Differences in fluorescence of doxycycline in chicken bone depending on dosage and treatment time

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Introduction

Doxycycline is an antibiotic that is widely used in poultry and other production animals. Animals can be treated therapeutically using the prescribed dosage, treatment period and withdrawal time, but also illegal treatment with low dosages for growth promotion can occur. Moreover due to cross-contamination with medicated feed, animals can be exposed to low levels of doxycyline. Treatment with tetracyclines such as oxytetracycline and doxycycline can be detected in bone due to their auto-fluorescence. In this experiment we investigated if therapeutical treatment could be distinguished from treatment with low dosages, such as occur in sub-therapeutical treatment and cross-contamination.

Materials and methods

In an animal experiment we treated broiler chickens (7 days old) with 3 levels of doxycycline in the drinking water (10 animals/group);

- 1) therapeutic 20 mg/kg BW for 7 days, withdrawal time 14 days
- 2) sub-therapeutic 2 mg/kg BW for 14 days, withdrawal time 7 days
- 3) cross-contamination 0.5 mg/kg BW for 21 days and no withdrawal time (fig. 1).

The animals were kept and fed according to practice
The animals were sampled at day 10, 17, 24 and 29.

Day 0	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
A				S							S							S					S
1	Therapeutic dose level																						
2	Sub-therapeutic dose level																						
3	Cross-contamination dose level																						

Figure 1. Overview of treatments and sampling A= arrival of birds, S = sampling.

Cross sections of tibia bone were evaluated with fluorescence microscopy and scored for intensity and localisation of fluorescence. Tibia bone was cleaned from flesh and sections of approximately 0.5 mm were cut using a electrical sawing machine.
For evaluation of the fluorescence the bone was divided into three regions; central, medial and peripheral (figure 2). The intensity was scored as weak, moderate or strong.

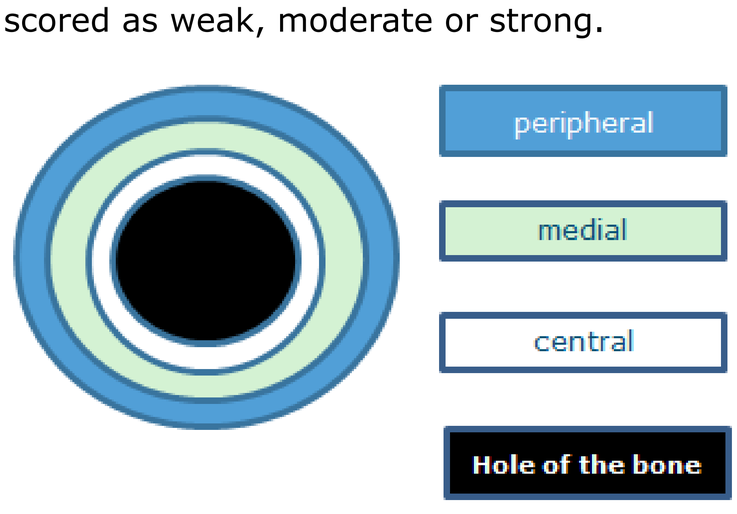
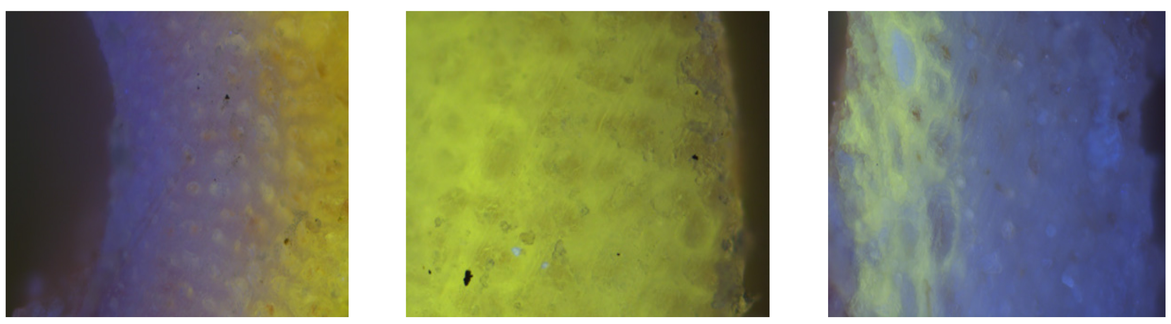


Figure 2. Overview of a cross section of the tibia bone and the different regions.

Results

In therapeutically treated animals bright fluorescence was first seen at the periphery of the bone, forming a band that moved to the central part of the bone in time during treatment and after withdrawal. In the other two groups the intensity was weaker and in time the whole bone was fluorescent. There was no difference between the fluorescence of the sub-therapeutic and cross-contamination groups.

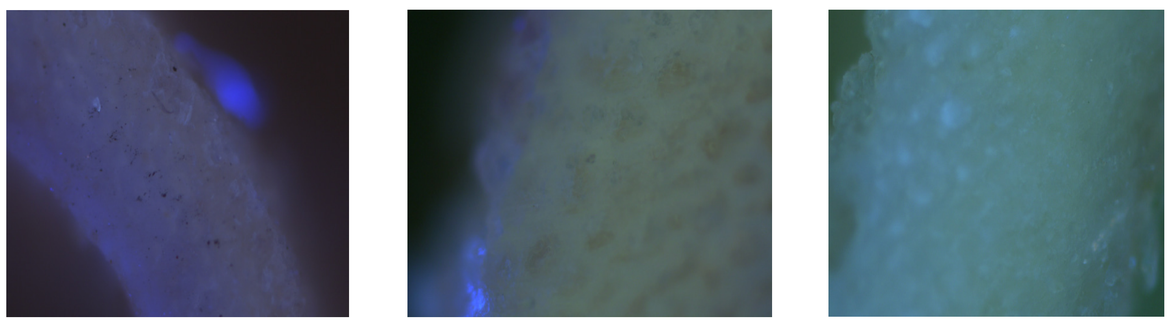


Day 10: only peripheral fluorescence

Day 17: the whole bone is fluorescent

Day 29: only central fluorescence

Figure 3. Pictures of fluorescence in tibia bone of therapeutically treated broilers at different sampling times (resp. during treatment and 3 and 15 days after withdrawal).



Day 10: only peripheral weak fluorescence (during treatment)

Day 17: peripheral and central weak to moderate fluorescence (during treatment)

Day 29: weak to moderate fluorescence of the whole bone (1 day after treatment)

Figure 4. Pictures of fluorescence in tibia bone of sub-therapeutically treated broilers at different sampling times.

Conclusions

- Therapeutical as well as non-therapeutical treatment leads to changes in the region of fluorescence in time
- Therapeutical treatment leads to brighter fluorescence than non-therapeutical treatment
- Subtherapeutical dose and cross-contamination dose do not differ in fluorescence pattern
- The value of this method to distinguish therapeutical treatment from non-therapeutical treatment has to be further investigated

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