Animal Drug Residues in Food

Food Toxicology
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Learning Objectives
• Define animal drug residues.
• Explore the relationships between food animals and drugs.
• Understand the major classes of drugs used in food animals.
• Understand the hazards associated with food animal drug use and how risk assessment is used to determine tolerances.

Learning Objectives
• Review the role of pharmacokinetics in the development of withdrawal times.
• Review food animal drug testing results.
• Understand the potential adverse effects of drugs that appears as residues in food animals.
• Discuss the issue of development of antibiotic resistant bacteria.

Animal Drug Residues
• “Residues of veterinary drugs include the parent compounds and/or their metabolites in any edible portion of the animal product, and include residues of associated impurities of the veterinary drug concerned.”

Food Animals - Food Animal Health - Drugs
• Food animals convert one source of nutritional energy (grass, grain, hay) into another (meat, milk, eggs).
• Food animal production practice requires management of animal health and this can require the use of drugs.
  – Animal health management
  – Animal industry economics
• Human health effects of food animal drugs can arise from drug residues.
• Human health effects can also arise when food animal drugs are not used (pathogens).

**Meat, Fish, and Dairy Consumption**
• The average American consumes 200 pounds of meat and fish, 67 pounds of poultry, 30 pounds of eggs, and 600 pounds of dairy products each year.
• Drug residues are analyzed, regulated, and monitored by FDA-CFSAN/CVM, USDA-FSIS, state milk ordinances, JEFCA (Joint FAO/WHO Expert Committee on Food Additives), international food agencies.
• Veterinary drugs are used by veterinarians and by food animal producers.
  – Pre-market drug tests
  – Residue avoidance testing
  – Drugs labels

**Food-Producing Animal Species**
• Major species
  – Cattle, cows
  – Swine
  – Chickens
  – Turkeys
• Minor species
  – Sheep
  – Goats
  – Llamas/Alpacas/Camels
  – Deer and other wildlife
  – Others

**Major Classes of Drugs Used in Food Animals**
• Topical antiseptics, bactericides, and fungicides
• Ionophores
• Steroid anabolic growth promoters and peptide production enhancers
• Antiparasite drugs
• Antibiotics
Other Food Animal Drugs

- Drugs that modify the gastrointestinal environment to reduce the likelihood of rumen foaming and bloat in cattle.
- Organic and inorganic water treatments that reduce the chances for water or fish infection in aquaculture.
- Miscellaneous drugs and compounds used with the advice of veterinarians to treat specific conditions.

Topical Antiseptics-Bactericides-Fungicides
- Used to treat surface skin, or hoof infections, cuts, and abrasions.

Ionophores
- Alter rumen microorganisms to provide more favorable and efficient energy substrates from bacterial conversion of feed.
- Impart some protection against some parasites.

Steroid Anabolic Growth Promoters and Peptide Production Enhancers
- Mechanism of action resides in the interaction of estrogen-, progesterone-, or testosterone-like compounds with specific classes of hormone receptors in animal cells.
  - Recombinant bovine somatotropin (BST) for increased milk production in dairy cows.

Antiparasite Drugs
- Used to control fleas, ticks, mange (mites), worms, giardia, coccidia and other intestinal parasites.

Antibiotics
- Used to control overt and occult (sub-clinical) diseases.
- Used to promote growth in sub-therapeutic doses.

Human Health Risk Issues

- Drug residue allergy
- Cancer, reproductive, and developmental effects
- Hormones
- Development of antibiotic resistant microbes
- Drug misuse

**Animal Drug Residue Tolerance Levels**

**Tolerance: Hazard Identification**

- Short term
  - Allergenicity
  - Toxicity
- Long term
  - Microbiological effects
  - Carcinogenicity
  - Reproductive effects
  - Teratogenicity

**Toxicity: Clenbuterol**

- Non-steroidal anabolic and metabolism accelerator.
- Spain, 1990 outbreak: 135 people ill from eating contaminated liver.
  - Several hospitalizations: tachycardia, muscle tremors, headaches, nausea, fever, chills
- Jalisco Mexico, December 2005: at least 225 people ill after consuming beef/liver.
  - Trembling, headache and malaise

**Allergenicity: β-Lactam Antibiotics**

- Anaphylactic reactions have been reported to result from consumption of beef or pork containing penicillin.

**Microbiological Effects**

- Disruption of normal human flora in the intestine.
  - Bacteria that usually live in the intestine act as a barrier to prevent incoming pathogenic bacteria from getting established and causing disease.
- Antibiotic residue might reduce total numbers of these bacteria or selectively kill some important species.

**Carcinogenicity: Nitrofurans, Nitroimidazoles**
• Furazolidone and its metabolites have been shown to induce cancer in animals.
• Had been labeled and approved for anti/protozoal and other uses for a wide variety of conditions in poultry and swine.
• FDA approval withdrawn 1991.
• FDCA Delaney Clause.

Reproductive and Teratogenic Effects: DES
• Diethylstilbestrol: a synthetic estrogen formerly used commercially as a growth promoting agent in livestock.
• Drug used in pregnant women in 1940’s.
  – Vaginal clear-cell adenocarcinoma in female off-spring exposed in utero (1 in 1000)
  – Structural abnormalities of uterus (69%)

Dose-Response and Exposure Assessment
• Toxicological tests in laboratory animals.
  – Part of pre-clinical drug development.
• Development of NOAEL.
• Safety factors.
• Acceptable daily intake (ADI).
• Sub-population sensitivity.
• Exposure assessment.
  – Food consumption.
  – Aggregate exposure.
• Use to develop Tolerance Level.

Tolerance Level
• The maximum permissible residue level which may be present in tissues or food animal products.
• Tolerances are specific for species and tissue (liver, kidney, fat, muscle) or product (milk, eggs).

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

Animal Drug Withdrawal Time

• Experimentally determined.
• Time required that concentrations in all food animal tissues or products are below tolerance.
• Margin of safety (MOS) increased to 95% confidence interval for 99% of population.
  – MOS = LD₁/ED₉₉
• Expensive
  – Limited products
  – Healthy animals

Animal Drug Withdrawal Time

• Other considerations
  – Aesthetic considerations
  – Risks perceived by public
  – Sensitive populations and issues
  – International relations and trade barriers

Extralabel (Off-Label) vs. Label Drug Use

• Higher dose than label
• Different route than label
• Different species than label
• Different disease indication than label

Extralabel (Off-Label) vs. Label Drug Use

AMDUCA 1996

• Animal Medicinal and Drug Use Clarification Act 1996
• Permits extralabel drug use in animals
  – Does not apply to feed and water additives
  – Several drug classes have been excluded
• Some drugs are prohibited from use in food animals
  – Clenbuterol, chloramphenicol, nitroimidazoles, nitrofurans
• Withdrawal time extrapolutions from other known applications
  – WDT = 10 x T₁/₂

Over-The-Counter (OTC) Veterinary Drugs
Majority of animal drugs sold in US
Can still cause residues if not used according to label.

Drug Residue Testing

Target tissues tested
- Milk
- Kidneys often tested at slaughter
STOP
- Swab test on premises
FAST
- Fast antimicrobial screen test
SOS
- Sulfa-on-site
CHARM II; SNAP
- Milk residues
Lab tests
- HPLC/GC/Mass Spectrometry

FDA Milk Drug Residue Database 2003
• 4,382,974 samples were analyzed for animal drug residues.
• 2,945 were positive for a residue.
• Samples:
  – Bulk Milk Pick-Up Tanker
    • Bulk raw milk from a dairy farm
  – Pasteurized Fluid Milk and Milk Products
    • Finished product in package form or bulk
  – Producer

FDA Milk Drug Residue Database 2003
Milk Residue Screening by Drug Family
2003 FSIS Meat Residue Monitoring
• Penicillin and sulfonamide drugs were most commonly detected at violative levels in swine and cattle.
• Neomycin and gentamicin were also detected in a number of cattle, particularly calves.
• Other drugs detected in cattle and swine included tilmicosin, flunixin, and tetracyclines.
• Arsenicals were detected in poultry.

Drugs Most Likely to be Detected in Meat
• Penicillin (including ampicillin)
• Tetracycline (including chlortetracycline and oxytetracycline)
• Sulfonamides (including sulfadimethoxine and sulfamethazine and sulfamethoxazole)
• Neomycin
• Gentamicin
• Flunixin
• Streptomycin
• Arsenicals

Penicillin
• Penicillin derivatives (β-lactam antibiotics) are widely used in cattle, swine and poultry to treat infections and as feed or drinking water additives to prevent some diseases.

Potential Adverse Effects: Penicillin
• Usually cleared rapidly from the blood via the kidneys and into the urine (kidney, liver about 100x higher than muscle).
• Allergic reactions determining factor for safety evaluation of residues.
  – Allergy to penicillin in different populations 3–10%.
  – No evidence that penicillin residues in food caused sensitization.
  – Some cases of persons with known sensitivity suffering allergic reaction
• Estimated that 10 IU (0.6 μg) could cause an allergic reaction in a sensitive individual.
  – 0.01 IU/ml of milk in a very sensitive individual.
• 2 cases of anaphylactic reactions with known hypersensitivity to penicillin, steak (in 1984) and pork (in 1972).
• JECFA estimated that if residues in meat (including liver and kidney) were at the MRL of 0.05 mg/kg and for milk were 0.004 mg/kg, the maximum daily intake of benzylpenicillin from residues would total 29 μg.

Tetracyclines
• Oxytetracycline is a broad-spectrum antibiotic used to treat a variety of infections and is also used as a growth promoter in animals.

Potential Adverse Effects: Tetracyclines
• Humans, ~ 60% of an ingested dose absorbed from GIT and widely distributed in the body.
  – Particularly to liver, kidney, bones and teeth.
  – Little metabolism of this drug in humans or animals and it was primarily excreted in the urine
• Not mutagenic, carcinogenic, or teratogenic in animal studies; some toxic effects were observed at high doses.
  – NOAEL 18 mg/kg body weight/day.
• Therapeutic doses occasionally associated with discolored teeth, allergic reactions, or peripheral blood changes
• Oxytetracycline did induce antibiotic resistance in coliforms in the human intestine; JECFA used this for MRL.
  – NOEL 2 mg/person/day
• There have been reports of allergic reactions but no cases that have involved exposure to residues in foods.
• JECFA estimated that if OTC residues in meat, milk and eggs were at the MRL, residues would total 260 μg.

Sulfonamides
• Sulfonamides are generally used to treat a wide variety of bacterial and coccidial infections in food producing animals and are used as growth promoters in swine.

Potential Adverse Effects: Sulfonamides
• Metabolized by numerous pathways with the major metabolite in humans, swine and cattle being an acetyl derivative.
• Data cited by JECFA indicate that the primary mechanism of toxicity of sulfonamides is associated with the thyroid–hypothalamus
  – Toxicity should be measured by parameters of thyroid and pituitary function.
• NOAEL 2.2 mg/kg bw/day.
• Hypersensitivity reactions (primarily skin rashes) to therapeutic levels of sulfonamides have been reported but there have been no cases that involved exposure to residues in foods.

Neomycin
• Neomycin is an aminoglycoside antibiotic that is used to treat intestinal, respiratory, and wound infections and mastitis.

Potential Adverse Effects: Neomycin
• Neomycin is not readily metabolized in animals or in humans.
• Not genotoxic. Like streptomycin and gentamicin, it has been reported to cause damage to the kidney and to hearing.
  – Recent data indicate that people with a rare mutation in their mitochondrial DNA may be more susceptible to deafness caused by aminoglycosides and other environmental factors than the general population.
• JECFA based its recommendation for a maximum daily intake of 3.6 mg/kg bw on results on hearing loss in guinea pigs.
• JECFA calculated that the estimated dose of neomycin from veterinary drug residues was 3 mg/day, primarily from milk (2.25 mg), kidney (0.5 mg), and muscle (0.15 mg). This was 3000 times less than the recommended oral therapeutic dose of neomycin.

Gentamicin
• Gentamicin is an aminoglycoside antibiotic

Potential Adverse Effects: Gentamicin
• Like streptomycin and neomycin, gentamicin has been reported to cause damage to the kidney and to hearing.
• Depleted rapidly from muscle and fat but tends to persist in kidney and liver.
• Not readily metabolized in animals or in humans.
• JECFA estimated that if residues in meat were at the recommended MRL, the maximum daily intake of gentamicin from residues would total 785 μg.
− 30 μg from muscle, 200 μg from liver, 250 μg from kidney, 5 μg from fat, 300 μg from milk.

Flunixin
• Flunixin is a non-steroidal anti-inflammatory drug (NSAID) and analgesic and is the only such drug allowed for use by veterinarians.

Potential Adverse Effects: Flunixin
• Flunixin inhibits prostaglandin synthesis apparently by a mechanism similar to aspirin.
• Since NSAIDs are commonly used in human medicine, it is believed that flunixin is a relatively safe drug and residues should not be very harmful.
• However it appears that this drug has not been tested adequately on humans, particularly for hypersensitivity reactions.

Streptomycin
• Streptomycin is an aminoglycoside antibiotic used for treating bacterial infections in food producing animals.

Potential Adverse Effects: Streptomycin
• Not readily absorbed from the GIT because of its high molecular mass and not metabolized significantly w/ inj.
• Oral doses of the drug are eliminated unchanged in the feces.
• Animal studies indicate most sensitive end point was a decrease in weight; used to set ADI of 30 μg/kg bw.
• Reports of allergic reactions to streptomycin
  − No cases that have involved exposure to residues in foods.
• One significant adverse effect in humans that occurred during treatment of pregnant women with TB.
  − Infants of women treated IM 1 g BIW 1st trimester: damage to a cranial nerve and congenital deafness.
• Streptomycin may also have adverse effects on kidney fn.
• No other evidence of effects on fertility or reproduction.
• It is not expected that low food residues/low abs. would affect fetal development.

Arsenicals
• Arsenical compounds are used in swine and poultry as growth promoters and to prevent bacterial enteritis.
• The most commonly used arsenic compound for poultry is roxarsone.

Potential Adverse Effects: Arsenicals
• Most of the roxarsone is excreted unchanged, but some metabolites have been detected in hen urine.
• Roxarsone is poorly retained in poultry meat (FDA limit is 0.5 mg/kg in chicken muscle).
• Inorganic arsenic is a known carcinogen and may adversely affect the circulatory and nervous systems.
• Organic arsenic is generally less toxic and some arsenic compounds are considered harmless.
• Diets containing 800 mg/kg roxarsone caused decreased body weight in mice; rats were more sensitive, showing lower body weights on diets containing 200–400 mg/kg roxarsone.
• There was equivocal evidence for carcinogenicity in male rats fed 100 mg/kg roxarsone for 2 years, but no evidence of carcinogenicity in female rats and both sexes of mice.

Development of Antibiotic-Resistant Bacteria
• Bad bugs → no drugs
• A major issue of drug use in food animals as well as over-use of antibiotics in humans

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

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

Animal Drug Residue Concerns
• Consumer health risk
  – Environmental concerns
• Consumer preference
• Production loss for the producer
  – Lost milk product ($6,000 to $80,000)
  – Lost animal ($500 to $2000)
• Legal action against
  the producer
  – Violative (illegal) residues