
  - heat
  - multiple passage on lab media
  - passage in cells
  - through a different host
  - sublethal chemicals

- Non pathogenic forms of the infecting organism
- Retain many or all of the surface antigens from which they are derived
- Replicate in the host but cause no disease
- Stimulate CMI and antibody both locally and systemically
- Not genetically defined

Live Vaccines

- Live vaccines stimulate immunity at mucosal surfaces, the entry points for most pathogens (GI tract, Respiratory tract)
- Living vaccines colonise and replicate on the surface of appropriate mucosa
- Immunity long lasting usually but generally less than that following natural infection
- Maternal antibody and antibiotic treatment may inhibit vaccine replication

***Bordetella bronchiseptica*, Attenuated strain, INTRANASAL FOR KENNEL COUGH (Intrac, Schering Plough)**

***Bacillus anthracis*, Capsule deleted, INTRAMUSCULAR FOR CATTLE (Anthrax spore vaccine, DEFRA, Licence only)**

***Chlamydophila abortus*, Freeze dried attenuated strain 1B, intramuscular for ENZOOTIC ABORTION IN SHEEP (Tecvax Chlamydia, Vetoquinol)**

***Salmonella enteritidis*, Naturally-attenuated oral vaccine for poultry (Lohmann)**

***Trichophyton verrucosum*, Attenuated live freeze dried vaccine given intramuscularly FOR RINGWORM IN CATTLE (Ringvac Bovis LTF-130, Intervet)**

Administration of Live Vaccines

- Parenterally for systemic humoral and cellular immunity
- Intranasally for the respiratory tract
- Orally for intestinal and lactogenic/transovarian immunity

Passive immunity

- Provided by dam
- Requires first administration and boost
- Booster given before parturition
- Egg/placental transmission in some species
- Colostral antibody in most farm species
- Milk antibody

Veterinary immunotherapy and prophylaxis

- Hyperimmune polyvalent antisera used traditionally
- Raised in horses
- Monoclonals now available
- Colostrum/immunoglobulin from other species
- Given before disease/after exposure

Current Hyperimmune Antisera

- Commercially available preparations of antisera produced by immunising horses or cattle
- Sera contain the appropriate antibodies or antitoxins
- Provide passive protection when unimmunised and exposed e.g. tetanus
- May produce hypersensitivity in recipient of sera

Tetanus Antitoxin Behring, (Intervet), 1000 units antitoxin/ml derived from horses and preserved using 0.5% phenol, subcutaneous or intramuscular

Lambisan, (Intervet), Lamb dysentery, struck and pulpy kidney, Antiserum raised in horses to *C. perfringens* β and ε toxoids given subcutaneously

Fig. 4
The production of an antiserum [illegible] immunized horse from the

NOVEL BACTERIAL VACCINES

Problems with traditional technology for making bacterial vaccines

- **Killed vaccines (bacterins)**
  - Usually good antibody response (no CMI, mucosal IgG)
  - May have poor efficacy – response against irrelevent antigen
- **Subunit vaccine**
  - Critical epitope(s) destroyed by inactivation
  - Poorly immunogenic
  - May still be toxic if insufficiently treated
- **Live vaccines**
  - Produced by non-specific methods
  - Basis for attenuation usually unknown
  - Control of attenuation difficult
  - May revert to virulence *in vivo*

Approaches for developing new or improved bacterial vaccines

Use genetic engineering to make:-

- Rationally attenuated live vaccines
- Genetically detoxified virulence factors
- Live vectors expressing foreign antigens
- Novel adjuvants
- Naked DNA vaccines

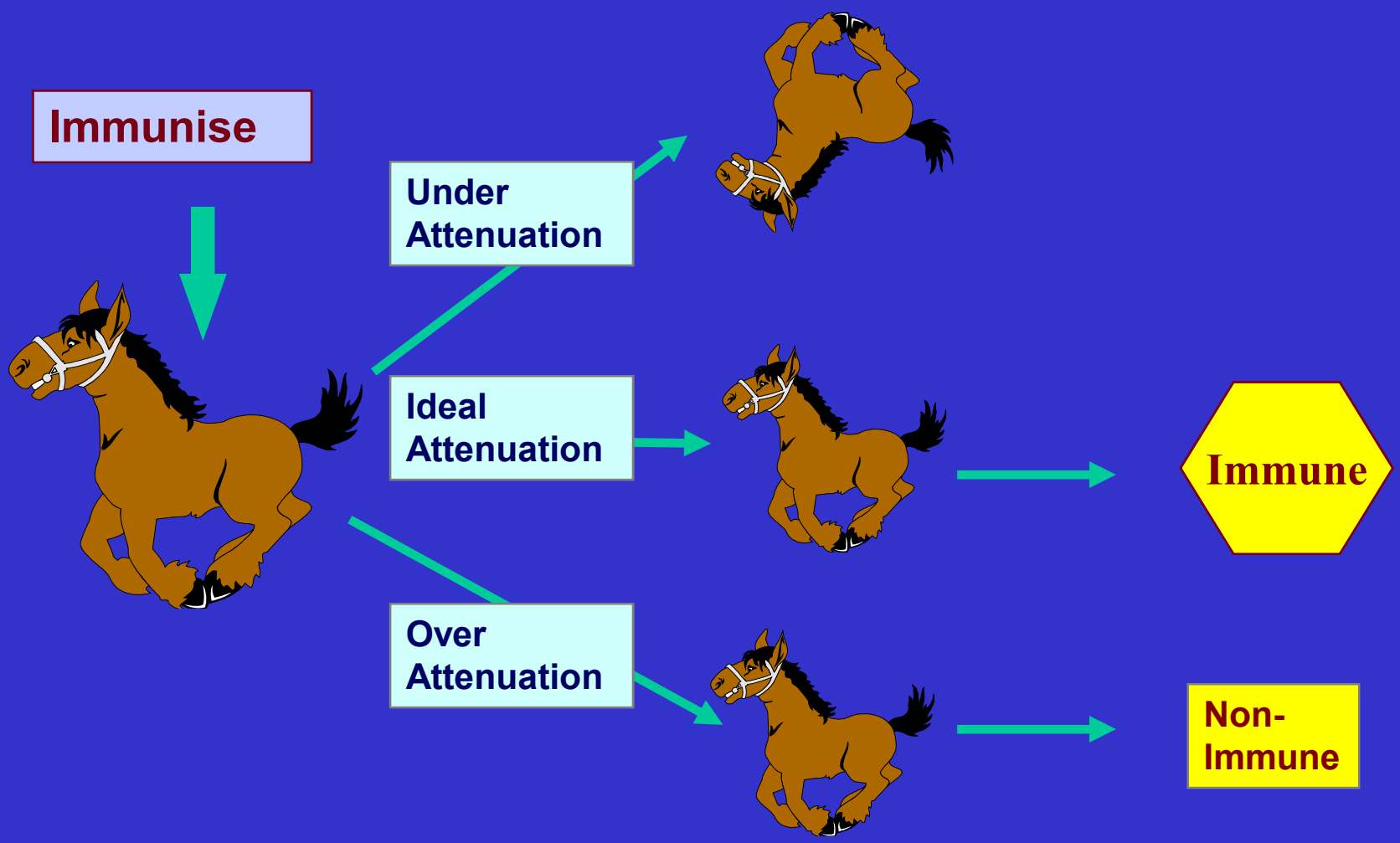
Target genes for attenuating bacteria

- Genes coding for virulence factors
  - Toxins eg. *V. cholerae*, *A. pleuropneumoniae*
- Regulatory genes
  - *phoP, Q Salmonella* sp, *bvg Bordetella* sp
- Metabolic pathway genes
  - aromatic pathway genes - many bacteria
  - purine biosynthesis genes - many bacteria
- Stress-response genes
  - *htrA Salmonella* sp

Rational attenuation of bacterial pathogens

- Clone target gene
- Inactivate gene, eg delete portion of gene
- Introduce mutated gene into bacteria
- Homologous recombination replaces wild type gene with mutated gene
- Check genotype and phenotype of mutant bacteria
- Check attenuation and immunogenicity

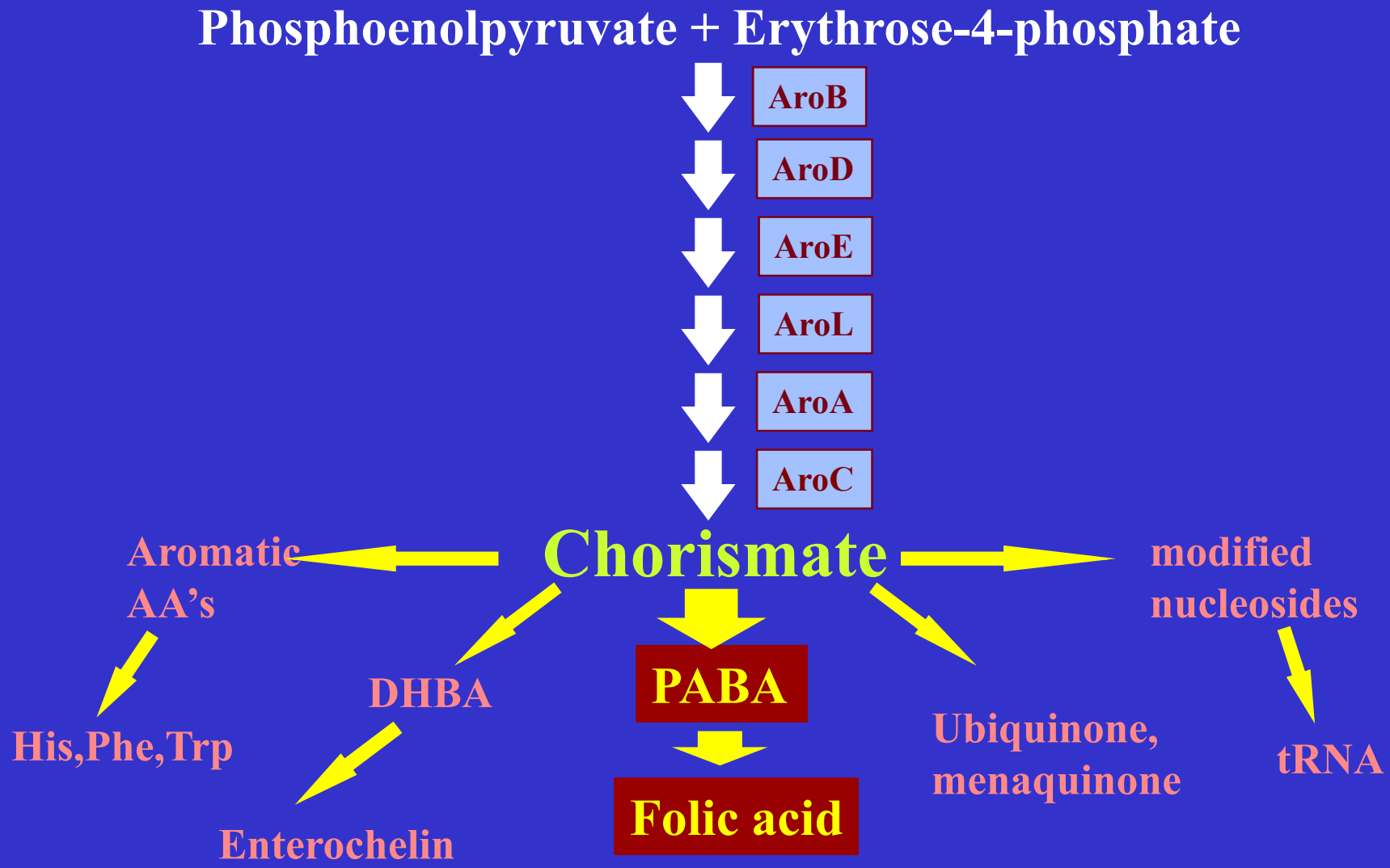
Levels of Attenuation



Pre-chorismate (aro) pathway

- Required for synthesis of aromatic compounds in bacteria, fungi and plants
- Precursors:
  - Phosphoenol pyruvate + erythrose - 4- PO4
- Chorismate, branch point for the biosynthesis of:
  - Aromatic amino acids: Trp, His , Phe
  - PABA - folate
  - DHBA - enterochelin
  - vitamins - ubiquinone, menaquinone
  - modified nucleosides - tRNA

Aromatic (pre-chorismate) pathway



Aro mutants of bacterial pathogens

- Mutation in one or more *aro* gene is highly attenuating
- *Aro* mutants undergo limited replication *in vivo*
- PABA not produced by vertebrate:
  - must obtain folate from diet
  - **bacteria cannot use exogenous folate**
- *aro* mutants of a number of bacteria are good experimental live vaccines
- ? virulent in immunocompromised individuals

Salmonella aro mutants

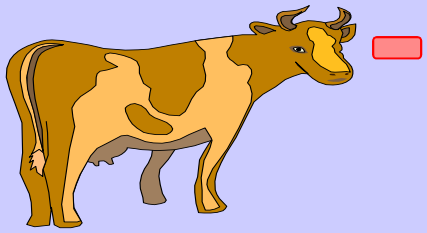
- Salmonella strains with mutations in one or more *aro* gene are highly attenuated
- *Salmonella aro* mutants undergo limited replication *in vivo*
- *S.typhi aro* mutants are safe and immunogenic in humans.
- *S.typhimurium aro* mutants are safe and immunogenic in a number of animals.

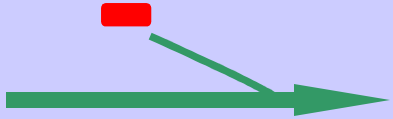
Oral vaccination of calves with a live recombinant salmonella vaccine

- *S.typhimurium aroA aroD*
- Give orally (10^{10} CFU) to 7 day old calves
- 3 weeks later challenge immunised and control animals with virulent *S.typhimurium*
- 7/8 immunised animals fully protected
- 4/4 control animals severe scour, humanely killed

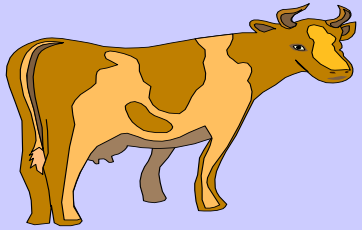
Oral vaccination of calves with a live recombinant salmonella vaccine

Immunise 1x oral with
S.typhimurium aroA aroD



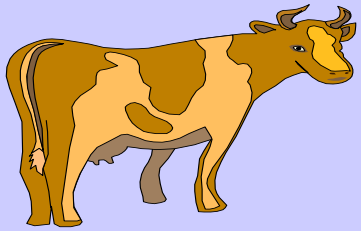


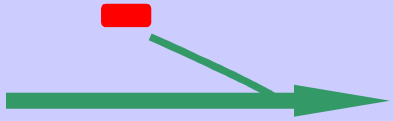
Challenge with wild type *S.typhimurium*



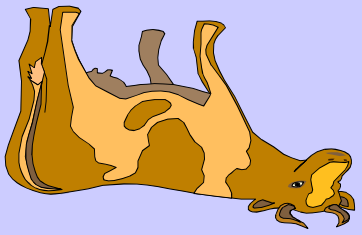
7/8 immune

Control





Challenge with wild type *S.typhimurium*



4/4 Severe scouring

Horse with strangles: swollen lymph nodes at neck

Strangles vaccine

Intervet Equilis StrepE.

Live genetically modified *S.equi* vaccine, *S.equi aro* mutant

Administer inside upper lip.

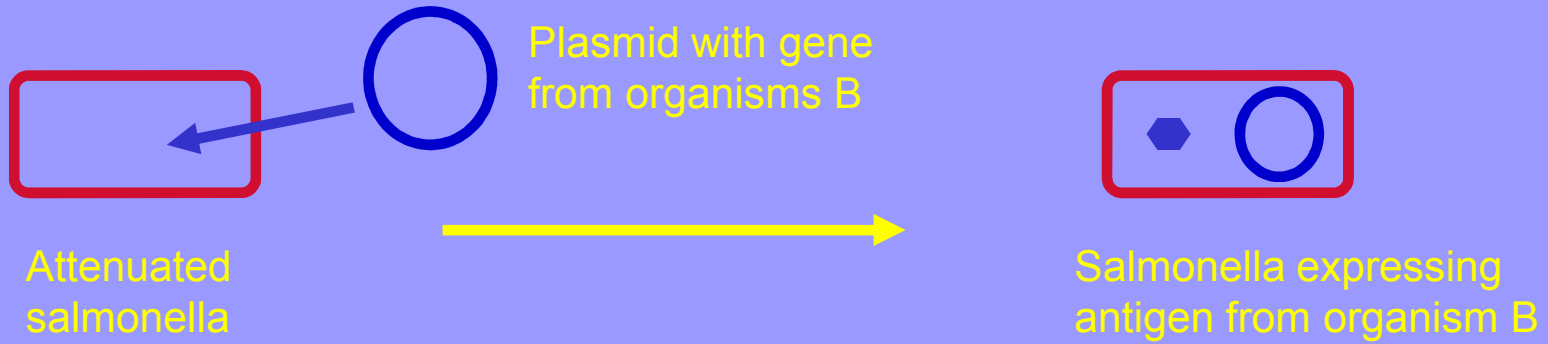
	Initial vaccination	**Re-vaccination**	**Outbreak situation**
High risk horses	Two vaccinations, four weeks apart	Every three months	No additional vaccinations required
Option for medium risk horses	Two vaccinations, four weeks apart	Every six months	Revaccinate if more than three months since the last vaccination

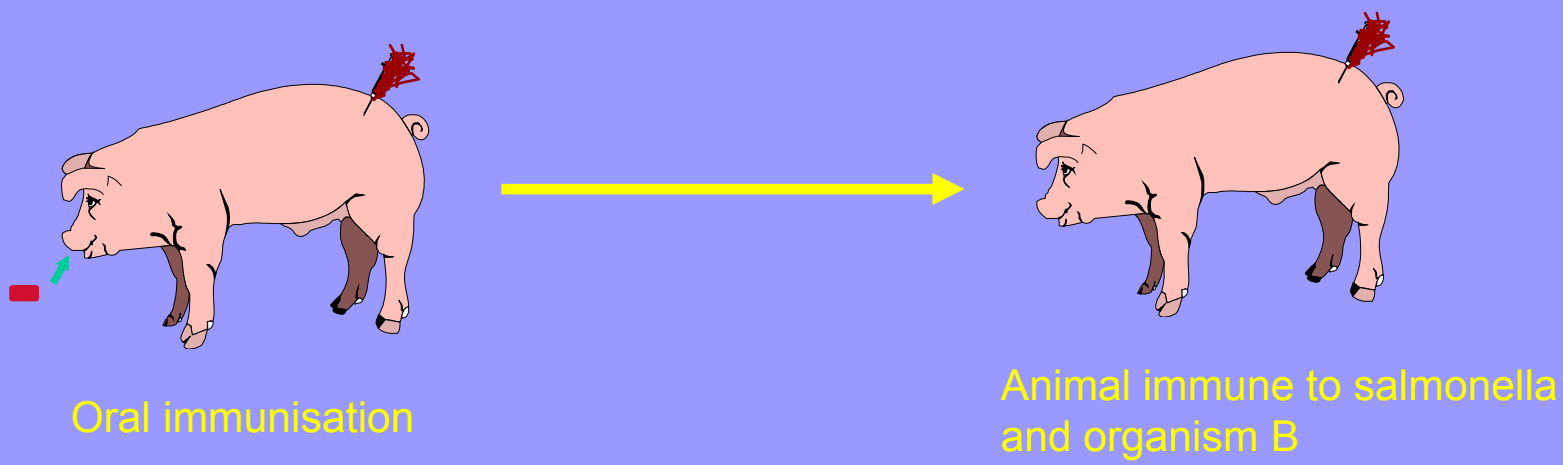
First live genetically modified bacterial vaccine licensed for use in UK

Advantages of using *Salmonella* as a live vector

- Stimulate all arms of immune system:
  - Antibody (secretory and circulating)
  - cell-mediated immunity
  - cytotoxic T cells
- Reside in antigen presenting cells
- Oral immunisation
- Genetics advanced
- Express antigens from organisms that are difficult or impossible to culture.

Using attenuated salmonella as live vectors for oral delivery of foreign antigens





Live bacteria as carriers of foreign antigens

- Advantages
  - Improves immunogenicity of vaccine
  - Stimulate all arms of immune system
  - No need for injection for immunisation
  - Express antigens from organisms that are difficult or impossible to culture
  - Multivalent vaccines
- Most work *Salmonella* sp then BCG
- Genes from bacteria, viruses, parasites and humans have been expressed in these systems

Genetically inactivated toxins

Advantages:

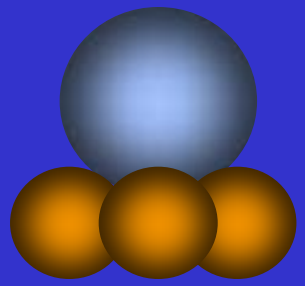
- Conformation and hence critical epitopes preserved
- Improved immunogenicity
- Safe - no chance of incomplete inactivation or reversion
- Use as novel adjuvants

Shiga-like toxin

Shiga-toxin = *Shigella dysenteriae,* Shiga-like toxin (SLT)/ Verotoxin (VT) produced by some *E.coli* strains (VTEC)

Pigs: Oedema disease. SLT-IIe. O138, 0139, 0141.

A = Enzymatic, N-glycosidase



B = Binding. Gangliosides Gb3 and GB4

Inhibits protein synthesis in eucaryotic cells. Removes an adenine residue from the 3' end of 28S rRNA. Prevents binding of aminoacyl t-RNA to the A site of the ribosome.

Vaccines based on inactivated SLT-IIe

Traditional technology

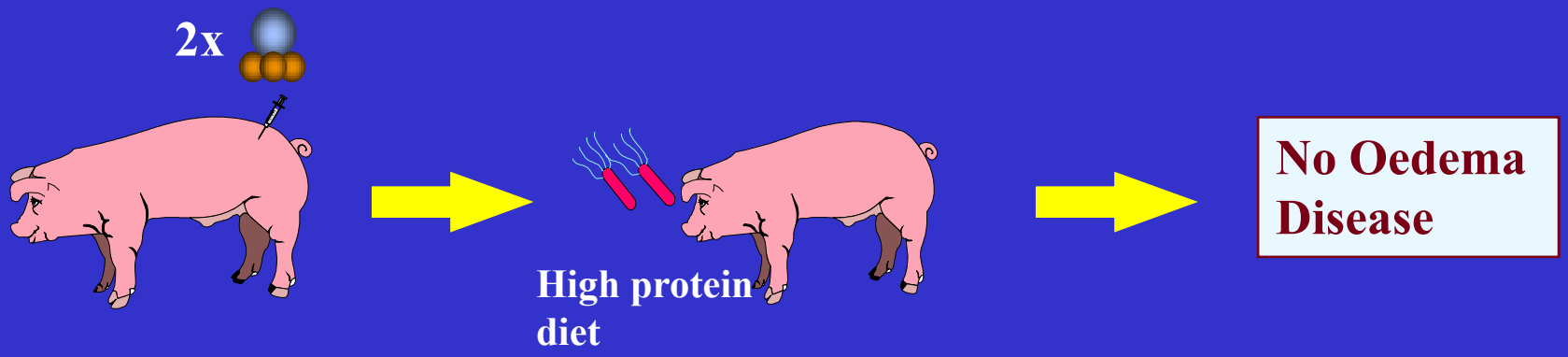
- Prepare SLT-IIe by treatment with formaldehyde
  - Inactive *in vitro*
  - Vaccinated pigs had reduced growth weight compared to non-immunised controls

Molecular biology

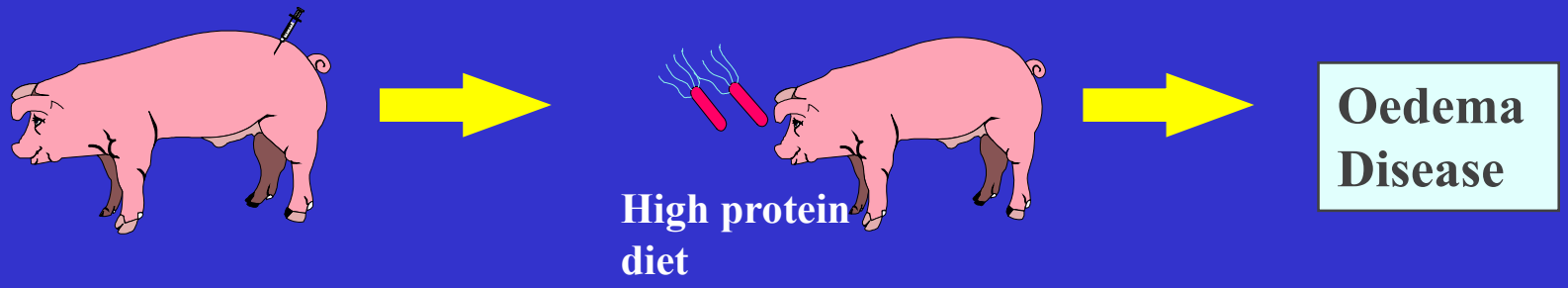
- Change amino acid in active site of A subunit
  - glutamate 167 to glutamine (E167Q) = inactive
  - inoculate pigs: no effect on weight gain

Immunisation of pigs with SLT-IIe E167Q

Immunisation **Challenge**



Control



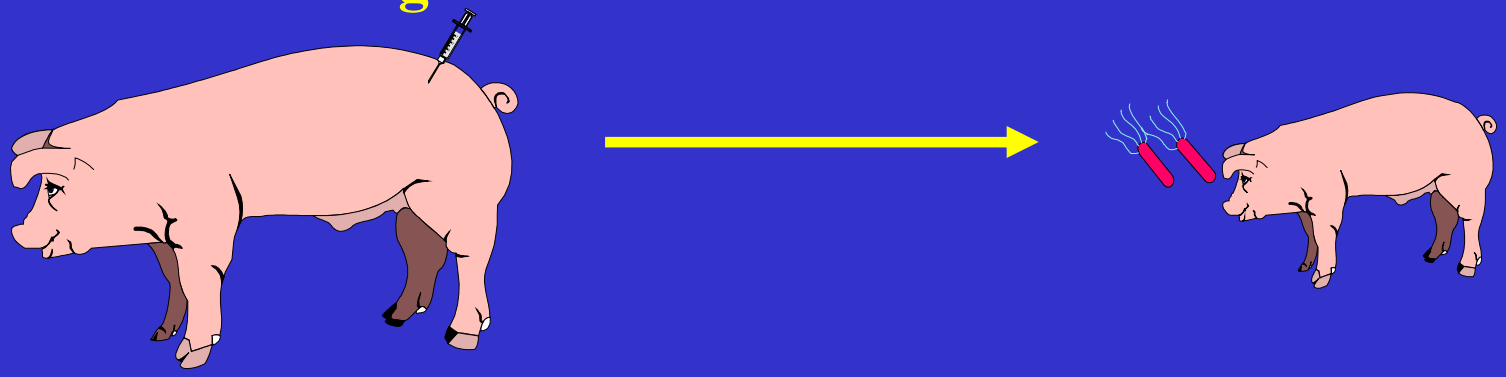
Recombinant *Pasteurella multocida* toxin vaccine

- PMT 147 kD protein responsible for the severe snout deformity seen in clinical (progressive) atrophic rhinitis
- 363 bp in frame deletion in 5' end of gene
- 133 kD non-toxic PMT molecule (dO)

Protection of piglets with dO PMT

Immunise sow 2x before farrowing

Challenge piglet with *B.bronchiseptica* and *P.multocida*



Compared with controls, piglets immunised with dO exhibit:

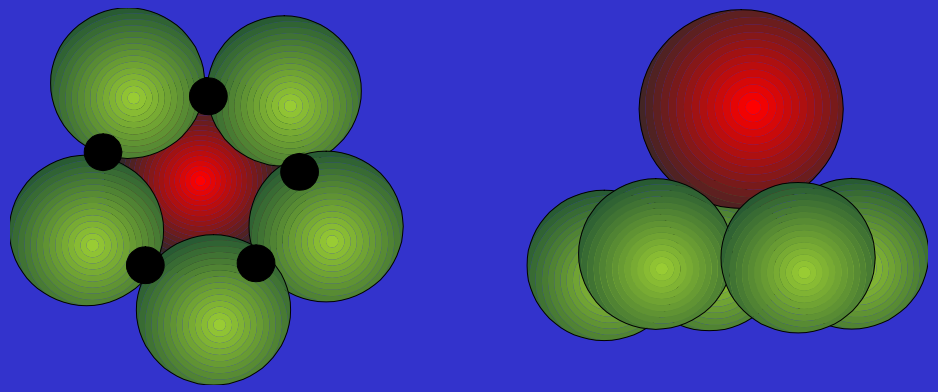
↓ snout deformity ↑ weight gain ↓ *P.multocida* recovered

Cholera toxin+ *E.coli* heat labile toxin

1. Most potent soluble oral immunogens known
2. Mucosal adjuvants.
3. Can overcome oral tolerance

Unacceptable for human use.

- 25ug CT orally produced purging diarrhoea indistinguishable from cholera in volunteers

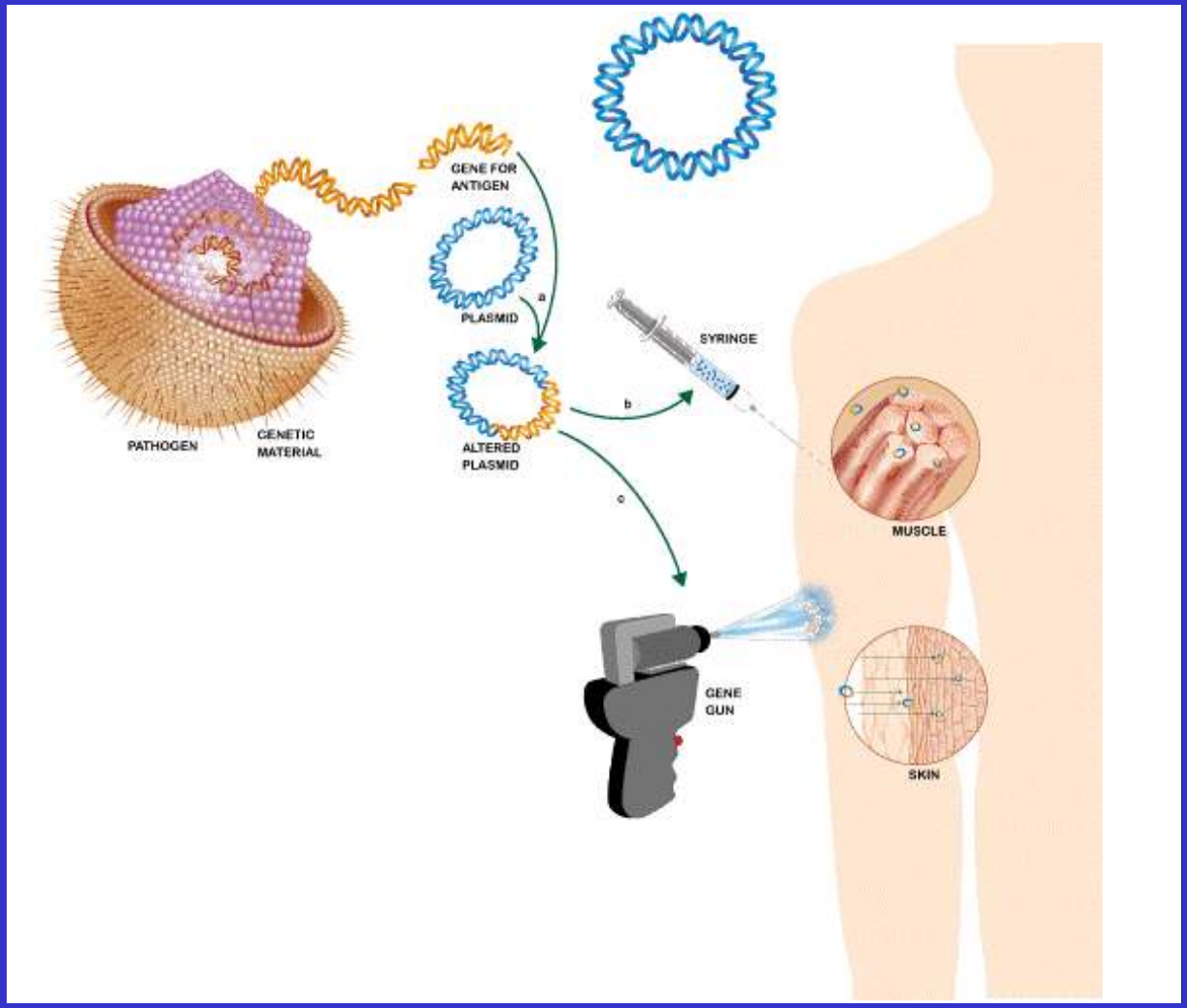


A subunit (CTA) = ADP-ribosyltransferase

B subunit (CTB) = pentamer cell binding subunit

Naked DNA Vaccines

- Injection of naked DNA using plasmids encoding genes with functional eukaryotic promoters
- Results in expression of the gene in striated muscle at site of injection
- Great potential for livestock/animal vaccines
- Safety issues?



Be careful when vaccinating us!! (we don't like it)