USAGE OF ANTIBIOTICS IN ANIMAL AGRICULTURE AND ITS ROLE IN INCREASING ANTIMICROBIAL RESISTANCE

The role of veterinary drugs in increasing of antimicrobial resistance

Antibiotics and Agriculture

- Antibiotics have growth-promoting and disease-fighting capabilities. Antimicrobials are used in everything from apples to aquaculture.

- Only half of all antibiotics produced are slated for human consumption. The other 50% are used to treat sick animals, as growth promoters in livestock

- These alternative uses result in the development of resistance in bacteria in or near livestock.

Antimicrobial resistance

- **1940s**: growth enhancement properties of antimicrobials identified
- **1950s**: widespread use of antimicrobials as feed additives
  - Usage without veterinary prescription
- **1960s**: Resistance in *Salmonella* from calves lead to ban in penicillin and tetracycline as feed Additive

1970s: reports of multidrug resistant bacteria

- **1978**: WHO defines rules for monitoring bacterial resistance in veterinary and human origin organisms
- **1980-90s**: Emergence of antimicrobial resistance

– Vancomycin resistance *Enterococci*

– Mulitdrug resistance *S. Typhimurium* DT104

– Floroquinolone resistance *Campylobacter*

Resistance to antimicrobials

- **Beta-Lactams**
  - 1998: Extended spectrum beta-lactamases (ESBLs)
    - Point mutations develop resistance to cephalosporins
  - 2000: ESBLs from food producing animals in Canada
  - 2001: Resistance extended to cephamycins (cefoxitin)
  - 2001: Beta-lactam resistant *Salmonella* found in meat
  - 2002: Nosocomial outbreaks of ESBLs reported in America, Asia, and Europe

Why use antibiotics for animals?

- Therapeutic

  Use of antimicrobial in animals with diagnosed disease.

- Prophylactic

  Use of antimicrobial in healthy animals in advanced of expected exposure or after an exposure to an infectious agent, before laboratory diagnosis

- Non therapeutic

  Any use of antimicrobial in the absence of disease or documented exposure to microbial disease

Non therapeutic use of antibiotics

- Growth promotion
- Feed efficiency
- Weight gain
- Preventing illness caused by bad sanitation

Antimicrobials used as growth promoters

- Antimicrobials, when used in low subtherapeutic doses in feed and water, are called "growth promoters". They are used by industry to:
- Reduce subclinical populations of pathogenic microorganisms in gut mass, lessening metabolic drain.
- Prevent irritation to the intestinal lining.
- Increase food passage through gut, allowing increased daily gain (4-16%) and feed utilization (2-7%).

Antimicrobial Growth Promoters (AGP)

Antimicrobial substances used as a supplement in animal feed in sub-therapeutic concentrations

Avoparcin (G+)

Spiramycin (G+)

Bacitracin (G+)

Avilamycin (G+)

Virginiamycin (G+)

Flavomycin (G+)

Tylosin (G+)

Carbadox (G-)

Olaquindox (G-)

Antimicrobial Growth Promoters (poultry)

- Penicillin
- Tetracycline
- Neomycin
- Glycopeptides
- Streptothricins
- Macrolides
- Virginiamycin
- Arsenical compounds
- Nitrofurans
- Sulfonamides
- Streptomycin
- Roxarsone
- Carbadox

Non therapeutic use of antibiotics

- These uses involve long-term, low dose treatment through feed and water to whole flock or herds.
- Many drugs are in the same class as human drugs
- Prolonged bacterial exposure to appropriate and low doses of antibiotics increase the resistance to those drugs
- Because of a steep rise in antibiotic use, bacteria in livestock are increasingly resistant to drugs.
- bacteria can transfer resistance to each other. bacteria resistant to animal drugs can become resistant to similar human drugs

Animal antibiotics often identical or belong to the same classes as human antibiotics.

Antibiotic class	U.S.-approved feed-additive antibiotic(s)
Penicillins	Penicillin G procaine
Tetracyclines	Chlortetracycline, Oxytetracycline
Amingoglycosides	Spectinomycin
Streptogramins	Virginiamycin
Macrolides	Tylosin, Erythromycin, Tilmicosin, Oleandomycin
Clindamycin (lincosamides)	Lincomycin, apramycin
Polypeptides	Bacitracin
Sulfonamides	Sulfanitran, Sulfadimethoxine, Sulfamethazine, Sulfaquinoxaline, Sulfathiazole, Neomycin sulfate
Arsenicals	Roxarsone, arsanilic acid, nitrarsone

Aarestrup et al. 2008; FDA *Green Book* 2006

Feed additives for performance enhancement, 70/524/EEC

- spiramycin (-99)
- tylosin (-99)
- virginiamycin (-99)
- avoparcin (-97)
- ardacin (-97)
- bacitracin (-99)
- avilamycin
- carbadox (-99)
- olaquindox (-99)
- flavofosfolipol
- monensin
- salinomycin

Antibiotics approved for use in bovines and/or porcines (Sweden 2001)

Individual medication

- penicillin-G
- penicillin-DHS
- amoxicillin
- ceftiofur
- macrolides (16-M)
- pleuromutilins
- trim/sulph.
- tetracyclines
- fluoroquinolones
- aminoglycosides

Group medication

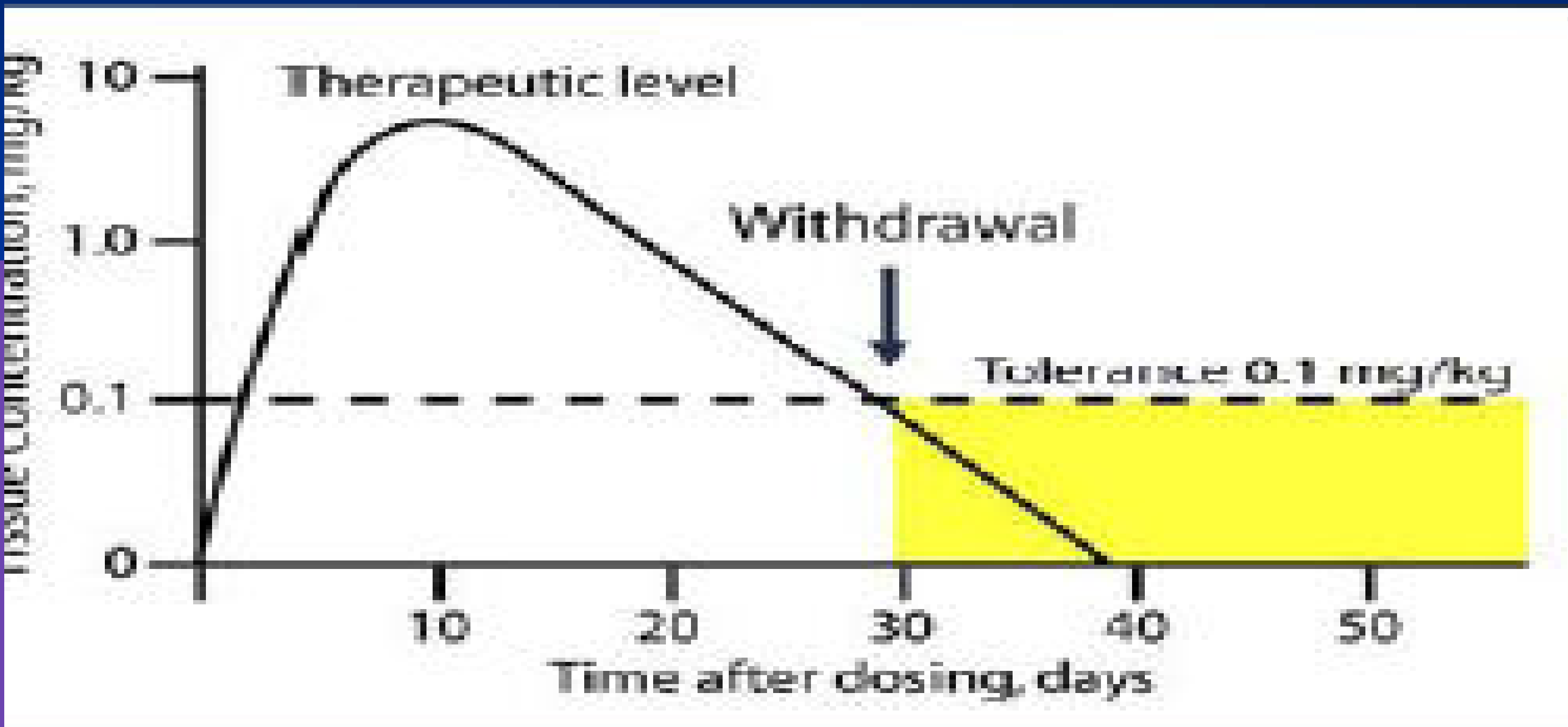
- macrolides (16-M)
- pleuromutilins
- tetracyclines
- fluoroquinolones

From: FASS VET. 2001

Withdrawal Time

- Time required for a drug or chemical concentration to fall below the *Tolerance Level* established in a specific target animal tissue.
  - Dependent upon drug, dose, formulation, route of administration, species, target tissue and disease / management factors.
  - Pharmacokinetics-toxicokinetics of the drug is the main factor.
- – Therapeutic level vs. elimination
  - PK of elimination can be different for different tissues.

Animal drug withdrawal time



Residues of all veterinary drugs are higher in liver and/or kidney tissue as compared to muscle tissue.

Drugs most likely to be detected in Meat

- Penicillin (including ampicillin)
- Tetracycline (including chlortetracycline,oxytetracycline)
- Sulfanamides (including sulfamethoxine ,sulfamethazine and sulfamethoxazole)
- Neomycin
- Gentamycin
- Streptomycin
- arsenicals

Foodborne Pathogens

- Animals used in food production
  - Cattle – *E. coli* O157:H7 (EHEC), *Salmonella*, *Campylobacter*
  - Swine – *Salmonella*, *Campylobacter*
  - Poultry – *Campylobacter*, *Salmonella*

Antimicrobials in livestock feed

- Studies show that up to 75% of antibiotics pass through unaltered in feces.
- Routine use in livestock feed increases antibiotic resistant pathogens being excreted by livestock.
- Antibiotic resistant pathogens in excreta become available in the environment to wildlife and grazing livestock, and can contaminate crops.
- Many pathogens have long survive after excretion, e.g. Salmonella and Avian influenza virus can survive for months after excretion.

"Overuse and misuse of antimicrobials in food animals contribute to the emergence of resistant forms of disease-causing bacteria. Such resistant bacteria can be transmitted from food animals to humans, *primarily via the food.*" World Health Organization Global Guidelines, 2000

What Factors Promote Antimicrobial Resistance?

- Exposure to sub-optimal levels of antimicrobial
- Exposure to microbes carrying resistance genes

Antibiotics in Foods

- Antibiotics are used in animal feeds and sprayed on plants to prevent infection and promote growth
- Multi drug-resistant *Salmonella typhi* has been found in 4 states in 18 people who ate beef fed antibiotics

Antibiotic resistant pathogens

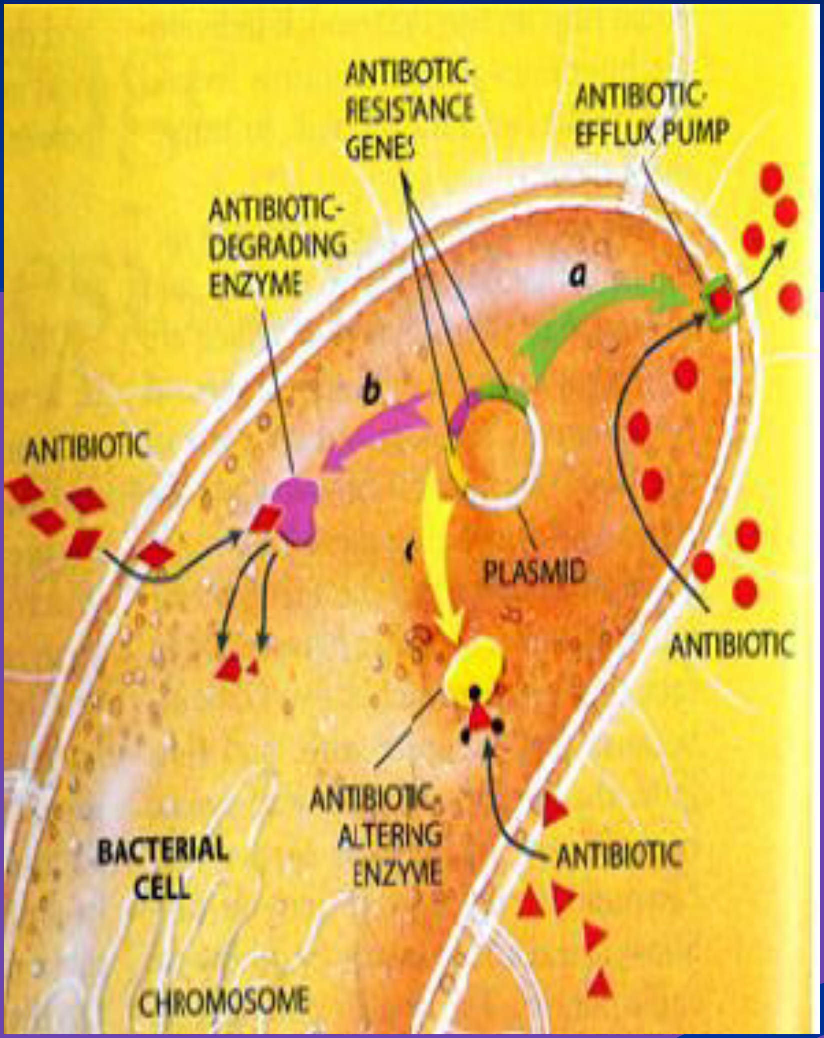
- Studies show there is horizontal gene transfer of antibiotic resistant genes in farm animal colons and there is stable maintenance of resistance transferred genes.(e.g. tetracycline, erythromycin, ampicillin, vancomycin, clindamycin resistance common)

- Studies show that antibiotic resistance genes in animals and humans contain identical elements, enabling spread from animal microflora to human microflora through the fecal-oral route.

Genetic Basis For Antimicrobial Resistance

- Chromosomal mutation (s)
- Acquisition of exogenous genes (or DNA)
- Conjugation (plasmids, transposons ±integrons)
- Transformation (acquisition of foreign DNA)
- Transduction (bacteriophage-mediated transfer of genes or DNA)

Mechanisms of Antimicrobial Resistance

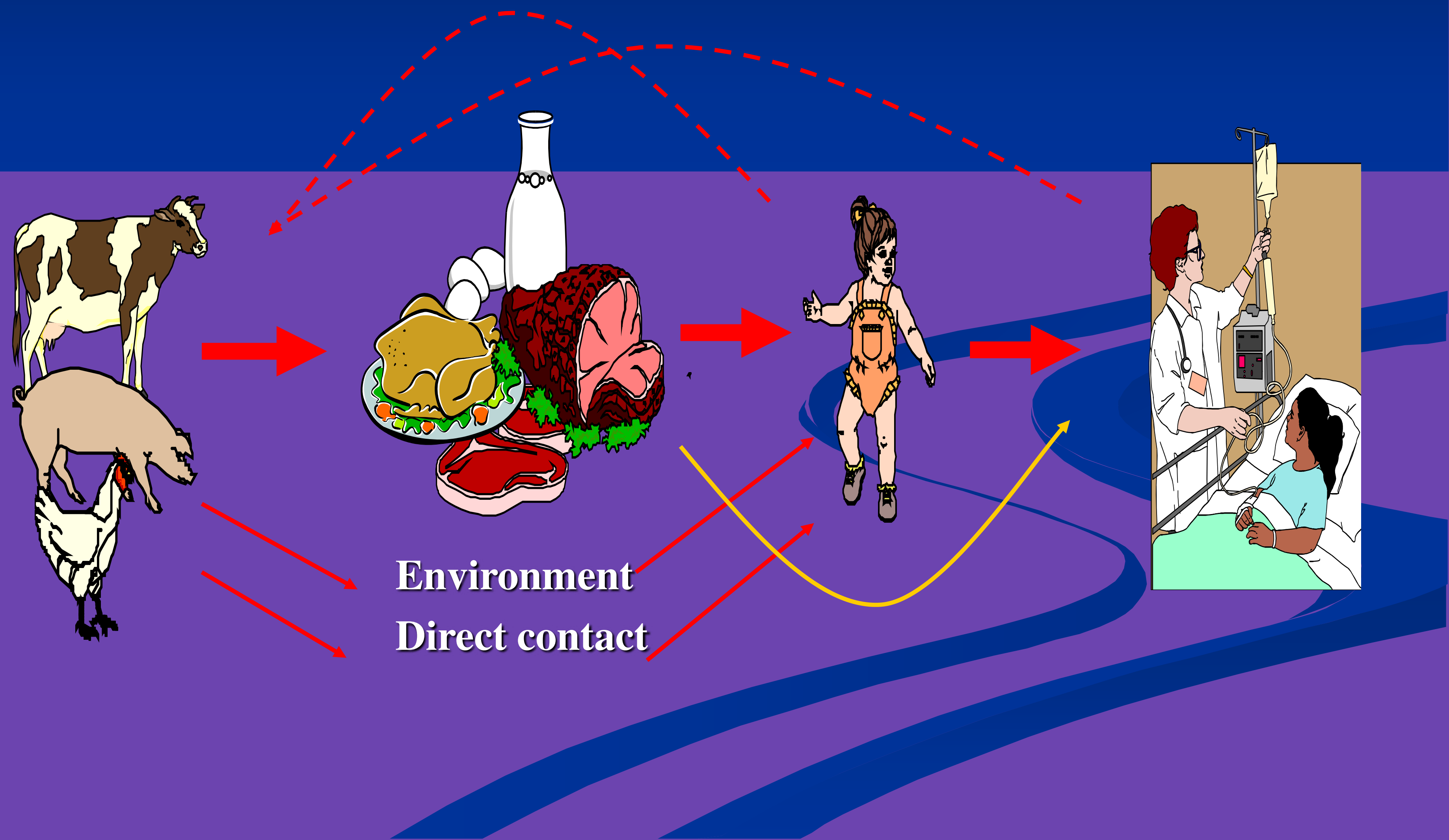


- **Enzymatic modification**
  - Beta lactamases
- **Decreased accumulation of antibiotic**
  - Permeability barriers - outer membrane Gram negatives
  - Porin mutations - carbapenems
  - Antibiotic efflux pumps - tetracyclines, macrolides
- **Alteration of the drug target**
  - Methicillin, vancomycin, macrolides

Antimicrobial resistant Pathogens

- *Campylobacter* spp
  - –Macrolide and fluoroquinolone resistance
- *Salmonella* spp
  - –Fluoroquinolone resistance
- Enterococci
  - –Vancomycin and macrolide resistance in chickens
- Methicillin-resistant *Staphylococcus aureus* in pork and chicken
- *E. coli* and *Salmonella* spp
  - –Quinolone resistance (*qnr*)
  - –Extended-spectrum β-lactamases
  - - gentamycin resistant E.coli in chickens

Transfer of antimicrobial resistance between animals and humans



Less Antibiotic Use In Food Animals Leads To Less Drug Resistance In People

Clinical Infectious Diseases (2006)42:1368-1374

- *Campylobacter jejuni* is a leading bacterial cause of foodborne illness in industrialized countries.
- Drug resistance can make Campylobacter infections difficult for to treat, and can result in longer bouts of and a higher risk of serious or even fatal illness.
- Australia prohibited the use of fluoroquinolones, in food animals such as poultry.
- Researchers examined *C. jejuni* isolates collected from 585 patients in five Australian states.
- Only 2% of the locally acquired Campylobacter isolates were resistant to ciprofloxacin, a type of fluoroquinolone (29% in countries w/o ban).
  - Sweden prohibited the use of fluoroquinolones for food animals in 1986
  - Norway has never licensed their use in food animals
- FDA proposed banning fluoroquinolones in poultry in 2000; finally enacted in September 2005.

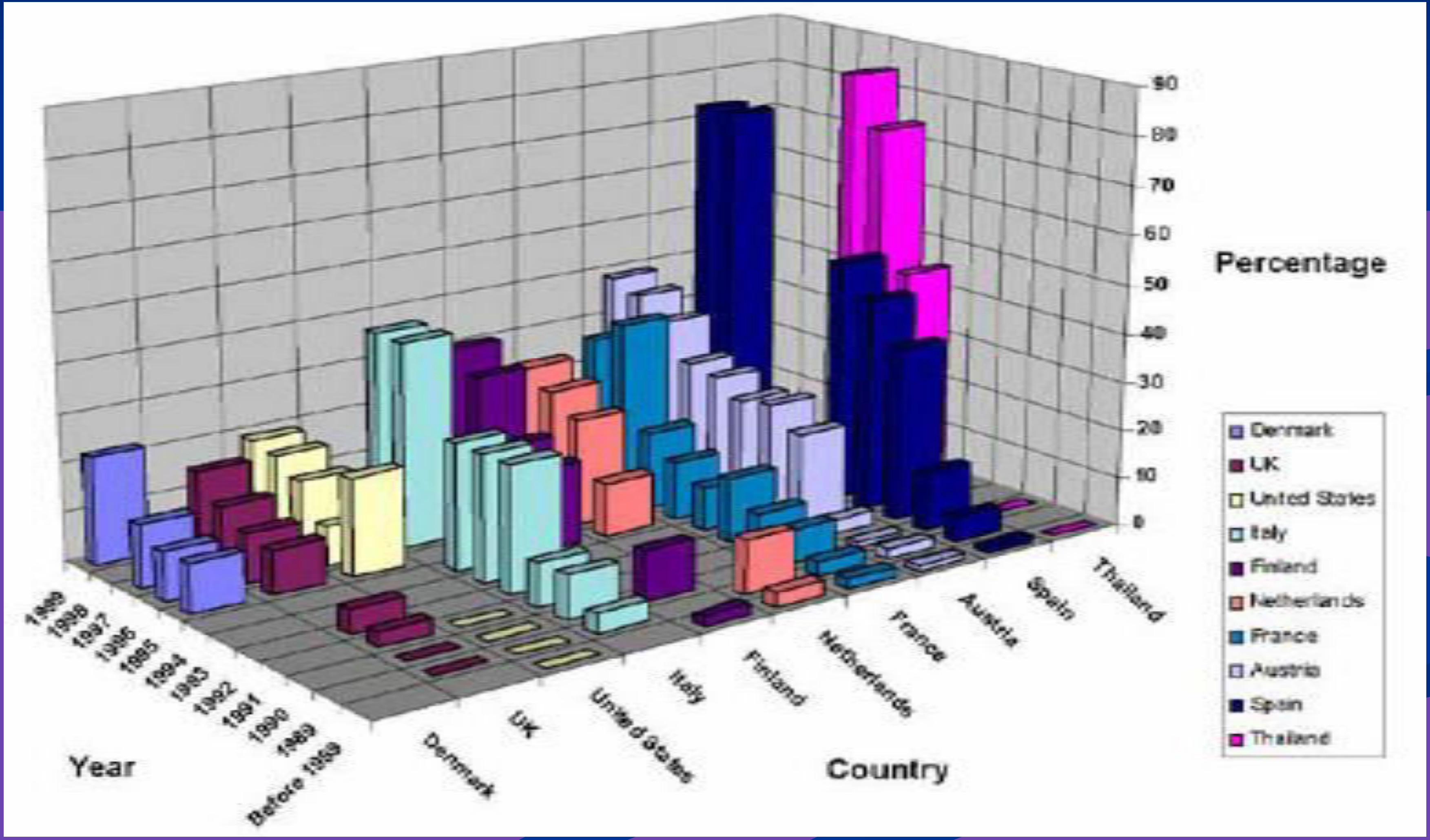
Bans on antimicrobial use

- EU banned 5 antibiotics from in-feed livestock use (i.e., zinc bacitracin, avoparicin, spiramycin, tylosin phosphate, virginiamycin) in 1999.
- Wider bans on antimicrobials from in-feed livestock use have been implemented in Sweden, Denmark, Netherlands and Switzerland.
- US banned fluoroquinolones from any livestock use in 2005.
- EU and New Zealand banned arsenicals from in-feed livestock use.

Agricultural Antibiotics

- 1/06: European Union bans antibiotics as growth promoters in animal feed
- 2008: USDA allows E. coli-tainted meat to be sold as pre-cooked hamburger patties, taco meat, pizza toppings, etc.

Quinolone resistance rates among *Campylobacter coli* and *C. jejuni* combined from humans, for both quinolones and fluoroquinolones



Multidrug Resistant Salmonella Typhimurium

- Idaho - Small animal veterinary clinic with 20 employees
  - Sept-October 1999
    - 10 employees had bloody diarrhea
    - Index case
      - Employee caring for several kittens with diarrhea 1-2 days before onset of diarrhea
    - All 10 employees ate meals at the clinic and had no common exposure outside the clinic
    - Salmonella Typhimurium from 5 patients
      - Isolates with similar DNA fingerprint type
      - Resistant to ampicillin, ceftriaxone, cephalothin, chloramphenicol, amoxicillin/clavulinic acid, gentamicin, kanamycin, streptomycin, sulfamethoxazole and tetracycline

MRSA in Dairy Cows

- 15 dairy farms in North West England(2006)
- 1043 samples
- 189 milk samples
- 101 nasal swabs
- 753 udder swabs
- No MRSA
- 17/818 cows positive for MR-staphylococci
- 12 udder swabs
- 5 nasal swabs

MRSA in Dairy Cattle in England and Wales

- Bovine clinical mastitis samples (2006-8)
  - 940 confirmed *S. aureus* isolates
  - All screened for *mecA* gene
  - All negative, but
  - 43% penicillin resistant
  - 1% augmentin resistant (synulox)
  - 6% ciprofloxacin
  - 2% tetracycline
  - 0.3% gentamicin

MRSA in food

- 1293 raw meat retail samples, England (2007)
- 11% of meat positive
- 84% MRSA
- Mostly <10 CFU/g

ESBL s in food-producing animals in England/Wales

- Nov 2004 (Visit 1)
- *E. coli* with CTX-M-14 (one clone) found
- Resistant to amp, amoxi-clav, ceftiofur, cefuroxime, cefotaxime, ceftriaxone, cefoperazone, cefpodixime, chloram, streptomycin, sulfa-trim, tet, and cip
- Prevalence in calves
- 64.6% (visit 1)- 92.7% (visit 3)
- Lactating cattle
- 50.0% (visit 1) – 15% (visit 2)
- Lots of *E. coli* clones CTX-M positive
- Some persisted over 3 visits (7 months)
- Plasmid transferable

ESBLs

- From 2006 *E. coli* diagnostic submissions screened for ESBLs at VLA
- All positive *E. coli* from cattle
- One farm was also positive for ESBL *E. coli* in sheep, horses and wild bird faeces
- CTX-M-1, 3, 14, 15 and 20 found
- **None in *Salmonella***
- **Farms positive for ESBL's all using 3rd generation cephalosporins**

ESBL's in food-producing animals

- France (Madec *et al* (2008), 46(4);1566-7)
  - •CTX-M-1 and 15 *E. coli* in cattle, pigs and poultry
  - •Cattle (n=1264)
  - •ESBL *E. coli* 6.3% sick, 5.8% healthy
  - •Poultry
  - •112 samples (10 slaughterhouses)
  - •12 ESBL-producers
  - •**ESBL *E. coli* also identified in Netherlands, Spain, Portugal, Belgium, Denmark**
  - •**Poultry, Pigs and cattle**

E.coli

- Drug resistance pattern in E.coli isolates from chicken colibacillosis in shahrekord (homaei,2003-2008)
- Erithromycin :75.36%
- Enrofloxacin:64.46%
- Flumequine:75.51%
- Lincospectin:52.8%
- Tetracycline :89.81%
- Difloxacine:59.92%
- Ciprofloxacin:59%
- Doxycycline:70.06%

Poultry farm waste water

- Evaluation of antibiotic resistance in some of bacteria isolated from poultry farm waste water (bakhshi,1387)
- Isolates: E.coli, E.faecalis and Stapylococcus
- Resistance: tetracycline ,gentamycin 100%
- E.coli:amoxicillin,ampicillin,nalidixic acid(70%), Enterococci and staphylococci : cipro, erythromycin

Salmonella (Iran)

- Salmonella as a potential pathogen in poultry and cow carcasses slaughtered in isfahan (shakerian,1387)
- Samples of cow and poultry meat, liver, external surface
- Salmonella entertidis 3.33% ,S.typyimurium 5.6%
- Resistant to penicillin, tetracycline, streptomycin, ampicillin, cephalothin
- Sensitive to ciprofloxacin and ceftriaxon

Salmonella

- Isolation and determining of antimicrobial resistance of salmonella strains isolated from food products in Tehran (Rafiei,1387)
- Resistance: tetracycline, SXT, streptomycin , ampicillin

Antibiotic-Resistant Bacteria Isolated From Meat

- Hypothesis was that the greater the amount of a drug used, the more likely bacteria would develop resistance to it.
- Beef:
  - Tetracycline > streptomycin = sulfamethoxazole > ampicillin > chloramphenicol > cephalothin
- Pork:
  - Tetracycline > streptomycin = sulfamethoxazole > ampicillin > chloramphenicol > gentamicin
- Chicken:
  - Tetracycline > sulfa > streptomycin = cephalothin > ampicillin > chloramphenicol > gentamicin
- Turkey:
  - Sulfamethoxazole > tetracycline > streptomycin > ampicillin > cephalothin > gentamicin

Antibiotic resistance in fish

- Vietnam study of bacteria from 3 catfish ponds showed antibiotic resistance, rates were (2006):

  Ampicillin 69%

  Oxytetracycline 61%

  Trimethprim/Sulphamethoxazole 61%

  Nalidixic acid 51%

  Nitrofurantain 37%

  Chloramphenicol 33%

- South African fish pond isolates had high levels of resistance to(2006) :

  Tetacycline 78%

  Amoxicillin 89%

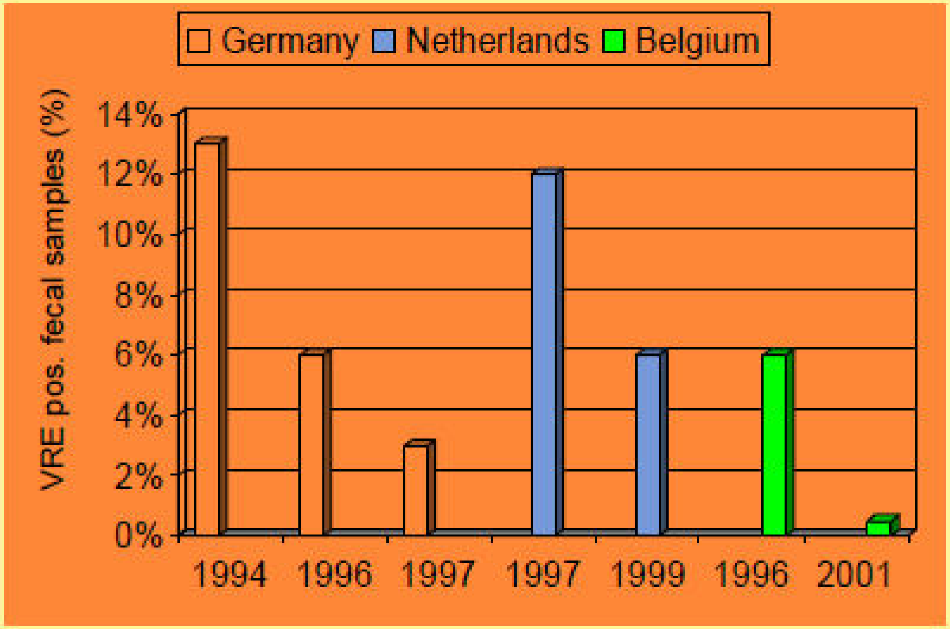
  Augmentin 86%

Bioaerosol risks

- Studies of bioaerosols inside intensive pig farms have shown more than 90% had multi-drug resistance.*,**
- Antibiotic resistance bacteria have been recovered 150 meters downwind from intensive pig farms.**
- Swine workers and veterinarians have elevated carriage of MRSA (methicillin-resistant *Staphyloccoccus aureus*).*, ***

*A.Chapin, et.al, Airborne Multidrug-Resistance Bacteria Isolated from Swine CAFO, 2005.
**S.G. Gibbs, et.al. Isolation of Antibiotic-Resistant Bacteria Downwind of Swine CAFO, 2006
*** Wulf, M, et.al. MRSA in Veterinary Doctors and Students in Netherlands, 2006

Reduced Antibiotic Resistance



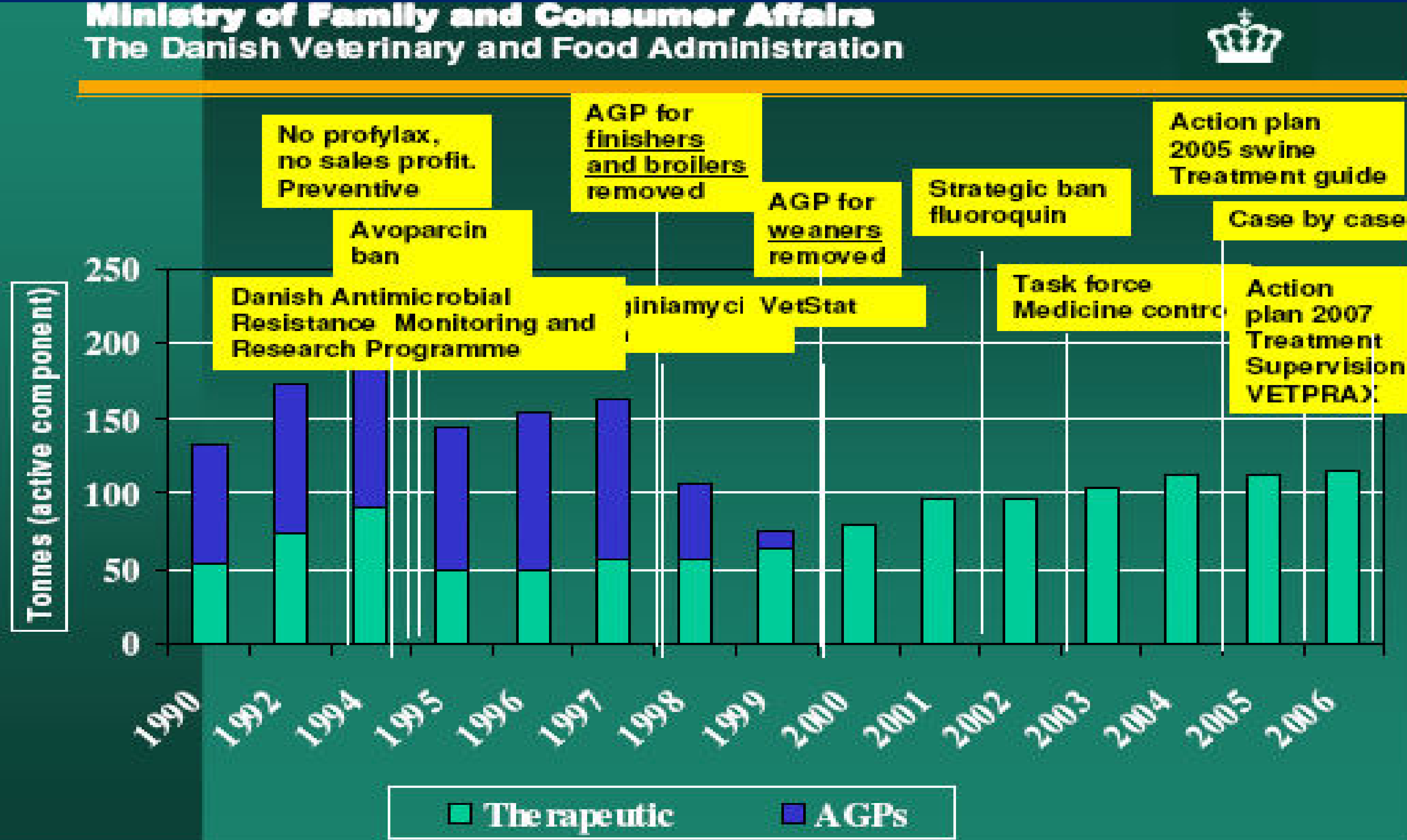
Human carriage of resistant pathogens, post-ban, in 3 European countries

***Denmark's phase –out* WHO Experts' Evaluation (2003)**

- Dramatic decrease in resistant bacteria in animals, in meat, and in humans
- Eliminating the routine use of antibiotics in livestock reduces human health risks without significantly harming animal health or farmers' income

Sources: Wegener HC. Current Opinion in Microbiology 2003; World Health Organization. Impacts of antimicrobial growth promoter termination in Denmark: The WHO international review panel's evaluation of the termination of the use of antimicrobial growth promoters in Denmark. 2003.

Trends in consumption of antimicrobials in food animals in Denmark



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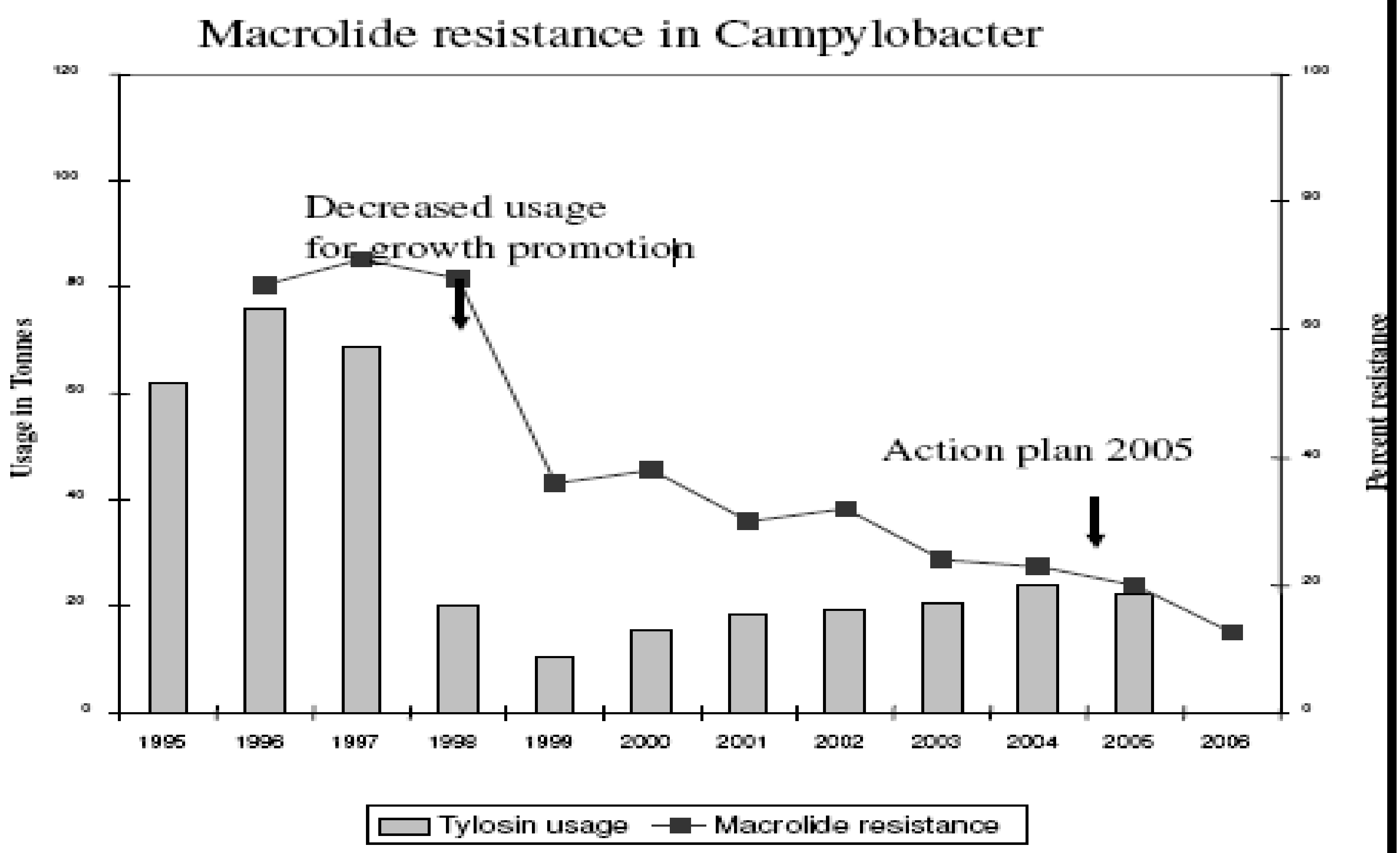

FDA Milk Drug Residue Database 2003

Source of Sample	Total Samples	Number Positive	Percent Positive	Regulatory Disposition (Pounds)
Bulk Milk Pick-Up Tanker	3,571,834	1,899	0.053%	70,108,000
Pastur. Fluid Milk and Milk Prod.	54,932	8	0.015%	64,000
Producer	665,627	1,009	0.152%	4,881,000
Other	90,581	29	0.032%	1,319,000
Totals	4,382,974	2,945	'	76,370,000

Regulatory Disposition = not for human consumption

Milk Residue Screening by Drug Family

Family/Drug 2003	Total Tests	Total Positive
AMINOGLYCOSIDES	1,290	1
Neomycin	1,858	2
AMPHENICOLS	201	0
BETA lactams	4,354,087	3,207
Cloxacillin	317	3
MACROLIDES	64	0
SULFONAMIDES	68,124	23
Sulfadimethoxine	4,478	3
Sulfamethazine	17,468	3
TETRACYCLINES	10,138	4
Tetracycline	118	0
TOTALS	4,458,141	3,248

- Antimicrobial therapy is not necessary for recovery from most cases of foodborne illness

▲ Most foodborne pathogens typically cause mild to moderate self-limiting symptoms that resolve without treatment

Recommendation to Reform antibiotic use in food animals

1-Restrict the use of antimicrobials to reduce the risk of antimicrobial resistance:

Phase out and ban the use of antibiotics for non-therapeutic uses.

2- Clarify definitions,estimates for drug use:

Non-therapeutic and therapeutic antibiotics.

3- Improvement of monitoring and reporting of antimicrobial use in food animal productions.

4- Improve monitoring and surveillance of antimicrobials in food supply, environment , animals and human population

5- Basic and applied research

– Mechanisms and risk factors

– New antimicrobials, alternatives to antimicrobials, vaccines

WHO Global Principles for Prudent use

- Antimicrobials should be prescribed only when indicated.
- Use of antimicrobials for prevention of disease can only be justified where it can be shown that a particular disease is present on the premises or is likely to occur. The routine prophylactic use of antimicrobials should never be a substitute for good animal health management.

THANK YOU