Anthelmintic drugs and coccidiostats: anti-parasitic drug residues in meat

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Globally, livestock play a vital role in the economy, which includes meat, milk, eggs and fibre. Cattle and poultry production are two of the most important agricultural industries worldwide. As incomes rise, people in both developed and developing nations have an increased appetite for animal protein, and therefore the demand on the industries to increase productivity is also intensifying.

This article will provide a brief overview of some common infections being battled throughout our worldwide cattle and poultry industries and also the drive to keep our meat and eggs drug-residue free.

Helminth infection

There has been a noted success in the last 60 years in controlling helminth parasites in cattle using anthelmintics. However, helminth infection continues to be a looming threat to our global livestock production with increased anthelmintic resistance.

Parasitic helminth infection affects food producing animals worldwide. The word 'helminth' is a general term meaning 'worm', but there are many different types of worms. The most widely prevalent helminths are the nematodes (round worms), which inhabit the gastro-intestinal tract. Larvae for these parasites are most commonly ingested during grazing.

Usually the development time of these larvae then depends on the specific type of nematode and the conditions they inhabit. Under optimal conditions the developmental phase takes up to 10 days.

Most trichstrongyles (type of nematode) moult into adults three weeks after infection. Eggs produced by female adults are present in the faeces of infected animals.

In Brazilian Beef herds, Cooperia infection has been found to be one of the most prevalent. The Cooperia species live in the small intestine and can penetrate the mucosa during larval development causing changes similar to those of the intestinal species trichostrongylus. Symptoms of this helminth infection include diarrhoea, weight loss, anorexia and insufficient weight gain.

Anthelmintic drugs are used to treat helminth infection in cattle. Despite the prevalence and economic impact of helminth infection in the global livestock industry, little attention is given to the development of new anthelmintics.

The most widely successful drug over the last 20 years has been Ivermectin (an Avermectin) which has decreased research into new drugs.

Below are some of the most commonly used veterinary anthelmintic drugs:

- **Avermectins:**

The avermectins are a group of chemically related compounds originally isolated from the actinomycete Streptomyces avermitilis. They are macrocyclic lactone derivatives with potent anthelmintic activity, but lack antibacterial or antifungal activity.

The avermectins also have insecticide activity and include the compounds ivermectin, abamectin, doramectin, emamectin, and eprinomectin.

- **Benzimidazoles:**

Albendazole is a benzimidazole carbamate, which is used as an anthelmintic in veterinary and human medicine. Albendazole is metabolised to albendazole sulphoxide and albendazole sulphone; the carbamate moiety is then cleaved to produce albendazole 2-aminosulphone.

Albendazole binds strongly to tubulin in the cells of nematodes, especially intestinal cells, resulting in a loss of absorptive function which causes starvation of the nematodes. Albendazole-containing products are available in liquid form or as pellets, both of which are administered orally.

- **Thiabendazole:**

Thiabendazole is an anthelmintic, which is used in the treatment and control of gastrointestinal roundworms in horses, cattle, goats and sheep, and for the control of lungworms in sheep. It is also used for the control of fungal diseases affecting plants, animals and man.

Thiabendazole binds strongly to tubulin in the cells of parasitic worms, especially intestinal cells, resulting in interference with absorptive function and starvation.

Thiabendazole is rapidly metabolized and excreted in cattle, pigs, sheep, goats; its major metabolite is 5-hydroxythiabendazole. The sum of unaltered thiabendazole and 5-hydroxythiabendazole is estimated to represent 50-90% of total residues and are the most persistent residues detected in liver.

- **Triclabendazole:**

Triclabendazole is a benzimidazole anthelmintic used in food animals, where it is mainly employed in the control of liver fluke in sheep and cattle.

Triclabendazole has a narrow spectrum of activity concentrated against a limited number of fasciolides. Triclabendazole is not active against nematodes

- **Levamisole:**

Levamisole is a synthetic imidazothiazole derivative that has been used extensively as an anthelmintic agent. The racemic mixture tetramisole is also used as an anthelmintic, although the anthelmintic activity is due to levamisole. Levamisole is commonly used in cattle, sheep, pigs, goats, and poultry to treat nematode infections, but has no activity against flukes and tapeworms. It is thought that its anthelmintic activity is achieved through interaction with the nicotinergic acetylcholine receptor, preventing males from successfully copulating as they lose their ability to control their reproductive muscles.

- **Moxidectin:**

Moxidectin is a semi-synthetic, macrocyclic lactone, structurally similar to abamectin, ivermectin and milbemycin. Moxidectin is intended for the treatment of endo- and ecto-parasites in cattle and sheep by oral or sub-cutaneous route and in horses by oral administration.

Residues and resistance

In 2011 46 border rejections were raised in the EU for residues of veterinary medical products, 25 of which were for the anthelmintic drug Ivermectin. These rejections have highlighted the need for a rapid and reliable screening method for many border inspection points and import authorities as vigilance must be increased to ensure that foods containing these drugs are not allowed to enter the food chain.

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Treatment of the infected animal with the wrong drugs, incorrect dosage or insufficient withdrawal period of the drugs are contributing to anthelmintic resistance as well as residues of these compounds remaining in the milk or tissue of treated animals.

Maximum Residue Levels (MRLs) have been established for the majority of these compounds.

Coccidiosis in poultry

Just like helminth infection in the cattle industries, coccidiosis is causing economic losses in the poultry industry. The most common species of coccidia that are prevalent in chickens are Eimeria tenella and Eimeria necatrix which both cause bloody intestinal coccidiosis.

In chickens, a coccidia oocyst is excreted in the droppings and then develops into what is called a sporulated oocyst, infecting other chickens. This sporulated oocyst facilitates many offspring inside the cells of the infected chicken and large amounts of intestinal cells may be destroyed or damaged.

After the developmental phase, male and female cells are produced which enables production of an oocyst. This oocyst ruptures from the intestinal cell wall and is excreted in droppings.

Poultry are generally raised in cramped, overcrowded conditions which make coccidiosis infection easily spreadable.

Coccidiosis is usually characterised by bloody droppings, weight loss and mortality in young chickens. Unsanitary conditions usually increase the prevalence of this infection in the poultry industry.

Anticoccidial drugs

Coccidiostats are antiprotozoal agents that act upon coccidia parasites in livestock. The EU has implemented testing guidelines (outlined in group B2b of Annex I to Council Regulation 2377/90) to test for veterinary medicines in food. This would encompass the treatment of coccidiosis – a protozoal disease that can cause diarrhoea and dysentery in the affected animal.

Control of coccidiosis is particularly important in the poultry industry, where the prophylactic use of coccidiostats prevents the disease from developing, thus increasing the risk of drug residue carry over into meat and eggs. Maximum Residue Limits have been set in place for a number of coccidiostat residues.

Below are some commonly employed coccidiostats:

- **Clopidol:**

Clopidol is a member of the pyridinol group of anti-coccidial compounds. It is coccidiostatic in action and is most effective against the sporozoite stage of Eimeria. It is the only member of the pyridinol group to be commercially successful and is one of the few drugs used to control coccidiosis in rabbits. It is administered in feed at a dose of 200ppm for rabbits and 125ppm for chickens, with a withdrawal period of five days. Clopidol is generally used in combination with nequinate and is also used with methylbenzoquate in chickens and turkeys.

The United States-Food and Drug Administration (US-FDA) has also approved the use of clopidol in medicated feed in combination with bacitracin zinc and roxarsone.

- **Decoquinate:**

Decoquinate is a quinolone derivative, active against eimeria and toxoplasma species. The quinolones are a family of synthetic antibiotics structurally related to nalidixic acid that inhibit the action of bacterial DNA gyrase enzymes, although the exact mode of action is not understood. Decoquinate inhibits the development of coccidia in the small intestine in the early part of the infective cycle, resulting in lower morbidity and mortality. Decoquinate is administered in feed at 20-40ppm for the prevention of coccidiosis. Decoquinate is also administered in combination with other antimicrobials such as chlortetracycline or roxarsone and bacitracin methylene disalicylate.

- **Diclazuril:**

Diclazuril is a benzeneacetonitrile derivative used as an oral anticoccidial agent. Its mode of action is not known, although it interrupts the life-cycle of parasites by blocking the excretion of oocysts. Diclazuril is administered as a medicated feed premix, at a recommended dose of 1ppm, to major poultry species, rabbits and lambs, although it is not intended for use in laying hens.

- **Halofuginone:**

Halofuginone is derived from febrifugine and is a member of the quinazolone group of chemicals. The active form is trans-halofuginone and the cis- form is present as an impurity. Halofuginone lactate is used for the prevention of diarrhoea due to Cryptosporidium parvum in non-ruminating calves, whereas halofuginone hydrobromide is used as an anticoccidial feed additive for broilers and turkeys.

Halofuginone has a steep dose-response curve and at high doses is a growth depressant, which can cause difficulties when administered in feed. Halofuginone hydrobromide is also administered in combination with other antimicrobials such as lincomycin and bambermycin.

- **Imidocarb:**

Imidocarb is a carbanilide derivative administered via a subcutaneous injection as the dipropionate salt to cattle and sheep for the treatment of anaplasmosis and babeiosis.

- **Lasalocid:**

Lasalocid is a polyether ionophore antibiotic produced by Streptomyces lasaliensis. Lasalocid is a mixture of several closely related substances termed lasalocid A-E. Lasalocid A constitutes 90% of the active substance. Lasalocid is mainly active against Gram positive micro-organisms and it is used in veterinary medicine as antiprotozoal agent.

Lasalocid is also administered as a medicated feed additive, either alone or in conjunction with other antimicrobials such as virginiamycin. Lasalocid sodium binds monovalent and divalent cation and alters ionic transport across lipid membranes. Although the use of lasalocid in laying hens is prohibited in Europe, one in eight samples of eggs tested by UK government chemists are reported to contain residues of lasalocid.

The contamination of eggs is thought to occur due to poor cleaning systems at feed mills and the accidental use in layers of lasalocid-medicated feed destined for young birds.

- **Maduramicin:**

Maduramicin is a polyether ionophore antibiotic produced by Actinomadura yumaense. It is often confused with maduramycin, a distinct compound isolated from Actinomadura rubra as reported by Fleck et al. (1978). Maduramicin has been approved for use for the prevention of coccidiosis in broiler chickens caused by various Eimeria strains.

Maduramicin is administered in feed at a dose of 5ppm, with a seven day withdrawal period. It is reported to be 12-24 fold more potent as a coccidiostat than other polyether antibiotics.

- **Monensin:**

Monensin is a polyether antibiotic and is the major component of the antibiotic complex produced by Streptomyces cinnamonensis. Monensin is used as a coccidiostat in poultry and a growth promoter in cattle.

- **Nicarbazin:**

Nicarbazin is an antiprotozoal agent used in veterinary medicine as a coccidiostat but also as a feed additive in animal husbandry. Nicarbazin interferes with mitochondrial metabolism and inhibits oocyst production. It consists of a 1:1 molar mixture of N,N'-bis(4-nitrophenyl) urea and 4,6-dimethyl-2(1H)-pyrimidinone and the complex is essential for coccidiostatic properties.

Nicarbazin is often administered in conjunction with other coccidiostats or antimicrobials for example narasin and bacitracin.

- **Nifursol metabolite:**

Nifursol is an anti-protozoal agent used in veterinary medicine. It belongs to the nitrofuran group of compounds, which are thought to be genotoxic due to the presence of a 5-nitro group.

Nifursol was used as an approved feed additive in the European Union (EU), for the prevention of histomoniasis in chickens and turkeys. However, its use has now been prohibited in the EU.

- **Robenidine:**

Robenidine is administered to chickens in medicated feed in combination with bacitracin and roxarsone.

- **Salinomycin/narasin:**

Salinomycin is a polyether antibiotic produced by Streptomyces albus and used in veterinary medicine as antiprotozoal agent to treat and prevent coccidiosis. It disrupts the growth and reproduction of certain intestinal bacteria and was the first licensed growth promoting polyether in swine. It is used in broiler chickens at 50-70ppm in feed, with a five day withdrawal period. Salinomycin is commonly administered in combination with other antimicrobials such as roxarsone and bacitracin, roxarsone and bambermycins, roxarsone and chlortetracycline, and tylosin.

Narasin is a polyether ionophore isolated as the major compound in an antibiotic complex produced by Streptomyces aureofaciens. It is used in veterinary medicine as an antiprotozoal agent but also in animal husbandry as a growth promoter.

It is effective against all intestinal and caecal coccidia if administered continuously in feed, but it should not be administered to laying hens. It is administered in feed at doses between 27 and 72ppm, often in conjunction with other antimicrobials such as nicarbazin and bacitracin, or bambermycin and roxarsone.

- **Toltrazuril:**

Toltrazuril is a triazinetrione derivative used as a coccidiostat in food producing animals. It is a suspected teratogen and has an extended withdrawal period.

Toltrazuril is effective against Eimeria at all intracellular development stages and is thought to interfere with nuclear division and mitochondrial activity. Toltrazuril is administered in drinking water at a dose of 10-25ppm in chickens, turkeys and rabbits.

Coccidiostat toxicity

In recent years concerns of chemical residues in the food stream has become very topical, and this includes concerns over coccidiostats which may be present in poultry tissue. It is thought feed contamination could also be a contributing factor.

The problems related to coccidiostats being present in our foodstuffs. Maximum Residue Levels have been established for the majority of these compounds. It is highly recommended that with these drugs, an appropriate withdrawal time is observed prior to slaughter and consequently this should result in no risk of toxicity in the consumer. Testing for these compounds in foodstuffs should be carried out.

Testing platforms

Biochip array technology based on ELISA test principles allows for the simultaneous detection of multiple compounds of drugs from a single sample. The anthelmintics testing array can detect 25 anthelmintic compounds from a single sample detecting both parent and metabolite forms of the compounds.

These include albendazole sulphoxide and albendazole–2-aminosulphone, both of which are the main residues detected in tissue matrices irrespective of whether animals were dosed with netobimin, albendazole or albendazole sulphoxide.

The Evidence Investigator Coccidiostats Array will quantitatively test for lasalocid, nicarbazin, imidocarb, toltrazuril, maduramicin, nifursol metabolite, salinomycin, clopidol, monensin, robenidine, decoquinate, halofuginone and diclazuril simultaneously.

Data indicates applicability of this biochip array to the multiplex determination of coccidiostat and anthelmintic residues, which is relevant for the screening of these compounds in foods to ensure compliance with regulatory MRLs. This multiplex screening approach reduces time and cost spent testing samples for these residues.

The biochip testing platform uses the world's only multi-analyte semi-quantitative drug residue analyser; the Evidence Investigator. This multiplex platform is also available for an extensive list of antibiotics and growth promoting compounds using sample matrices including muscle, liver/kidney, seafood, milk, honey, urine and animal feed.

The multiplex testing format will reduce cost, run time and labour requirements – all of which guarantee a faster turnaround time for results along with a higher sample throughput. ■