

DETECTION OF ANTIMICROBIAL RESIDUES IN SLAUGHTERED ANIMALS IN AUSTRALIA

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INTRODUCTION

Antimicrobial drugs are used extensively in food producing animals as therapeutic and prophylactic agents to maintain optimum health, as growth promotants and to increase feed efficiency. From a veterinary public health point of view these applications may lead to unacceptable residues of the parent drug and/or metabolites in edible products derived from these animals. This is especially so if the recommended usage, dosage and withholding periods are not observed.

To reduce the incidence of violative residues in edible animal products, it is essential that regulatory authorities legislate to govern the use of drugs; provide monitoring and surveillance testing programs to ensure these regulations are obeyed; implement deterrents for malpractice; and restrict the entry of adulterated products into the food chain. These measures should be coupled with education and awareness programs for veterinarians, animal husbandry extension officers, producers and others associated with the livestock and processing industries.

MONITORING PROGRAMS

Monitoring programs obtain information on the frequency, levels and trends of residues in various classes of livestock. They identify and predict specific residue problems where education or other action may be required. These programs are also used to identify producers who market animals with violative levels of residues. Programs are generally established using a statistically based sample size with samples collected at random from apparently healthy animals at slaughter and analysed for various residues. The sampling rates may be increased if a problem area exists or is perceived to exist. A similar sampling procedure may be used by countries importing meat products. This involves the random sampling and testing of product at the port of entry.

In Australia, the National Residue Survey monitors for a wide range of residues in food products destined for export and domestic markets. This program is conducted by the Commonwealth Department of Primary Industries and Energy (DPIE). Residues of antimicrobial drugs in slaughtered animals has been monitored at export registered abattoirs since 1975. Each month the DPIE abattoir inspection staff collect a number of samples from certain categories of livestock and forward the samples to the Australian Government Analytical Laboratories (AGAL) for testing. During 1988 approximately 5,400 samples of cattle, sheep, pig, poultry and horse kidneys will be tested for inhibitory substances; 1,500 samples of cattle and pig livers for sulphonamides; and 1,160 samples of cattle, sheep, pig and poultry meats for chloramphenicol. Because of time constraints in sampling, transport and testing, sampled carcasses are not

retained until results are available, but are allowed to enter the food chain.

Kidney samples are screen tested for a broad spectrum of bacterial growth inhibitory substances by the agar diffusion method on agar plates seeded with suitable antimicrobial susceptible organisms. Positive samples are further tested with various combinations of media, susceptible and resistant bacteria and penicillinase. Characteristic profiles of results indicate the residues are consistent with particular drug groups. The samples are then bioassayed on agar plates with standard concentrations of a drug from that group and the results are expressed as units of activity for that drug. Liver samples are screen tested for sulphonamides by the qualitative thin layer chromatography (TLC) method. Positive liver samples are further quantified by high pressure liquid chromatography (HPLC). Meat samples are tested for chloramphenicol by HPLC.

Test results and all identifying information will be entered in the National Residue Database. The relevant State regulatory authorities are notified of residues detected at levels near to or exceeding the maximum residue level (MRL). All samples identifiable to the property of origin are traced back and appropriate action taken. The program is regularly modified according to results of monitoring during previous years. @SUBTITLE = SURVEILLANCE PROGRAMS

Surveillance programs are designed to test samples from slaughtered animals suspected or known to have been recently exposed to antimicrobial agents and to prevent products containing violative levels of residues from entering the food chain. The sampling is biased and is directed at particular carcasses or categories of animals in response to information from the national monitoring program, importing countries monitoring programs, research projects, or from observations during ante-mortem or post-mortem inspection.

The DPIE has recently introduced surveillance testing of calves at export abattoirs. During each day's slaughter a minimum of 50 per cent of calves up to 70 kg dressed weight is required to be randomly sampled. However, processors may sample at a higher rate. Samples of urine from the bladders are collected in evacuated containers, identified with the carcasses and tested by the processor using the agar diffusion method at on-plant laboratories. Calves which are positive to the urine test are retained and meat samples are tested on-plant by the same method. Livers and kidneys from carcasses testing positive to the urine test are condemned or diverted to animal food unless tested to prove that they do not contain residues.

Carcasses and correlated remaining offal are passed for human consumption if negative to the meat test. If the meat test is positive then all tissues are condemned or diverted to animal food.

The surveillance program will soon be extended to all categories of pigs and other at risk animals such as dairy cows, emergency and restricted (suspect) slaughter animals. This program will change with prevalence of violations in the various categories of animals and with international trade requirements.

The identification of animals condemned on the meat test, and the urine screen test results if requested, are forwarded to the relevant State authorities for traceback and follow up action to the producer. In certain instances the positive tissue sample will undergo confirmatory testing by physicochemical methods to identify and quantify the residue present.

RECENT DEVELOPMENTS

1. Animal Identification

An efficient animal identification system is essential in any testing program at abattoirs. Efficient identification allows rapid traceback of animals and carcasses to the property of origin. Since 1987, the DPIE has required all bovine and porcine animals to be identified by before entry to a registered establishment for slaughter. Each cattle property in Australia has a registered property number and cattle delivered from that property are identified with tail-tags bearing that number. All pig producers are issued with a tattoo number for animal identification. Property identification for other classes of livestock such as sheep and horses is being investigated and will be introduced as soon as practicable.

2. Sample Collection

(a) Urine

Urine samples are collected in sterile evacuated containers from the bladder of animals during or after slaughter evisceration. The evacuated containers are 10 ml plain or silicone coated. Experienced operators may obtain samples (minimum 3 drops) from up to 97 per cent of calves. The labelled containers are correlated with the carcass identification and placed in a polystyrene stand which contains 100 samples. This collection method is simple, rapid and efficient.

(b) Tissue

For the monitoring program, a DPIE computer generated random sampling system creates a monthly program for abattoir selection, number and type of samples within each category of livestock, date of collection and time period within the day for sample collection. The tissue samples are identified with the property of origin where this information is available. The frozen samples are forwarded to AGAL in Sydney, Melbourne and Adelaide for analysis of inhibitory substances, to Perth for sulphonamides, and to Hobart for chloramphenicol.

In the surveillance program, if the urine screen sample is positive then a meat tissue sample, approximately 30 g, is collected in a plastic bag as hygienically as possible. To assist in the production of meat fluid, the sample may be frozen then thawed in an incubator or water bath with a maximum temperature of 37°C. Samples of meat fluid are tested as soon as practicable to overcome the problem of bacterial contamination.

3. On-Plant Antibacterial Residue Testing

The DPIE in conjunction with Medical Diagnostics, a commercial laboratory, has developed separate on-plant test kits for testing antibacterial residues in urine and

meat fluid. Each kit is self-contained, capable of testing 200 samples and has a shelf life of 6 months. A freeze dried pellet of *Bacillus subtilis* is reconstituted in nutrient broth and swabbed on the surface of the plate. Sterile filter paper discs (13 mm diameter) are impregnated by touching the surface of urine or meat fluid. Four sample discs are placed on the plate together with a 10 µg neomycin control disc in the centre. The plates are incubated at 30°C for up to 18 hours. The concentration of bacteria in the reconstituted broth and agar media is standardised to produce a constant zone of inhibition for the control disc thus ensuring constant sensitivity of the test. The test kits are capable of detecting most commonly used veterinary antimicrobial drugs, including sulphonamides, in spiked samples of urine and meat fluid at or below 1 µg/ml.

Processors may use the same test kits or equivalent test kits or methods. Australian Quarantine and Inspection Service (AQIS) veterinary officers at export abattoirs use the DPIE test kits as part of a quality assurance program to monitor random duplicate samples previously tested by the processor. In future, veterinary officers will conduct residue differentiation tests on urine samples correlated with positive meat fluid tests. Differentiation tests for penicillins, sulphonamides and chloramphenicol will be conducted by the addition of penicillinase, para-aminobenzoic acid (PABA) and beta-glucuronidase solutions to sample discs on agar plates.

4. National Residue Database

This consists of a computer database conducted by DPIE which will record residue information on all species and properties throughout Australia. Also included will be a wide range of residue technical information. At present the database contains all the information on the recently intensified organochlorine pesticide testing program and in future will contain the National Residue Survey information. In future the database will also include information on the on-plant surveillance testing program.

CONCLUSION

The Department of Primary Industries and Energy has been conducting an efficient and effective monitoring program for antimicrobial residue detection which has indirectly reduced the violations of maximum permitted residue levels in animal food products destined for both domestic and export markets. The recently introduced on-plant surveillance testing program will be an integral part of an efficient and scientifically based inspection service.

The monitoring and surveillance programs have been aided by recent developments in animal identification, sample collection, on-plant test kits and data recording systems.

The relevant State regulatory authorities are notified when residues close to or in excess of legal limits are detected by the monitoring and surveillance programs so that each case can be investigated and appropriate action taken.