Monitoring of Domestic and Imported Eggs for Veterinary Drug Residues by the Canadian Food Inspection Agency

Dugane J. Quon[†]

Calgary Food Laboratory, Canadian Food Inspection Agency, 3650 36 Street N.W., Calgary, Alberta, Canada T2L 2L1

The Canadian Food Inspection Agency (CFIA) routinely monitors the Canadian food supply to ensure that the levels of antibiotic residues are below the stated regulatory guidelines. Over a two-year period, both domestic and imported eggs in Canada were analyzed for a number of veterinary drugs. These included chloramphenicol, β-lactams, fluoroquinolones, halofuginone, macrolides, sulfonamides, tetracyclines, decoquinate, and coccidiostats. More than 99% of the samples screened were found to be free of any veterinary drug residue. The remainder were found to contain tetracyclines, sulfonamides, ciprofloxacin, macrolides, streptomycin, clopidol, ethopabate, and nitromide. Current methods used for the analysis of these residues are discussed.

Keywords: *Antibiotics; eggs; chloramphenicol; lactams; fluoroquinolones; sulfonamides; tetracyclines; coccidiostats; decoquinate*

INTRODUCTION

The use of veterinary drugs is widespread in the agriculture industry of today. These drugs are used for a number of reasons, including to prevent or cure disease, to reduce the potential for disease, or as a growth promoter to increase feed conversion. With the prevalent use of antibiotics in food-producing animals, there is a significant risk of drug residues remaining in the final product. The level of any residue remaining in the edible portion is dependent on a number of factors, such as excretion, metabolism, adsorption, and distribution of the drug. The rate at which these occur determines the concentration and the chemical nature of the drug (Turnipseed, 1998). Other major concerns arising from the presence of antibiotic residues in the food supply include the following: adverse reactions, such as allergic reactions to β-lactams (Ponvert et al., 1999) or streptomycin (Tinkelman and Bock, 1984); drug-resistant bacteria transferring from food to humans (Wegener et al., 1999); or illness in humans, such as aplastic anemia from chloramphenicol (Settepani, 1984). In addition to immediate adverse effects, there are also long-term effects to the exposure of low levels of residues that remain unknown.

The need exists to monitor for the presence of veterinary drug residues in animal food products, such as eggs. The Canadian Food Inspection Agency (CFIA), as part of its mandate, monitors for the presence of antibiotic residues in the egg supply available for consumption in Canada, both domestic and imported. The monitoring program is set up to screen for the presence of chloramphenicol, β-lactams, fluoroquinolones, halofuginone, macrolides, sulfonamides, tetracyclines, decoquinate, and coccidiostats. The CFIA has screened >3500 samples of eggs and egg products for these veterinary drugs over a period covering April 1997 through July 1999.

[†] E-mail quond@em.agr.ca; telephone (403) 299-7633; fax (403) 221-3293.

Chloramphenicol is a broad spectrum antibiotic with high activity against several microorganisms. The majority of reported cases of drug-induced aplastic anemia, one of the few life-threatening reactions to drugs, have been associated with chloramphenicol (Malkin et al., 1990). The mortality rate may be as high as 50−70%, and patients who survive may develop acute leukemia; therefore, chloramphenicol is completely banned for use in all food-producing animals.

The β-lactam class of antibiotics includes a large variety of natural and semisynthetic compounds and reportedly have the most frequent cases of adverse allergic reactions in children (Kamada, 1991). Penicillin G is widely used in the treatment of farm animals, primarily because it is widely available and inexpensive. There have been a number of documented cases of an allergic reaction from penicillin residues in milk being triggered in previously sensitized individuals (Dewdney, 1991). Due to the potential widespread use and the reactions of hypersensitive individuals, regulatory requirements for the residues of β-lactams must be kept very stringent to minimize the potential exposure of low-level residues to these people. The macrolides class of antibiotics are primarily bacteriostatic and most effective against Gram-positive organisms. Erythromycin and tylosin, for example, may also be used as growth promoters in animal feed. Very few of these drugs have elicited allergic reactions in individuals (Dewdney, 1991).

Sulfonamides and tetracyclines are used extensively in food-producing animals, both therapeutically and prophylactically. Adverse effects from the use of the sulfonamide class of drugs has been observed, including allergic reaction and blood and liver disorders (Bjorkman, 1991). Widespread resistance to these drugs is quite common, and their residues need to be limited in the food supply.

Halofuginone, alkomide, nitromide, zoalene, ethopabate, dinsed, clopidol, and decoquinate are coccidiostats that are most commonly used prophylactically via

10.1021/jf000144x CCC: $19.00 Published 2000 by the American Chemical Society
Published on Web 12/01/2000

animal feeds to prevent a costly outbreak of coccidiosis. In poultry, coccidiosis may lead to poor weight gain, reduced egg production, and untimely death (Kennedy, 1996). The quinolones are a group of drugs with a broad antibacterial spectrum; enrofloxacin has been bound to be effective for the treatment of various bacterial infections of food-producing animals (Jordan et al., 1993).

MATERIALS AND METHODS

Chloramphenicol and 1-heptanesulfonic acid were obtained from Aldrich (Milwaukee, WI). Penicillin G, streptomycin sulfate, sodium sulfathiazole sequihydrate, sulfamerazine, tetracycline, oxytetracycline, and chlortetracycline were obtained from Sigma (St. Louis, MO). Enrofloxacin and ciprofloxacin were obtained from Bayer (Etobicoke, ON). Halofuginone hydrobromide was obtained from Hoechst Roussel Vet (Clinton, NJ). Sulfamethazine was obtained from Pfizer (London, ON). Clopidol and erythromycin were obtained from Rhone Merieux (Baie d'Urfé, PQ). Alkomide, dinsed, ethopabate, nitromide, zoalene, and decoquinate were gifts from Lab Services Ottawa, Canadian Food Inspection Agency (Ottawa, ON). CHARM II test kits were obtained from Charm Sciences Inc. (Malden, MA). Ethyl acetate, methanol, hexane, cyclohexane, acetonitrile, chloroform, ethyl alcohol, NaCl, glacial acetic acid, hydrochloric acid, and ammonium hydroxide were obtained from Caledon Laboratory Chemicals (Edmonton, AB). Ammonium acetate was obtained from Fluka (Oakville, ON). Sodium acetate, formic acid, and metaphosphoric acid were obtained from BDH, Inc. (Toronto, ON). Orthophosphoric acid was obtained from Fisher Scientific Canada (Nepean, ON). Fluorescamine was obtained from Regis Technologies, Inc. (Morton Grove, IL). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) was obtained from Supelco, Inc. (Oakville, ON).

Egg samples for β-lactams, macrolides, tetracyclines, streptomycin, and sulfonamides residues were screened using the CHARM II test (Charm Sciences, 1998). Ten-gram sample portions were weighed into 50-mL polypropylene centrifuge tubes. These samples were heated to 100 °C for 6 min followed by the addition of 30 mL of extraction buffer. After the samples had been homogenized for 60 s (Polytron, Brinkmann Instruments Canada Ltd., Mississauga, ON) and centrifuged 10 min at 2500*g* using a Mistral 3000E (Fisher Scientific Ltd., Nepean, ON), the supernatant was transferred to a clean tube. Binding tablet were added to a set of 13 × 100 mm test tubes, followed by 0.3 mL of water. For β-lactams, tetracyclines, streptomycins, and sulfonamides, 2 mL of extract was added (4 mL for macrolides) and heated for 2 min (55 °C for β-lactams and macrolides; 35 °C for streptomycins and sulfonamides). A tracer tablet was added to each test tube and heated for 2 min (5 min for tetracyclines) at the same temperature. The extracts were centrifuged at 1500*g* and decanted. A 3-mL portion of scintillation fluid was added to the test tube after the pellet was broken up by mixing with 0.3 mL of water. Test tubes were read on a scintillation counter (Charm Sciences Inc.) for 1 min.

Chloramphenicol analysis (Health of Animals Laboratory, 1996a) was done by the addition of 0.1 mL of *m*-chloramphenicol (500 ng/mL), as an internal standard, to 5 g of egg in a 50-mL polypropylene centrifuge tube. Following the addition of 30 mL of ethyl acetate, the samples were mixed vigorously and centrifuged for 6.5 min at 1400*g*. The ethyl acetate was transferred to another centrifuge tube, while the aqueous portion was re-extracted twice with 15 mL of ethyl acetate. The combined organic extracts were evaporated to dryness at 60 °C. The residue was dissolved in 4% aqueous NaCl, followed by the addition of 10 mL of hexane. Samples were shaken for 2 min and centrifuged for 5 min at 1400*g*. Hexane was removed, discarded, and extracted twice more. Next, 10 mL of ethyl acetate was added to the aqueous portion, shaken, and centrifuged. The ethyl acetate was transferred to another centrifuge tube and the aqueous portion extracted twice more with ethyl acetate. The combined extracts were evaporated to <1 mL at 60 °C and derivatized using 0.2 mL of BSA for 20 min at 65 °C. After excess reagent had been evaporated off, the extract was made up to 1 mL with 60:40 cyclohexane/hexane. A 1-μL aliquot was injected onto an HP 5890 GC with electron capture detector (Agilent Technologies, Montreal, PQ).

Analysis of clopidol (Health of Animals Laboratory, 1996b) was performed by weighing 5 g of an egg sample, mixed with 25 mL of acetonitrile, and the extract was centrifuged for 10 min at 2500*g*. The supernatant was passed through a 1-g basic alumina Mega Bond Elut column (Varian Canada, Inc., Mississauga, ON) and a Bio-Rad AG1-X8 anion exchange column [Bio-Rad Laboratories (Canada) Ltd., Mississauga ON] in series. The columns were washed with 10 mL of methanol and allowed to run dry. The alumina SPE column was discarded, and the anion exchange column was eluted with 12 mL of 1% acetic acid in methanol and collected. The eluate was taken to near dryness at 65 °C and reconstituted with 2 mL mobile phase. The aliquot was filtered through a Millex-HV 0.45-μm filter (Millipore, Bedford, MA), and 50 μL was the injection volume. The HPLC mobile phase was 10:90 methanol/water with a Supelcosil LC-8-DB column (5 μm, 50 mm × 4.6 mm i.d., Supelco, Inc., Bellefonte, PA) and ran under isocratic conditions with a flow rate of 0.5 mL/min. The HPLC system was an HP1090L system controlled by HP ChemStation software (Agilent Technologies) with the detector set 267 nm.

Alkomide, nitromide, zoalene, ethopabate, and dinsed were analyzed together by HPLC (Laboratory Services Division, 1995). Samples were prepared by weighing 1 g of sample, mixed with 10 mL acetonitrile, and centrifuged for 10 min at 2500*g*. The supernatant was passed through C18 SPE tubes (500 mg, Mega Bond Elut, Varian Canada, Inc.) and collected in 15-mL centrifuge tubes. The eluate was evaporated to near dryness at 65 °C, reconstituted in 1 mL of 20% acetonitrile, and filtered through a Millex-HV 0.45-μm filter (Millipore). A 100-μL aliquot was injected onto an HP1090L system controlled by HP ChemStation software (Agilent Technologies). The initial mobile phase was 20:80 acetonitrile/water, which was ramped to 80:20 acetonitrile/water over 16 min and then returned to the initial conditions. Separation was achieved on a Zorbax SB-C18 (3.5 μm, 150 mm × 4.6 mm i.d., Agilent Technologies) with a flow rate of 1 mL/min and detection at 260 nm.

The determination of decoquinate (Health of Animals Laboratory, 1996c) was performed by weighing 5 g of homogenized sample and mixing with 15 mL of 4:1 methanol.chloroform. After the sample had been centrifuged for 5 min at 2500*g*, the supernatant was transferred to another centrifuge tube. The extraction was repeated and the combined extracts were shaken with 15 mL of 5% metaphosphoric acid and 4 mL of chloroform, followed by centrifugation at 2500*g* for 5 min. The aqueous portion was discarded, and a 2-mL portion of the organic layer was transferred to a test tube and taken to dryness at 60 °C. The residue was dissolved in 2 mL of mobile phase and placed at −15 °C for 15 min to solidify unwanted fat. After the sample had been centrifuged, it was passed through a Millex-HV 0.45-μm filter (Millipore). A 50-μL aliquot was injected onto an HP1090L system controlled by HP ChemStation software (Agilent Technologies). The mobile phase was composed of 0.025 M calcium nitrate tetrahydrate, acetonitrile, and methanol in a ratio of 13:4:83. Chromatography was done on a Zorbax SB-C18 (5 μm, 250 mm × 4.6 mm i.d., Agilent Technologies) with an isocratic flow rate of 1.0 mL/min and fluorescence detection with an excitation wavelength of 328 nm and emission wavelength of 410 nm.

Halofuginone was analyzed using modifications of a method obtained from the U.S. Department of Agriculture (1991) and Beier et al. (1994). After 3 g of sample had been mixed with 9 mL of acetonitrile, the solution was centrifuged for 5 min at 2500*g*. Next, the supernatant was decanted into a flask containing 50 mL of NaCl (4%) solution and mixed. The extract was passed through a C18 column (500 mg, Bond Elut, Varian Canada, Inc.) preconditioned with 3 mL of methanol and 3 mL of H_2O. After the column had been dried by drawing air through it, 2 mL of ethyl acetate was slowly passed through

to waste. The column was again dried and eluted with 3 mL of methanolic buffer solution (2:1 methanol/0.25 M ammonium acetate). After the eluate had been evaporated to <1 mL at 60 °C, the volume was readjusted to 1 mL with mobile phase and filtered through a Millex-HV 0.45-μm filter (Millipore). The mobile phase used in the separation was made of 5:3:12 acetonitrile/0.25 M ammonium acetate buffer, pH 4.3/H_2O. A 100-μL volume was injected onto an HP1090L system controlled by HP ChemStation software (Agilent Technologies). Analysis was done on a Zorbax SB-C18 column (5 μm, 250 mm × 4.6 mm i.d., Agilent Technologies) with an isocratic flow rate of 1 mL/min and UV detection at 243 nm.

The monitoring of the quinolone class of antibiotics comprising enrofloxacin and ciprofloxacin (Health of Animals Laboratory, 1996d) was done by shaking 2 g of egg sample with 20 mL of acidic ethanol (1% acetic acid in ethanol) for 10 min, followed by centrifugation at 2500*g* for 5 min. The extraction was repeated, and the combined extracts were passed through an SCX SPE column (500 mg, BakerBond, J. T. Baker, Phillipsburg, NJ) that had been preconditioned with 5 mL of acidic ethanol. The SPE column was washed with 5 mL of methanol and dried with air for 5 min. Next, the analytes were eluted from the column with 5.5 mL of ammoniated methanol (30% ammonium hydroxide in methanol). The eluate was evaporated to <1 mL at 60 °C and adjusted to 2 mL with mobile phase and filtered through a Millex-HV 0.45-μm filter (Millipore). The mobile phase was made up of 20:80 acetonitrile/0.05 M sodium phosphate and 0.0025 M 1-heptanesulfonic acid, pH 2.4. Separation was done on a Zorbax SB-C18 column (5 μm, 250 mm × 4.6 mm i.d., Agilent Technologies) with an isocratic flow rate of 1.0 mL/min and fluorescence detection with an excitation wavelength of 278 nm and emission wavelength of 444 nm. A 50-μL aliquot was injected onto an HP1090L system controlled by HP ChemStation software (Agilent Technologies).

Samples that were found to be positive from the CHARM II test were further analyzed using two other HPLC methods. The first used HPLC using precolumn derivatization and fluorescence based on a method by Takeda et al. (1991). After a 5-g sample portion was shaken with 10 mL of 0.1 N HCl, a 0.4-mL aliquot was mixed with 0.6 mL of 24% methanolic trichloroacetic acid (TCA). After 1.0 mL of acetate buffer (0.8 M) had been added, the solution was filtered through an Acrodisc GHP filter (0.45 μm, 25 mm, Pall Gelman Laboratory, Mississauga, ON). Next, 0.2 mL of fluorescamine was added, and 100 μL was injected 30 min after derivatization onto a Waters HPLC system comprising a 717 autosampler, a 600E pump, and a 474 detector controlled by Millennium^{32} software (Waters Corp., Milford, MA). The mobile phase was made up of 3:5 acetonitrile/acetic acid (2%), and chromatography was done on a Supelcosil LC18DB column (5 μm, 250 mm × 4.6 mm i.d., Supelco, Inc.) with a flow rate of 1.0 mL/min and a column temperature of 55 °C. Detection was done at an excitation wavelength of 405 nm and an emission wavelength of 495 nm.

The next step was identifying the positive samples on the LC/MS, this was done by modifying a method by Takatsuki et al. (1990). A 5-g portion of egg sample was mixed with 5 mL of acetonitrile and internal standard (sulfamethoxypyridazine). Next, the extract was centrifuged for 5 min at 1000*g* and decanted into a 15-mL centrifuge tube. This was repeated with a second portion of acetonitrile, and the extracts were combined. The acetonitrile layer was then salted out using NaCl and subsequently passed through dried $NaSO_4$ and a silica Sep-Pak (500 mg, Waters Corp., Milford, MA) and washed with 5 mL of acetonitrile. The eluate was collected and evaporated to dryness at 40 °C and reconstituted with 1 mL of 0.1 M acetic acid. The extract was filtered with a Millex-HV 0.45-μm filter (Millipore), and 100 μL was injected onto an HP1100 LC/MSD (Agilent Technologies). The mobile phase consisted of 15:20:65 acetonitrile/0.4% formic acid/water with a flow rate set at 0.5 mL/min. The column temperature was 35 °C, and the mass spectrometer was set up to operate in positive atmospheric pressure ionization electrospray (API-ES) mode. The capillary voltage was set at 4500 V, fragmentor at 75 V, gain at 2.0, gas temperature at 350 °C, and the drying gas at 10 L/min. The mass spectrometer was operated in single ion monitoring (SIM) mode, and the ions monitored were 156, 265, and 287 for sulfamerazine and 156, 186, 279, and 301 for sulfamethazine. The internal standard (sulfamethoxypyridazine) was monitored for the 281 ion.

Table 1. Summary of Drug Residue Results

veterinary drug	no. of samples	no. of positives	no. of confirmed positives
chloramphenicol	203	0	0
β-lactams	386	0	0
enrofloxacin	262	0	0
ciprofloxacin	262	1	0
halofuginone	268	0	0
macrolides	386	2	–[a]
streptomycins	386	1	–
sulfonamides	383	10	1
tetracyclines	386	9	–
clopidol	110	2	–
alkomide	90	0	0
dinsed	90	0	0
ethopabate	90	2	–
nitromide	90	6	–
zoalene	90	0	0
decoquinate	87	0	0
total	3569	33	

[a] Not confirmed.

Table 2. Detection Limits of Charm II Test in Eggs

drug family	antimicrobial drug	concn in egg (ng/g)
β-lactams	penicillin g	50
macrolides	erythromycin	500
tetracyclines	chlortetracycline	200
streptomycins	streptomycin	500
sulfonamides	sulfamethazine	50

RESULTS AND DISCUSSION

The CFIA had screened >3500 samples over a two-year period encompassing April 1997 through July 1999 for veterinary drug residues, and 33 of them tested either presumptive positive or positive (See Table 1). This represents just under 1% of all the samples tested. Of these, 203 were analyzed for chloramphenicol, and none were found to be positive, whereas β-lactams, macrolides, streptomycins, and tetracyclines were tested in 386 egg samples. There were no positives found for β-lactams, but two samples for macrolides, one for streptomycin, and nine for tetracyclines were found. At the time of writing these were still awaiting further confirmation. Of the coccidiostats, halofuginone, alkomide, dinsed, zoalene, and decoquinate were all found to be negative. Two egg samples containing clopidol and ethopabate were found, in addition to six samples with nitromide, in the initial screen. Of the 262 samples tested for enrofloxacin and ciprofloxacin, only 1 was a presumptive positive for ciprofloxacin. This had not been analyzed by a confirmatory technique at the time of writing.

The antibiotic screen performed using the Charm II test included the analysis of β-lactams, tetracyclines, sulfonamides, streptomycin, and macrolides. The antimicrobial drug used and detection limits achieved for the Charm II test in eggs are listed in Table 2. For β-lactams and sulfonamides, the CHARM II test was able to detect positives as low as 50 ng/g while detecting chlortetracycline at 200 ng/g and streptomycin and

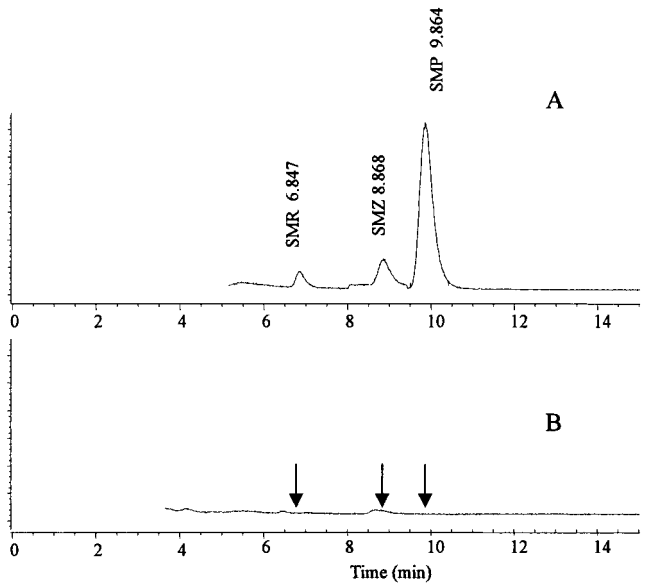

Figure 1. Typical chromatograms of a 100-μL injection of an egg sample: (A) egg spiked with 20 ng/g sulfamerazine (SMR) or sulfamethazine (SMZ) with sulfamethoxypyridazine as internal standard; (B) egg blank.

macrolides at levels of 500 ng/g. The high detection levels for streptomycin and macrolides, although sufficient for current monitoring purposes, may lead to a number of false negatives in the egg samples analyzed. Further work is required to develop a more sensitive method for the screening of these analyses. These detection levels need to be lowered to get a more accurate determination of the amount of drug residues being carried through the food supply. The CHARM II test did not detect any positives for β-lactams of the 386 samples that were analyzed. The tetracycline screen detected 9 positives, 2 were detected in the macrolides screen, and there was one sample that tested positive for streptomycin of 386 samples tested. None of these had been analyzed by a confirmatory technique at the time of writing. The CHARM II test for sulfonamide was positive in 10 samples of the 383 tested.

Sulfonamides showed the greatest number of potential positives in the egg samples analyzed during the reporting period. This was possibly due the prevalent use of the drug in the poultry industry. However, upon further analysis of these samples, only one was found to be positive following confirmation by two other methods. Samples found to be positive using the CHARM II test were subjected to further testing. Initially, these samples were analyzed using fluorescence detection based on a method using precolumn derivatization with fluorescamine. Although this method was able to determine that a majority of the egg samples did not contain any sulfonamide residue, a number were found to possibly contain some sulfonamide residues, namely, sulfamethazine and sulfamerazine. This was able to provide some preliminary identification of the possible residues present in the sample based on retention time. However, because a small number of sulfa drugs coeluted under these conditions, they required further identification; for example, it was found that sulfathiazole, sulfadiazine, and sulfapyridine coeluted with one another. To further evaluate, these samples were analyzed on a LC/MSD to positively confirm the presence of sulfa drugs in the egg samples. Figure 1 shows a chromatogram of a 20 ng/g spike of both sulfamethazine

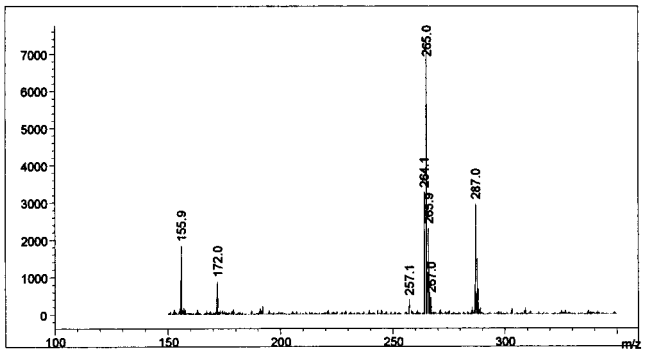

Figure 2. Mass spectrum of sulfamerazine.

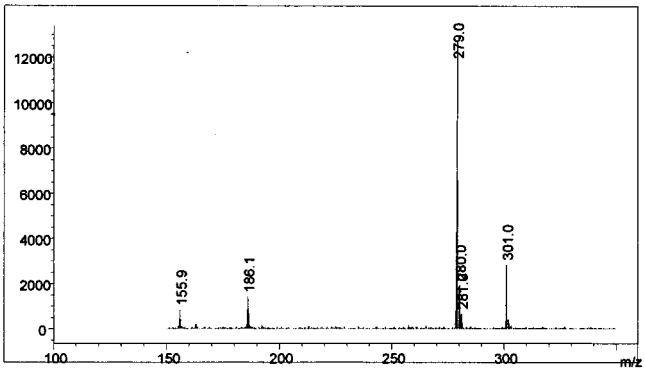

Figure 3. Mass spectrum of sulfamethazine.

and sulfamerazine in egg with sulfamethoxypyridazine as the internal standard. The 156 ion was the result of the $[H_3NPh - SO_2]^+$ group fragmenting off, and the 172 and 186 peaks were the result of the $[RSO_2]^+$ group for sulfamerazine and sulfamethazine, respectively. The other ions observed were the molecular ion $[M + H]^+$ and the sodium adduct of the molecular ion $[M + Na]^+$. Figure 2 shows the mass spectrum obtained from sulfamerazine; the major ions were found to be 156, 172, 265, and 287. For sulfamethazine, the major ions found were 156, 186, 279, and 301 (Figure 3). Following the analysis on the mass spectrometer, there was only one sample that was found to contain any sulfonamide residue above the detection limit of 5 ng/g. This sample contained sulfamethazine at a level below the quantitation limit of 15 ng/g.

There were no samples detected to contain any residues of halofuginone during the period of April 1997 to July 1999. Figure 4 shows a typical chromatogram of a blank egg sample and one with 100 ng/g of halofuginone. The limit of detection of the method is 14 ng/g with a quantitation limit of 43 ng/g. Over this same period, there have been no violations found for the presence of chloramphenicol. The limit of detection (LOD) is found to be 0.3 ng/g with a limit of quantitation (LOQ) of 1.0 ng/g. Figure 5 shows a typical chromatogram of a blank egg sample and one with 3.1 ng/g of chloramphenicol.

The determination of fluoroquinolones, specifically enrofloxacin and ciprofloxacin, is completed by HPLC with fluorescence detection. Their tolerance levels have not yet been established, so they are currently subjected to zero tolerance and no measurable amount of residues is permitted in eggs. The LOD and LOQ for this method are 24 and 73 ng/g for enrofloxacin, respectively, and 11 and 32 ng/g for ciprofloxacin, respectively. During the reporting period there were no samples found to contain any residues of these fluoroquinolones. There

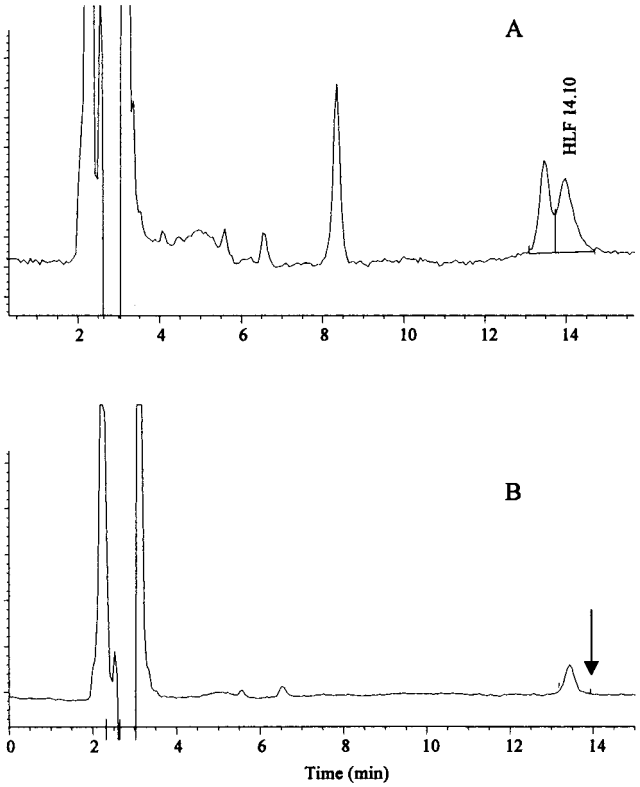

Figure 4. Typical chromatograms of a 100-μL injection of an egg sample: (A) egg spiked with 100 ng/g halfuginone; (B) egg blank.

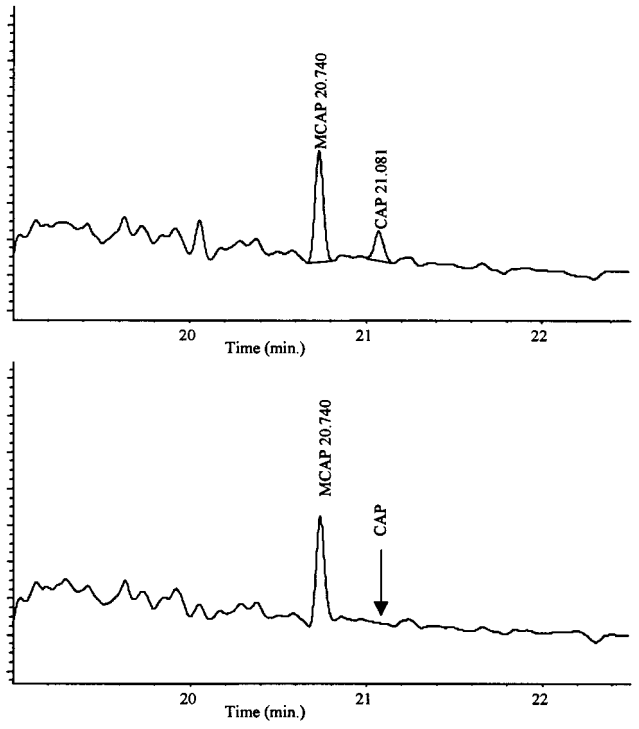

Figure 5. Typical chromatograms of a 1-μL injection of an egg sample: (A) egg sample spiked with 3.1 ng/g chloramphenicol (CAP) and internal standard *m*-chloramphenicol (MCAP); (B) egg blank.

was one sample that was initially found to be presumptive positive but was not confirmed by another analytical technique. Figure 6 shows a typical chromatogram of a blank egg sample and one with 100 ng/g of ciprofloxacin and 200 ng/g of enrofloxacin.

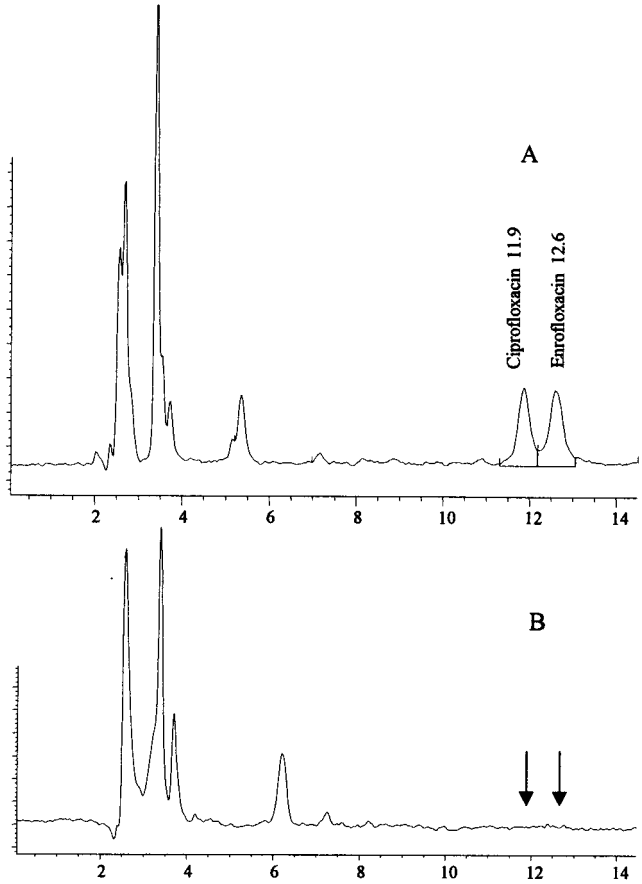

Figure 6. Typical chromatograms of a 50-μL injection of an egg sample: (A) egg spiked with 200 ng/g ciprofloxacin and 100 ng/g enrofloxacin; (B) egg blank.

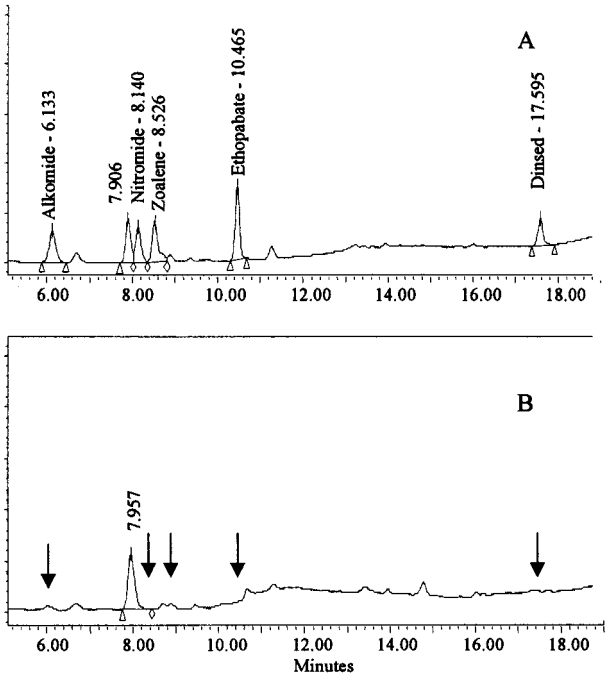

Figure 7. Typical chromatograms of a 100-μL injection of an egg sample: (A) egg spiked with 500 ng/g alkomide, nitromide, zoalene, ethopabate, and dinsed; (B) egg blank.

The five coccidiostat residues, alkomide, nitromide, zoalene, ethopabate, and dinsee, are analyzed in a multiresidue screening method by HPLC with UV detection. Figure 7 shows a typical chromatogram of a

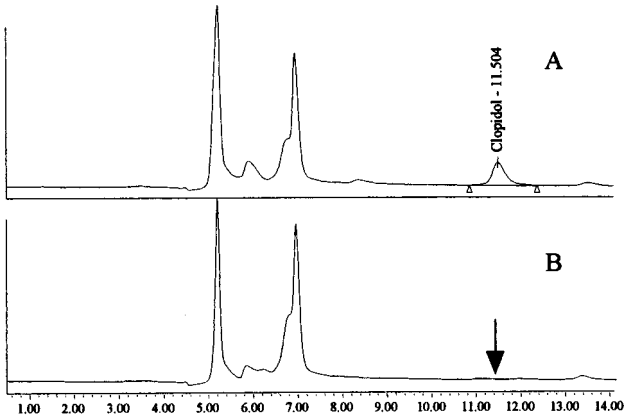

Figure 8. Typical chromatograms of a 50-μL injection of an egg sample: (A) egg spiked with 500 ng/g clopidol; (B) egg blank.

Table 3. LOD and LOQ for Drug Residue Analysis

drug	LOD (ng/g)	LOQ (ng/g)
alkomide	120	370
chloramphenicol	0.3	1
ciprofloxacin	11	32
clopidol	23	69
decoquinate	130	400
dinsed	120	350
ethopabate	110	320
enrofloxacin	24	73
halofuginone	14	43
sulfamethazine	5	15
sulfamerazine	5	15
nitromide	100	310
zoalene	100	310

blank egg sample and one with 500 ng/g of each coccidiostat drug. The LOD and LOQ for the coccidiostat drugs are given in Table 3. Of 90 samples analyzed, residues of ethopabate were found twice and nitromide was found 6 times.

Of the other coccidiostats, clopidol and decoquinate, there have not been any samples that have been found to contain these veterinary drug residues. Figure 8 shows a typical chromatogram of a blank egg sample and one with 500 ng/g of clopidol. The LOD for this method is 23 ng/g with an LOQ of 69 ng/g. The LOD and LOQ for the decoquinate method are 130 and 400 ng/g, respectively. The determination of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in eggs was performed by HPLC with photodiode array detection. Figure 9 shows a typical chromatogram of a blank egg sample and one with 600 ng/g decoquinate.

There were 33 samples that were potentially positive of 3569 samples analyzed during the period of April 1997 to July 1999. Upon further analysis, it was found that 55% (18 samples) of the positives detected were products imported from the United States (Table 4). There were no major differences observed in residues found in either the domestic or imported samples. The residues detected in the screening tests were virtually evenly split between samples that originated in Canada and those imported from the United States. The sample that was confirmed to contain sulfamethazine, albeit at an extremely low level (<15 ppb), originated in Manitoba. Other relationships concerning samples that were not confirmed at the time cannot be elaborated upon until the samples have undergone further analysis.

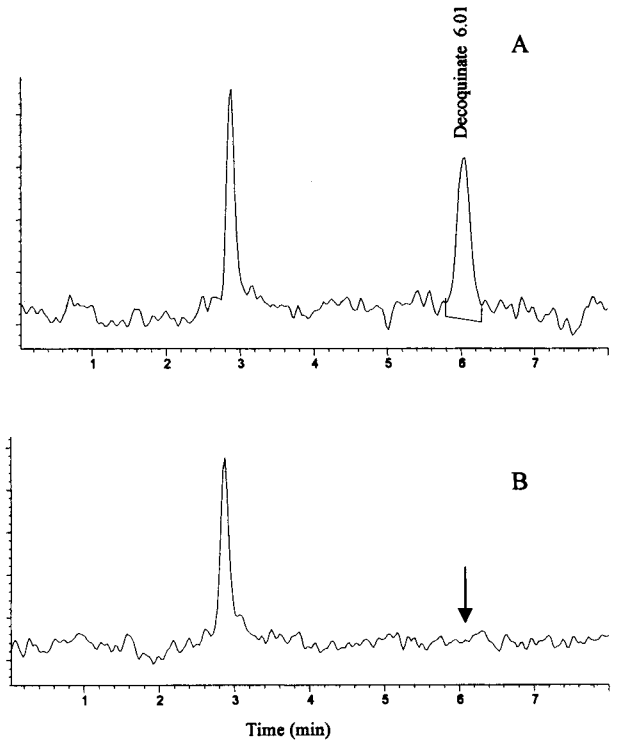

Figure 9. Typical chromatograms of a 50-μL injection of an egg sample: (A) egg spiked with 500 ng/g clopidol; (B) egg blank.

Table 4. Sample Origins of Positives and Presumptive Positives

drug residue	state of origin	drug residue	state of origin
clopidol	Maryland	ciprofloxacin	Alberta
ethopabate	Maryland	clopidol	Ontario
macrolides	Maine	ethopabate	Saskatchewan
macrolides	Minnesota	nitromide	Alberta
nitromide	Maine	nitromide	Alberta
nitromide	Maine	nitromide	Saskatchewan
nitromide	Minnnesota	sulfonamide	Alberta
streptomycin	Maine	sulfonamide	Alberta
sulfonamide	Maryland	sulfonamide	British Columbia
sulfonamide	Maryland	sulfonamide	Manitoba
sulfonamide	Maine	sulfonamide	Ontario
sulfonamide	Maine	tetracyclines	Manitoba
sulfonamide	not specified	tetracyclines	Manitoba
tetracyclines	Maryland	tetracyclines	Ontario
tetracyclines	Michigan	tetracyclines	Quebec
tetracyclines	Vermont		
tetracyclines	Vermont		
tetracyclines	Vermont		

ABBREVIATIONS USED

BSA, *N*,*O*-bis(trimethylsilyl)acetamide; CAP, chloramphenicol; SMR, sulfamerazine; SMZ, sulfamethazine; LOD, limit of detection; LOQ, limit of quantitation.

ACKNOWLEDGMENT

I am thankful for the contributions of Al Bass, Gwen Fernandes, and Dan Leung and for their dedication and hard work in analyzing the samples and their help in gathering the data. I thank the others in our unit, Fred Butterworth, Andrew Pearson, Rocke Wilton, and Peter Hartlieb, for their cooperation during this period of work.

LITERATURE CITED

Beier, R. C.; Rowe, L. D.; Abd El-Azaz Nasr, M. I.; Elissalde, M. H.; Stanker, L. H. Extraction and HPLC analysis of halofuginone in chicken serum. *J. Liq. Chromatogr.* **1994**, *17*, 2961−2970.

Bjorkman, A.; Phillips-Howard, P. A. Adverse reactions to sulfa drugs: implications for malaria chemotherapy. *Bull. W.H.O.* **1991**, *69* (3), 297−304.

Charm Sciences, Inc. CHARM II test for Antimicrobial Drugs in Eggs; Malden, MA, 1998.

Dewdney, J. M.; Maes, L.; Raynaud, J. P.; Blanc, F.; Scheid, J. P.; Jackson, T.; Lens, S.; Verschueren, C. Risk assessment of antibiotic residues of β-lactams and macrolides in food products with regard to their immuno-allergic potential. *Food Chem Toxicol*. **1991**, *29* (7), 477−483.

Health of Animals Laboratory. Determination of chloramphenicol in tissue by electron capture gas chromatography. *Methods Manual*; Centre for Veterinary Drug Residues, Health of Animals Laboratory: Saskatoon, SK, 1996a; version CHL-SP04 1996/11.

Health of Animals Laboratory. Determination of clopidol residues in poultry tissue. *Methods Manual*; Centre for Veterinary Drug Residues, Health of Animals Laboratory: Saskatoon, SK, 1996b; version CLP-SP05 1996/12.

Health of Animals Laboratory. Determination of decoquinate in animal tissue by HPLC. *Methods Manual*; Centre for Veterinary Drug Residues, Health of Animals Laboratory: Saskatoon, SK, 1996c; version DEC-SP05 1996/12.

Health of Animals Laboratory. Determination of enrofloxacin and ciprofloxacin in bovine and porcine tissues and sarafloxacin in poultry tissues by liquid chromatography with fluorescence detection. *Methods Manual*; Centre for Veterinary Drug Residues, Health of Animals Laboratory: Saskatoon, SK, 1996d; version FLQ-SP02 1996/12.

Jordan, F. T.; Horrocks, B. K.; Froyman, R. A model for testing the efficacy of enrofloxacin (Baytril) administered to turkey hens in the control of *Mycoplasma iowae* infection in eggs and embryos. *Avian Dis.* **1993**, *37*, 1057−1061.

Kamada, M. M.; Twarog, F.; Leung, D. Y. Multiple antibiotic sensitivity in a pediatric population. *Allergy Proc.* **1991**, *12* (5), 347−350.

Kennedy, M. *Coccidiosis in Chickens*; Alberta Agriculture and Rural Development: Edmonton, AB, 1996.

Laboratory Services Division. Determination of five coccidiostat drug residues in shell eggs by HPLC. *Food Laboratory Methods Manual*; Agriculture and Agri-Food Canada, Food Production & Inspection Branch, Laboratory Services Division: Ottawa, ON, 1995; version FLS-1995-003.

Malkin, D.; Koren, G.; Saunders: E. F. Drug-induced aplastic anemia: pathogenesis and clinical aspects. *Am. J. Pediatr. Hematol. Oncol.* **1990**, *12* (4), 402−410.

Perkins, John R.; Parker, Carol E.; Tomer, Kenneth B. Nanoscale separations combined with electrospray ionization mass spectrometry: Sulfonamide determination. *J. Am. Soc. Mass Spectrom*. **1992**, *3*, 139−149.

Ponvert, C.; Le Clainche, L.; de Blic, J.; Le Bourgeois, M.; Scheinmann, P.; Paupe, J. Allergy to β-lactam antibiotics in children. *Pediatrics* **1999**, *104* (4), e45.

Settepani, J. A. The hazard of using chloramphenicol in food animals. *J. Am. Vet. Med. Assoc.* **1984**, *184*, 930−931.

Takatsuki, K.; Kikuchi, T. Gas chromatographic-mass spectrometric determination of six sulfonamide residues in egg and animal tissues. *J. Assoc. Off. Anal. Chem*. **1990**, *73*, 886−892.

Takeda; Nobuyuki; Akiyama, Yumi. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products. *J. Chromatogr.* **1991**, *558*, 175−180.

Tinkelman, D. G.; Bock, S. A. Anaphylaxis presumed to be caused by beef containing streptomycin. *Ann. Allergy* **1984**, *53* (3), 243−244.

Turnipseed, Sherri B. Analysis of Animal Drug Residues. In *Analytical Procedures for Drug Residues in Food of Animal Origin*; Turnipseed, S. B., Long, A. R., Eds.; Science Technology System: West Sacramento, CA, 1998; pp 1−8.

U.S. Department of Agriculture. Halofuginone. *Midwestern Laboratory Manual*; Food Safety and Inspection Service: version July 1991 HLF.

Wegener, H. C.; Aarestrup, F. M.; Gerner-Smidt, P.; Bager, F. Transfer of antibiotic resistant bacteria from animals to man. *Acta Vet. Scand. Suppl.* **1999**, *92*, 51−57.

Received for review February 1, 2000. Accepted September 18, 2000.

JF000144X