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Verification of Compliance with Organic Meat Production Standards by Detection of Permitted and Nonpermitted Uses of Veterinary Medicines (Tetracycline Antibiotics)

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In the production of "organic" meat, one of the controlled processes is the use of veterinary drugs. Strict standards are in place as to when and how such drugs may be used. Therefore, the aim of this project was to determine whether it was possible to distinguish between a single therapeutic dose of a tetracycline (permitted under the standards) and both multiple therapeutic dosing and prophylactic dosing (not permitted). This comprised an evaluation of (i) pigs that were treated with oxytetracycline and (ii) chickens dosed with two different tetracycline antibiotics (oxytetracycline and chlortetracycline). The methodology described, using bone sectioning and examination under ultraviolet illumination (either direct observation or fluorescent microscopy), allows samples from animals that have been treated with different dosing regimes (a single therapeutic dose, two successive therapeutic doses, and long-term, low-level "prophylactic" dosing) to be assessed for compliance with organic farming regulations. Validation of the methodology by blind checks of unknown samples by a second operator has been successfully performed, and validation results are presented. The developed methodology has been shown to be applicable to a variety of species and a selection of tetracycline drugs.

KEYWORDS: Organic meat production; pigs; chickens; tetracyclines; oxytetracycline; chlortetracycline; fluorescence microscopy; bone deposition patterns

INTRODUCTION

Organic farming can be considered as a system that will produce food of good nutritional quality by using management practices that aim to avoid the use of agrochemical inputs and minimize damage to the environment and wildlife. It is a developing area of agricultural production.

Within the European Union, organic food production standards are regulated (*1*). Within the UK, the United Kingdom Register of Organic Food Standards (UKROFS) was formerly responsible for administering the EU Regulations (as amended). In 2003, responsibility passed to the Organic Strategy Branch of the Department for Environment, Food and Rural Affairs (Defra), and the Regulations were consolidated into the Compendium of UK Organic Standards (*2*).

In organic farming systems, animal health doctrine is based on the nurturing positive health and vitality to ensure the proper control of disease, hence encouraging positive animal welfare (*2*). Where treatment is necessary, phytotherapeutics, homeopathic products, and trace elements are preferred to chemically synthesized allopathic veterinary medicines (*2*).

Notwithstanding this, the regulations require that sick animals must be properly treated, and, if necessary, conventional veterinary medicines must be used (albeit under very strict control) to ensure the health and welfare of livestock. However, where an animal or group of animals receives more than one course of treatment, if their productive lifecycle is less than 1 year, the livestock concerned, or produce derived from them, may not be sold as organic (*2*). Also, if animals have received treatment with allopathic products, then the withdrawal time must be twice that stated by the manufacturer, or 48 h, whichever is longer (*2*). Prophylactic treatment with antibiotics is specifically forbidden under organic regimes.

Organic farming generally requires more effort from the producer, and organic produce commands a premium price. Consumers purchase organic produce in the belief that it is healthier and less harmful to the environment.

Given the price premium on organic food, there is a temptation for nonorganic producers to market their produce as organic. Such practices are clearly disadvantageous to organic food customers, producers, and suppliers.

Although many drugs are licensed for use in animals (*3*), certain groups of drugs predominate in their use. In the UK, the tetracyclines comprise around 50% of the antibiotics prescribed for veterinary use (*4*).

The tetracyclines are a group of bacteriostatic antibiotics (*5*). The first (naturally occurring) members of the class of the class were discovered in the late 1940s (*6*). Subsequently, over 1000

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natural, semi-synthetic, and synthetic analogues have been identified, but only seven have found wide use. The tetracyclines are active against a variety of both Gram-positive and Gram-negative bacteria, as well as *Mycoplasma*, *Chlamydia*, *Rickettsia*, and some protozoal parasites. Acquired resistance to the tetracyclines is an issue of concern (*5*, *6*).

Notwithstanding this, the tetracyclines are generally effective drugs. They are safe, having few major side effects, and are generally well tolerated. Importantly, they are both easy to administer and effective through oral dosing, for instance, via water and feed (*6*). This makes them extremely popular as veterinary antibiotics.

In 1957, Milch et al. (*7*) published research on the localization of tetracyclines into bones in rats, and the location and observation of the deposits by bone sectioning and direct fluorescence microscopy. The direct fluorescence meant that little sample pretreatment (e.g., decalcification and fixing) was needed, and indeed was often detrimental. The complexes in bone were found to be stable provided they were stored out of direct sunlight (*8*). The fluorescent complexing phenomenon has subsequently been widely used in medical, veterinary, and environmental research. Tetracycline labeling and biopsy is reported for the assessment of bone growth and the rates of bone remodeling (*9*), to distinguish wild and hatchery populations of sockeye salmon (*10*), and dosed bait uptake has been used as a tool in wildlife research (*11*). However, there have been no reports of the use of the phenomenon to distinguish between different drug dosing regimes.

Most methods for determining drugs in animal tissue have been concerned with the presence or absence of residues in edible tissues. Methods of this type are essential for ensuring compliance with residues regulations. However, if the drugs are used as directed, and normal withdrawal periods are observed, any residue levels will be below the maximum residue limit (MRL). Hence, if longer than normal withdrawal periods are observed, normal methodology will have even less chance of detecting the use of drugs. Also, such methods do not provide time-resolved information, only data about a single point in time, and therefore will not give any information about the historical patterns of drug administration. As such, they are unsuitable for the purpose of examining whether permitted or nonpermitted dosing, as defined in the Standards (*2*), has occurred.

Therefore, new methodology was necessary to be able to monitor dosing patterns in animals. Based on their market position (*4*), the tetracyclines were chosen as the first group of drugs for which methodology was devised.

The methodology used was derived from the methodology reported by Milch et al. (*7*), with changes and modifications. The aim of the project was to determine whether it was possible to distinguish between a single therapeutic dose of a tetracycline (permitted under the Standards (*2*)) and both multiple therapeutic dosing and prophylactic dosing (not permitted).

MATERIALS AND METHODS

Animals. *Pigs.* Fifteen Large White−Yorkshire cross piglets (3 weeks old) were obtained from a local pig breeder. The animals were selected at random from the breeder's stock, and no effort was made to select even numbers of each sex. The animals had not been organically reared, but their medical history prior to arrival was documented, and no tetracyclines had been used on the piglets or their dams during pregnancy. On arrival, the animals were randomly divided into four groups: one of three animals and three of four animals each. The animals were kept in secure pens, which prevented any crossover between the groups. Each pen had a straw-filled shelter, had access to a grassed area, and was provided with items for environmental enrichment. Animals were checked at least twice daily.

Animals were fed twice daily on a proprietary organic fattening pig ration and were provided with water ad libitum. The ration fed was screened for antibiotic activity by a zone of inhibition test (*12*) (limit of detection 25−50 μg/kg oxytetracycline). The animals were allowed to acclimatize for at least 5 weeks before the trial began. At the end of the trial, all animals were euthanized by barbiturate overdose following sedation. Weight at death was 35−45 kg.

Chickens. Day-old Cobb 500 strain broiler chickens (84 birds) were obtained from a local poultry dealer. The animals were selected at random from the breeder's stock, and no effort was made to select even numbers of each sex. The birds had not been organically reared, but their medical history prior to arrival was documented and no tetracyclines had been used on the chicks or the laying hens. On arrival, the birds were randomly divided into eight groups: six groups of 10 birds and two groups of 12 birds. The birds were kept in secure outdoor aviaries, which prevented any crossover between the groups. Each aviary had a grass floor, straw-filled nest boxes and shelters, a perch area, and was provided with items for environmental enrichment. Staff checked the birds at least twice daily for evidence of ill effects or stereotypic behavior.

Birds were fed twice daily on a proprietary organic broiler ration and were provided with water ad libitum. The ration fed was screened for antibiotic activity by a zone of inhibition test (*5*) (limit of detection 25 μg/kg oxytetracycline, 50 μg/kg chlortetracycline). The birds were allowed to acclimatize for at least 6 weeks before the trial began. At the end of the trial, the birds were euthanized by cervical dislocation. Weight at death was 4−5 kg, and all birds were at least 81 days old at the time of death, as required under the Standards (*2*).

Dosing. *Pigs.* Animals were dosed with OTC (Terramycin Soluble Powder Concentrate (oxytetracycline hydrochloride, 200 g/kg), Pfizer, Ramsgate Road, Sandwich, Kent, UK) via their drinking water. Animals receiving a therapeutic dose were provided with drinking water containing 500 mg/L of OTC, while those receiving a prophylactic dose were provided with drinking water containing 50 mg/L of OTC. These doses correspond to around 20 mg/kg bodyweight (BW) per day (therapeutic) and around 2 mg/kg BW per day (prophylactic). The exact dose received would depend on water consumption and animal body weight. During dosing periods, no other source of drinking water was provided. Dose concentrations and durations were derived from the Veterinary Formulary (*4*), in discussion with a veterinary surgeon.

Dosing periods were as follows:

One group ($n = 3$) was given OTC at a therapeutic dose for 5 days, followed by a 14-day withdrawal, ending in euthanasia.

One group ($n = 4$) was given OTC at a therapeutic dose for 5 days, followed by a 10-day abeyance from dosing. At this point, a further 5 days of therapeutic dosing were given, followed by a 14-day withdrawal, ending in euthanasia.

One group ($n = 4$) was given OTC at a prophylactic dose for 42 days, followed by a 14-day withdrawal, ending in euthanasia. The remaining animals ($n = 4$) were held as a control group. One control animal was euthanized with each of the first two dosed groups, and the remaining two animals were euthanized with the last dosed group.

Chickens. Birds were dosed with OTC (Terramycin Soluble Powder Concentrate (oxytetracycline hydrochloride, 200 g/kg), Pfizer, Ramsgate Road, Sandwich, Kent, UK) or CTC (Aureomycin soluble powder (chlortetracycline hydrochloride, 55 g/kg), Fort Dodge Animal Health Ltd., Flanders Road, Southampton, UK) via their drinking water. Birds receiving a therapeutic dose were provided with drinking water containing 330 mg/L of OTC or 138 mg/L of CTC, while those receiving a prophylactic dose were provided with drinking water containing 33 mg/L of OTC or 13.8 mg/L of CTC. These doses correspond to around 35 and 3.5 mg/kg bodyweight (BW) per day, respectively, for therapeutic and prophylactic dosings of OTC and 20 and 2 mg/kg bodyweight (BW) per day, respectively, for therapeutic and prophylactic dosings of CTC. The exact dose received would depend on water consumption and body weight. During dosing periods, no other source of drinking water was provided. Dose concentrations and durations were derived from the Veterinary Formulary (*4*), in discussion with a veterinary surgeon.

Table 1. Dosing Regimes for Oxytetracycline in Chickens

regime				
single therapeutic	dosing 5 days	withdrawal 14 days		
double therapeutic	dosing 5 days	abeyance 10 days	dosing 5 days	withdrawal 14 days
prophylactic	dosing 42 days	withdrawal 14 days		

Table 2. Dosing Regimes for Chlortetracycline in Chickens

regime				
single therapeutic	dosing 5 days	withdrawal 2 days		
double therapeutic	dosing 5 days	abeyance 10 days	dosing 5 days	withdrawal 2 days
prophylactic	dosing 42 days	withdrawal 2 days		

Dosing periods were as shown in Tables 1 and 2.

Sampling and Sectioning. Bones (tibia, fibula, femur, radius, ulna, humerus, and ribcage from pigs, and tibia and femur of chickens) were removed from the animals intact and freed of the bulk of the soft tissue with a filleting knife. Defleshed samples were stored deep-frozen at −20 °C pending further treatment. Bone samples selected for examination (humerus, femur, and rib) were carefully cleaned of all adhering tissue with scissors and scalpels, and the ends of the bones were removed with a fine bladed razor saw. Sections (0.5−0.8 mm thick) were cut through the bone perpendicular to the long axis using a low-speed diamond bladed saw (Buehler Isomet, fitted with a 12.5 cm HC15 wafer blade (Buehler UK, Milburn Hill Road, Science Park, University of Warwick, Coventry, UK)). Sections were stored in plastic containers in 70% ethanol. Prior to examination, sections were stored at room temperature in the dark.

Examination. Sections were mounted on slides using glycerol jelly prior to examination. Sections cut through large-diameter bones (femora and humeri) were examined using direct fluorescence, and data were captured using a macro photographic approach. Small diameter bones (ribs) were examined by fluorescence microscopy, with photomicrography to capture data.

The macro photographic setup was comprised of a Nikon D1X SLR digital camera with a 105 mm Macro lens, exposure 1/15−1/60 s at f5.0. The samples were viewed and photographed by placing the slide directly on top of a UV lamp (365 nm illumination), with the camera overhead.

Microscopy and photomicrography were performed using a Zeiss Axioplan 2 fluorescence microscope, operated in incidence mode, with a ×10 objective and ×10 eyepiece. The light source was a Zeiss HBO50 mercury lamp, and a Type 2 filter unit (excitation 450−490 nm, emission 515−565 nm) was fitted. Photomicrographs of ribs were obtained using a Nikon CoolPix 4500 digital camera, operated in manual mode, with an aperture setting of f2.6 and a shutter speed between 1/15 and 1/125 s.

Macro photographs and photomicrographs were saved in jpeg format, and then examined using Adobe Photoshop 7.

RESULTS AND DISCUSSION

The observations performed throughout the trial noted no illness, distress, or stereotypic behavior among the pigs or chickens. Although oral dosing was used in both species, and for both drugs, because tetracycline uptake and deposition is not affected by the mode of administration, there is no evidence to suggest that tetracyclines administered by injection would give different results.

Screening tests of the feed for antibiotic activity were negative. All relevant quality control criteria for the screening tests were met, indicating that the feeds were free of tetracyclines at the concentration noted above.

Quality of the bone sections was critical to successful diagnosis of the dosing regime. Poor quality sections, those that were ragged, uneven, or otherwise nonuniform, were in many cases impossible to examine effectively. Therefore, considerable care was needed in the removal of soft tissue from the surface of the bones. Insufficient removal made it more difficult to produce uniform sections, while overzealous treatment could damage the bone surface. This was especially critical for the chicken bones due to their narrower calcified section. It was also a key factor in producing reliable test samples from CTC dosed birds. These birds had undergone relatively short withdrawal periods, so that the fluorescent bands were close to the surface of the bone. In the case of the rib bone sections, the axis of the cut was found to be of critical importance. For a pig rib section to be useful, the cut must be made perpendicular to the long axis of the bone, and precisely normal to the curvature of the bone along both the lengthwise and the transverse axes. Sections cut "across" the axes did not provide the necessary diagnostic information. Although uneven sections from the larger bones could be examined with the naked eye, the very narrow depth of focus available in both the macro photographic equipment and the microscope made producing adequate pictures from these sections almost impossible. Difficulties with colored artifacts noted by other authors (*11*) were not observed. Although all bones examined were found to be diagnostic, certain bones types were used more frequently than others. This is a reflection of the intended use of the method. The methodology is intended for use at the regulatory level, on retail samples. In retail samples, certain bone types predominate. Bone-in chicken thighs and bone-in pig ribs (chops) are common and easier to obtain and would therefore be expected to predominate in market surveys. Hence, these bones were examined predominantly.

Pigs. Distinctive, diagnostic patterns of fluorescence could be observed in the pig bones. These patterns could be immediately related to the dosing regime the animal had undergone.

In the case of pigs that had received a single therapeutic dose, a single, highly intense band of yellow-green fluorescence was observed against the bluish background, as seen in Figure 1 (rib bone) and Figure 2 (femur). In some specimens, an autofluorescent artifact can be observed at the interface between the bone and the periosteum. This appears as a narrow, bright bluish-white line (shown by an arrow in Figure 2) and is readily distinguishable from the yellow-green fluorescence associated with the bound tetracycline residues.

Where an animal had received two therapeutic doses, two distinct, intense bands were observed as see in Figure 3 (rib bone) and Figure 4 (femur).

In prophylactically dosed animals, a single, wide, diffuse band was observed as shown in Figure 5 (rib bone) and Figure 6 (femur).

In nonexposed control animals, no fluorescent banding was observed as shown in Figure 7 (rib bone) and Figure 8 (femur).

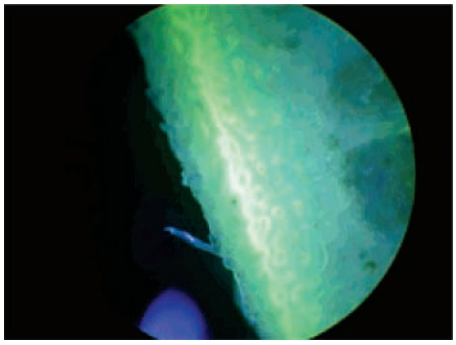

Figure 1. Section through a rib bone of a pig given a single therapeutic dose of oxytetracycline.

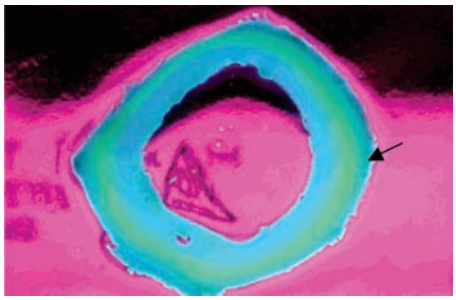

Figure 2. Section through a femur of a pig given a single therapeutic dose of oxytetracycline.

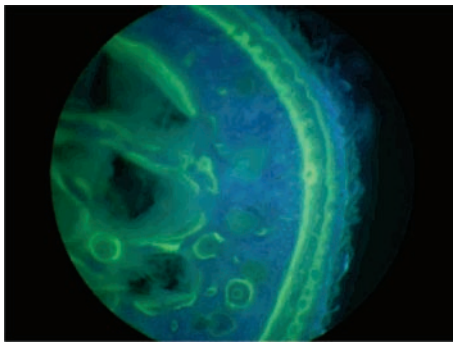

Figure 3. Section through a rib bone of an animal given two therapeutic doses of oxytetracycline.

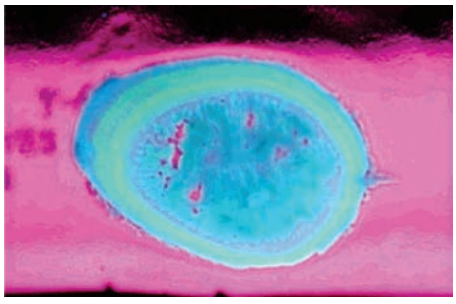

Figure 4. Section through a femur of a pig given two therapeutic doses of oxytetracycline.

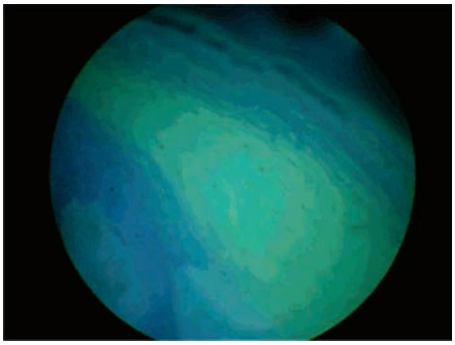

Figure 5. Section through a rib bone of a pig given a prophylactic dose of oxytetracycline.

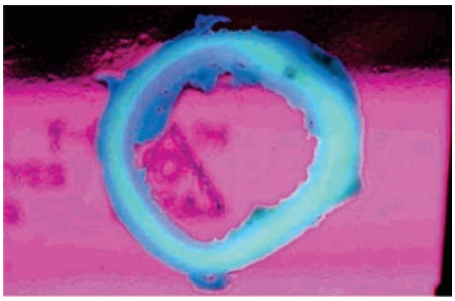

Figure 6. Section through a femur of an animal given a prophylactic dose of oxytetracycline.

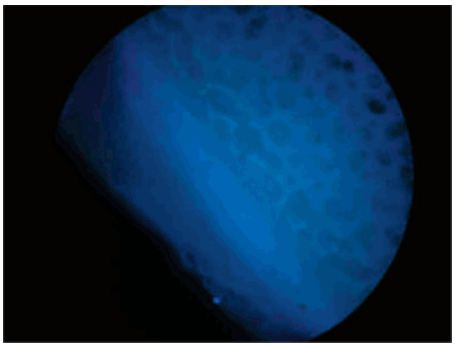

Figure 7. Section through a rib bone of a control pig not exposed to oxytetracycline.

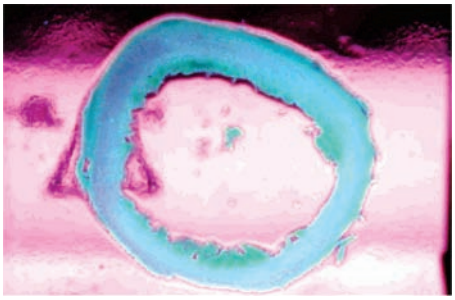

Figure 8. Section through a femur of a control pig not exposed to oxytetracycline.

Thus, dosed and nondosed animals were immediately distinguishable, and single-dosed animals could be distinguished from multiply dosed animals.

The consistency of the effect was assessed by preparing and examining single sections from each animal in each group. The same effect was observed in all cases. Multiple sections were also cut from one bone from one animal in each group and examined, to demonstrate that the effect could be seen throughout the bone. An example of this study is shown in Figure 9. Here, a montage of the photographs of six sections taken through a rib bone from one pig that had received two therapeutic doses of oxytetracycline is presented.

Although single therapeutic and prophylactic doses could be distinguished by the intensity of the fluorescent band (highly intense from therapeutic doses, weak from prophylactic doses), and by the band being discrete (for therapeutic doses) or diffuse (for prophylactic doses), further discrimination was possible by observing the ratio of the bandwidth to the bone width. In

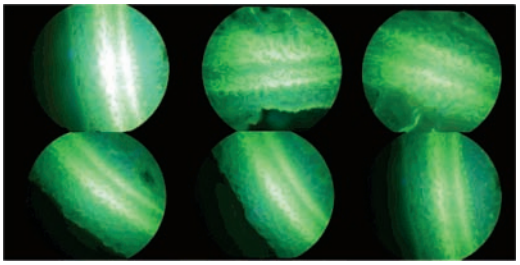

Figure 9. Photomontage showing sections through femora of three different animals given a single therapeutic dose of oxytetracycline.

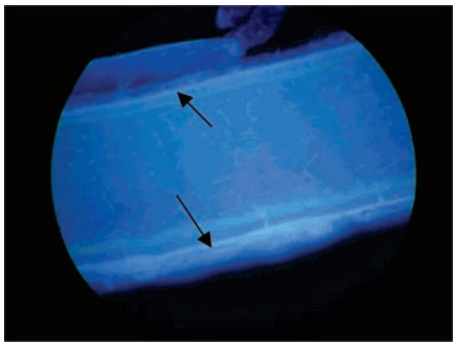

Figure 10. Section through a femur of a chicken given a single therapeutic dose of oxytetracycline.

therapeutic (short-term) doses, the ratio of the width of the band to the width of the calcified bone was small, whereas in prophylactic (long-term) doses, the ratio of the width of the band to the width of the calcified bone was large.

In most cases, when sections from large bones (humeri and femora) were mounted on a microscope slide and placed directly on a UV lamp, the bands were distinct enough to be distinguished with the naked eye, although the magnified images produced by macro photography were much more diagnostic. Small bone sections (ribs), when viewed through the microscope, were immediately diagnostic of the dosing regime.

Although the method (either direct visual examination or using microscopy) was fully capable of distinguishing between animals that had received permitted and nonpermitted doses of OTC, such a method would not provide a record of the observation that could be recalled, verified, or audited if necessary. Should the method have to be used to challenge the authenticity of organic meat samples, such verification and audit would be essential if the observation were to be credible. Therefore, obtaining photographs of the specimens of sufficient quality that the differences between the types of samples were readily distinguishable was an essential part of the method development.

Photographs and photomicrographs underwent very little manipulation because images were sufficiently sharp to not need additional resolving via the photographic manipulation package. After minor adjustment of the image contrast, the color representation in the images was found to be very "true" to that observed by the naked eye or through the microscope.

Although bones and sections were stored in the dark because of the issue of the loss of fluorescence (*8*), no diminution of intensity was noted even after femur sections had been on the surface of the UV lamp for over an hour.

Chickens. The applicability of the methodology was evaluated by extending the study to chickens treated with either OTC or CTC. Once again, a diagnostic pattern of fluorescence could be observed in the bones. These patterns could be observed for both of the drugs used, and as for pigs could be immediately related to the dosing regime the bird had undergone.

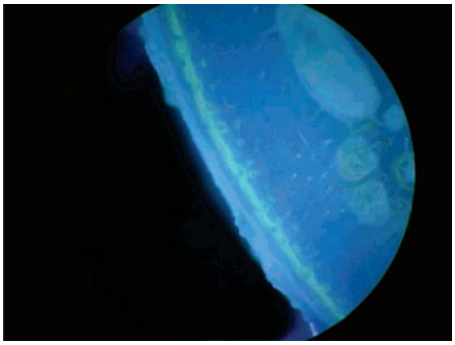

Figure 11. Section through a femur of a chicken given a single therapeutic dose of chlortetracycline.

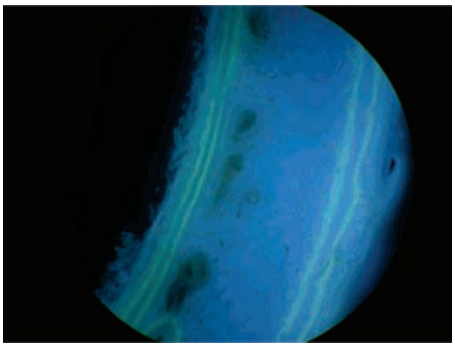

Figure 12. Section through a femur of a chicken given two therapeutic doses of oxytetracycline.

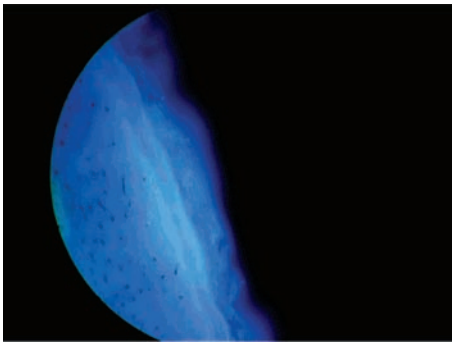

Figure 13. Section through a femur of a chicken given two therapeutic doses of chlortetracycline.

A single band of fluorescence was observed against the bluish background (Figure 10, dosed with oxytetracycline, Figure 11, dosed with chlortetracycline) in the case of birds that had received a single therapeutic dose of either drug. In some specimens, an autofluorescent artifact can be observed at the interface between the bone and the periosteum (indicated by arrows in Figure 10). This appears as a narrow, bright bluish-white line and is readily distinguishable from the yellow-green fluorescence associated with the bound tetracycline residues.

In birds that had received two therapeutic doses, two distinct bands were observed (Figure 12, dosed with oxytetracycline, Figure 13, dosed with chlortetracycline).

In prophylactically dosed birds, a single, wide, diffuse band was observed (Figure 14, dosed with oxytetracycline, Figure 15, dosed with chlortetracycline).

In nonexposed control birds, no fluorescent banding was observed (Figure 16).

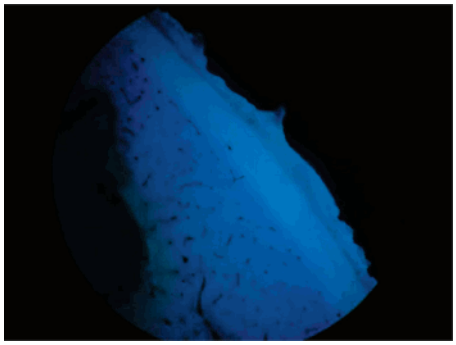

Figure 14. Section through a femur of a chicken given a prophylactic dose of oxytetracycline.

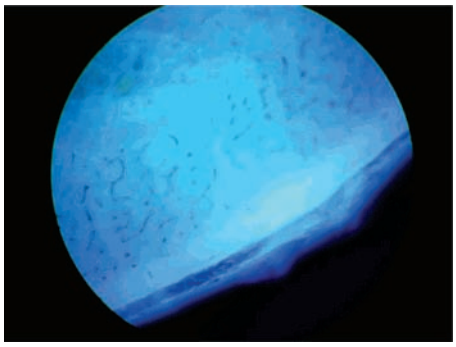

Figure 15. Section through a femur of a chicken given a prophylactic dose of chlortetracycline.

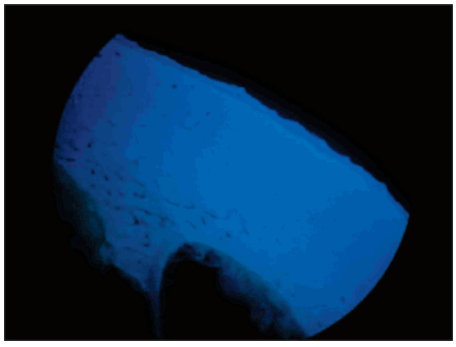

Figure 16. Section through a femur of a control chicken not exposed to tetracyclines.

As previously described for OTC in pigs, a single section cut through the femur of each bird provided the same diagnostic result. Further, one femur from one bird in each dosing population had multiple sections cut from different points along its length ($n = 6$) and examined. Again, the same result was observed for all sections, demonstrating the robustness of the technique.

To demonstrate the overall reliability of the method, a blind check, where bone sections were provided as unknown samples ($n = 24$) to another operator who had undergone basic instruction, was performed. The other operator was able to correctly identify the dosing regime in 96% of cases and to produce diagnostically useful pictures of each specimen. The results of the first blind trial are shown in Table 3.

After additional training, a second blind trial was undertaken on a different set of unknown samples ($n = 16$, chicken femora). The results of this trial (100% correct) are shown in Table 4.

It should be noted that these values are simply the proportion of correctly identified samples out of the total number examined by the second operator. No inference as to a "confidence level" for the method has been drawn from these data by the authors. The authors developed the validation criteria used, in the absence of any published guidelines for methods of this type. In a chromatographic technique, obtaining satisfactory data from the examination of 18 replicates would be considered sufficient initial validation. In this case, 40 replicates were examined.

The one sample that was not correctly identified in the first blind trial was a CTC prophylactic dosed chicken femur. These bones exhibit relatively weak fluorescence, and hence there is limited contrast between such dosed bones and untreated bones. The limited depth of focus and field of view available on the microscope made placing the unknown sample and a "control" (either untreated or known positive treated) on the stage together for simultaneous viewing impossible. Although a comparison microscope with a suitable ultraviolet source and filter system

Table 3. Results of the First Blind Method Check

sample	actual description	observation	correct identification
	Chicken Femora		
1	control	control	Y
2	prophylactic dosed OTC	prophylactic dosed	Y
3	**prophylactic dosed CTC**	**control**	**N**
4	double therapeutic dosed OTC	double therapeutic dosed	Y
5	single therapeutic dosed OTC	single therapeutic dosed	Y
6	double therapeutic dosed CTC	double therapeutic dosed	Y
7	single therapeutic dosed OTC	single therapeutic dosed	Y
8	control	control	Y
	Pig Rib		
9	double therapeutic dosed	double therapeutic dosed	Y
10	prophylactic dosed	prophylactic dosed	Y
11	prophylactic dosed	prophylactic dosed	Y
12	control	control	Y
13	control	control	Y
14	double therapeutic dosed	double therapeutic dosed	Y
15	single therapeutic dosed	single therapeutic dosed	Y
16	single therapeutic dosed	single therapeutic dosed	Y
	Pig Femora		
17	double therapeutic dosed	double therapeutic dosed	Y
18	prophylactic dosed	prophylactic dosed	Y
19	prophylactic dosed	prophylactic dosed	Y
20	control	control	Y
21	control	control	Y
22	double therapeutic dosed	double therapeutic dosed	Y
23	control	control	Y
24	single therapeutic dosed	single therapeutic dosed	Y

Table 4. Results of the Second Blind Method Check[a]

sample	actual description	observation	correct identification
1	double therapeutic dosed CTC	double therapeutic	Y
2	double therapeutic dosed OTC	double therapeutic	Y
3	control	control	Y
4	prophylactic dosed CTC	prophylactic	Y
5	prophylactic dosed OTC	prophylactic	Y
6	control	control	Y
7	single therapeutic dosed CTC	single therapeutic	Y
8	prophylactic dosed CTC	prophylactic	Y
9	control	control	Y
10	control	control	Y
11	single therapeutic dosed OTC	single therapeutic	Y
12	control	control	Y
13	single therapeutic dosed OTC	single therapeutic	Y
14	control	control	Y
15	double therapeutic dosed CTC	double therapeutic	Y
16	prophylactic dosed OTC	prophylactic	Y

[a] All samples are chicken femora.

might have been useful in dealing with this problem, no such instrument was available.

Not withstanding that the second trial exhibited a 100% success rate, it was considered that a less subjective means of determining the dosing regime was required. This subjectivity increases with the amount of processing the images undergo. The human eye coupled with a good microscope possesses tremendous discriminating power. As the images are captured by digital camera, undergo computer processing, are saved in different formats, and output in various forms, at each stage some resolution is lost. Therefore, work is currently underway to investigate techniques that can provide a semiquantitative assessment of the drug dosing regimes used. Initial approaches using image processing software have showed promising results.

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LITERATURE CITED

(1) Council Regulation (EEC) 2092/91, Official Journal No L 198, 22, *7*, 1991; p 1.
(2) Compendium of UK Organic Standards, Defra, March, 2003.
(3) *The Veterinary Formulary*, 4th ed.; Bishop, Y., Ed.; Pharmaceutical Press: London, UK, 1998.
(4) Ministry of Agriculture Fisheries and Food, Veterinary Medicines Directorate April 2000, Sales of antimicrobial products used as veterinary medicines or growth promoters in the UK from 1993−1998.
(5) *Martindale, The Extra Pharmacopeia*, 33rd ed.; Sweetman, S. C., Ed.; Pharmaceutical Press: London, UK, 2002.
(6) Chopra, I.; Hawkey, P. W.; Hinton, M. Tetracyclines, Molecular and Clinical Aspects. *J. Antimicrob. Chemother*. **1992**, *29*, 245−277.
(7) Milch, R. A.; Rall, D. P.; Tobie, J. E. Bone Localization of Tetracyclines. *J. Natl. Cancer Inst*. **1957**, *19*, 87−93.
(8) Buyske, D. A.; Eisner, H. J.; Kelly, R. G. Concentration and Persistence of Tetracycline and Chlortetracycline in Bone. *J. Pharmacol. Exp. Ther*. **1960**, *130*, 150−156.
(9) Frost, H. M. Tetracycline-based Histological Analysis of Bone Remodelling. *Calcif. Tissue Res*. **1969**, *3*, 211−237.
(10) Weber, D. D.; Ridgway, G. J. The Deposition of Tetracycline Drugs in Bones and Scales of Fish and its Possible Use for Marking. *Prog. Fish-Cult.* **1962**, *24*, 150−155.
(11) Linhart, S. B.; Kennelly, J. J. Fluorescent Bone Labeling of Coyotes With Demethylchlortetracycline. *J. Wildl. Manage*. **1967**, *31*, 317−321.
(12) Stead, S.; Sharman, M.; Tarbin, J. A.; Gibson, E.; Richmond, S.; Stark, J.; Geijp, E. Meeting maximum residues limits: an improved screening technique for the rapid detection of antimicrobial residues in animal food products. *Food Addit. Contam.* **2003**, *21*, 216−221.

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