

MATERIAL AND METHODS

The study was carried out on 215 five-month-old (Group A) and 565 six/seven-month-old (Group B) veal calves collected from 95 farms and reared according to standard programmes. Serum cortisol, ir-inhibin, osteocalcin, antioxidant capacity (SAC), and urea were measured using commercially available kits. Biomarker values (medians with interquartile ranges) were compared using the Mann Whitney test.

RESULTS AND CONCLUSION

After UNEQ model application, 24.2% ($n = 52$) Group A-, and 55.3% ($n = 284$) Group B calves were classified as non compliant (NC). In both age groups, median values of serum cortisol, ir-inhibin, and osteocalcin were significantly lower in NC than in compliant animals (Cortisol: Group A, 9.83 ng ml⁻¹ versus 16.41 ng ml⁻¹, $P < 0.0001$; Group B, 10.18 ng ml⁻¹ versus 16.37 ng ml⁻¹, $P < 0.0001$. Ir-inhibins: Group A, 0.98 ng ml⁻¹ versus 1.19 ng ml⁻¹, $P = 0.03$; Group B, 1.01 ng ml⁻¹ versus 1.16 ng ml⁻¹, $P = 0.01$. Osteocalcin: Group A, 8.55 ng ml⁻¹ versus 10.22 ng ml⁻¹, $P = 0.0009$; Group B, 8.61 ng ml⁻¹ versus 9.43 ng ml⁻¹, $P < 0.0001$). In Group A calves no differences in SAC and urea levels were observed between compliant and NC, whereas in Group B SAC values were higher in NC than in compliant animals ($P = 0.011$); urea was lower in NC than in compliant calves ($P = 0.033$). The recorded changes in biomarker values from calves classified as NC reflect those expected after the exposure to the different GP classes (1), suggesting that UNEQ model based on the selected serum biomarkers may represent a good approach to identify possible GP abuse in veal calves.

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5.7.

Evaluation of stress-related prednisolone biosynthesis in cows participating to 'Batailles des Reines'

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INTRODUCTION

Natural corticosteroids include two families of substances: mineralocorticoids and glucocorticoids. Several molecules simulat-

ing their structure and behaviour, with increased pharmacological activity, have been synthesized and are widely used in the clinical practice. Beside legal treatments, these drugs can also be misused. One of the more abused 'corticosteroid' is prednisolone. Until few years ago, it was considered exclusively synthetic, but nowadays a debate on its possible endogenous origin is under way. Many researchers have worked to ascertain the relation between stressful conditions, such as transportation and slaughterhouse environment, and endogenous production of prednisolone. In order to verify further allegedly stressful conditions, our laboratory analyzed urine samples collected from the cows participating to the 'Batailles des Reines' (a traditional contest based on ritual and spontaneous fights of pregnant cows), to verify if an endogenous prednisolone production may occur in these animals.

MATERIALS AND METHODS

We developed and validated a LC-MS/MS method for the simultaneous determination of cortisol, cortisone, prednisolone, prednisone, 20 α -dihydroprednisolone, 20 β -dihydroprednisolone, 20 β dihydroprednisone and 6 β -hydroxyprednisolone. Sample preparation includes an enzymatic deconjugation, followed by a SPE. The method was applied for the analysis of urine samples from 2012 and 2013 'Batailles des Reines' competitions, for a total of 114 samples.

RESULTS

The analytical method was validated following the 2002/657/CE Decision. Cortisol and cortisone were found in all but one urine samples, with average values of 8.35 \pm 5.17 ng ml⁻¹ and 4.93 \pm 3.10 ng ml⁻¹, respectively, and no significant differences between 2012 and 2013. Prednisolone was found in only one sample, at a concentration of 1.45 ng ml⁻¹, accompanied by cortisol and cortisone concentrations at the highest values found in these urine samples, 35.5 and 18.1 ng ml⁻¹ respectively. Traces of prednisolone were found also in three other samples. In these urines cortisol and cortisone concentrations were around average values.

CONCLUSIONS

The stress produced by the 'Batailles des Reines' fight appears to be present but lower than that caused by both transportation and slaughterhouse environment, as evaluated from cortisol and cortisone urine concentrations. This stress level is probably not sufficient to induce endogenous prednisolone biosynthesis, which was observed in only one case.

5.8.

Illicit administration of estradiol in cattle: case report

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INTRODUCTION

Estrogens, of which 17 β -estradiol is the most active molecule, constitute a group of steroid compounds known for their importance in the estrous cycle. Besides their function in the

reproductive cycle, they also play an important role in a number of other physiological processes, including mineral, fat, sugar and protein metabolism and sodium and water retention. Owing to their wide-reaching systemic effects, estrogens are also illegally administered to stimulate growth in calves and boost meat production.

In February 2014, a police investigation was conducted for alleged illicit cattle treatments, within a livestock in Piedmont, northern Italy. During the operations of judicial investigation, an undeclared animal treatment was observed by hidden cameras. The subsequent day, four anonymous liquids were impounded and so also serum of ten animals was rapidly collected and transferred to our laboratory.

MATERIALS AND METHODS

The anonymous liquids were properly diluted and injected into a triple quadrupole mass spectrometer API 4000 (ABSciex), equipped with an electrospray ionization source. Serum samples were analysed with a procedure validated according to 2002/657/CE Decision, using a LC-MS/MS (QQQ) equipped with a H-ESI, operating in negative multiple reaction monitoring (MRM) mode (Thermo Fisher). Sample preparation involved liquid/liquid extraction followed by centrifugation and SPE clean up.

RESULTS

In all four anonymous liquids 17 β -estradiol benzoate, a typical prodrug of 17 β -estradiol, was found at concentration ranging from 12.0 mg ml⁻¹ to 14.4 mg ml⁻¹. Nine out of ten serum samples were found positive to 17 β -estradiol at concentrations comprised between 0.059 μ g l⁻¹ and 0.208 μ g l⁻¹, which are much higher than physiological values.

CONCLUSIONS

The illicit drug treatment was found thanks to a very good collaboration between Police and the laboratory staff. The requisition of anonymous liquids permitted to identify the analyte to look for. The direct observation that an animal treatment had been carried out permitted to collect the animal serums immediately thereafter, making it possible to find strong evidence of the exogenous treatment. It is also interesting the finding of one compliant animal out of ten controlled. This practice of keeping one untreated animal in the same box with treated veals is quite typical in cattle breeding: it is suggestive of the corruptive intent of the breeder, addressed to obtain dishonest veterinary controls focused only on the untreated animal.

5.9.

A deltamethrin nil milk withdrawal time and good local tolerance after treatment of ewes with Deltanil[®] pour-on solution

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INTRODUCTION/OBJECTIVE

A GLP study was carried out according to EMA Guideline in order to follow the residue depletion of deltamethrin in ewe's milk and to estimate the milk withdrawal period after treatment with Deltanil[®] 1%_{w/v} deltamethrin pour-on solution. The local tolerance at the application site was also followed.

MATERIALS AND METHODS

Ten high and ten low yielding milking ewes were included in this study. Five mL of the product were applied once, topically on each animal, equivalent to about 50 mg of deltamethrin per animal. Clinical signs and application sites were observed daily for local tolerance up to 8 days post-treatment. Animals were milked twice daily (every 12 h \pm 30 min). Milk samples were regularly collected before treatment, 6 h, 8 h and 12 h post-treatment and twice daily until 8 days post-treatment for deltamethrin analysis. Deltamethrin levels in milk were determined by an adequate and validated routine analytical LC-MS/MS method according to criteria defined in Vol. 8. The lowest limit of quantification (LLOQ) was 10 μ g kg⁻¹, half the milk MRL set by the EMA for all ruminants. The withdrawal period was calculated using the EMA software MELK14 and a statistical method.

RESULTS

A good general and local tolerance was observed for the product. All deltamethrin individual concentrations were below the LLOQ (10 μ g kg⁻¹), with no difference between low and high yielding ewes from 6 h (first milking) after the treatment, except for one ewe which showed quantifiable values from 8 h to 24 h after treatment, but always under the MRL (20 μ g kg⁻¹). Using the SCPM approach, the statistically calculated withdrawal period was 0 days.

CONCLUSIONS

The results of this study allow a nil milk withdrawal time to be determined and show the good local tolerance of the Deltanil[®] Pour-on Solution when topically applied at the recommended dose in milking ewes for the control of ectoparasites.

5.10.

Residues of decoquinatate in eggs after feeding hens with compliant cross-contaminated feed

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INTRODUCTION

Decoquinatate is a quinolone derivative that can be used as feed additive or in-feed medication for the treatment or prevention of coccidiosis in poultry and ruminants. It is also regarded as an effective aid in the toxoplasmosis and cryptosporidiosis in ruminants. In EU countries decoquinatate is authorised for chicken broilers as feed additive.

As cross-contamination of feeds for non-target animals with decoquinatate is unavoidable, its maximum levels (ML) in feed and in food of animal origin have been established. It is not known however if undesirable residues of decoquinatate in hen eggs may occur after contaminated but compliant feed is administered to hens.

MATERIALS AND METHOD

Twenty laying hens received feed containing 0.34 \pm 0.081 mg kg⁻¹ of decoquinatate (ML for feed = 0.40 mg kg⁻¹) during 14 days. Then, for the next 14 days decoquinatate-free diet was applied. The eggs were collected daily during the whole experiment and stored in 6 \pm 4°C. The