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Accepted author version posted online: 24 Sep 2013. Published online: 20 Nov 2013.

To cite this article: Vincent Porphyre, Michel Rakotoharinome, Tantely Randriamparany, Damien Pognon, Stéphanie Prévost \& Bruno Le Bizec, Food Additives \& Contaminants: Part A (2013): Residues of medroxyprogesterone acetate detected in sows at a slaughterhouse, Madagascar, Food Additives \& Contaminants: Part A, DOI: 10.1080/19440049.2013.848293

To link to this article: http://dx.doi.org/10.1080/19440049.2013.848293

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(Received 4 July 2013; accepted 20 September 2013)

Keywords: pork; kidney fat; food safety; growth promoters; anabolic; medroxyprogesterone acetate; Madagascar

Introduction

Synthetic progestagens have been widely used in gynecologic practice. Progestin medroxyprogesterone acetate (MPA) is used in contraceptive medication and also in human hormone replacement therapy, but its side effects are commonly reported: MPA has a significant glucocorticoid activity that decreases bone density (Ishida & Heersche 2002), and it may also impair memory (Braden et al. 2010). MPA is associated with the development and maintenance of obesity, and it may also be considered as a risk factor for cardiovascular diseases (Clark et al. 2005). In animal husbandry, MPA is allowed for reproduction purpose in sheep. MPA also possesses anabolic activity and therefore has been used in livestock production to improve muscle gain (Meyer 2001). Within the European Union, the use of progestagens and other steroid hormones for the purpose of fattening farmed animals is prohibited (Courtheyn et al. 2002). According to the European Commission Decision 2003/181/EC, the minimum required performance limit in animal tissues is equal to 1 µg kg\(^{-1}\) (Commission of the European Communities 2003).

In European Union countries, the misuse of synthetic hormones in animal husbandry was reported in milk (Unusan 2008) and pork meat (Oksbjerg et al. 1995). MPA-contaminated feed was also incriminated (van Leengoed et al. 2002). While veterinary monitoring systems in developed countries allow an efficient and rapid alert to protect consumers, the situation is unclear in most of the developing countries regarding the use of hormones in animal farms and the hazard exposure for local consumers.

In Madagascar, the consumption of pork meat is traditional and the demand for pork meat is increasing in urban markets. The pig production sector is extensively developed with complex trade circuits from remote rural areas to urban abattoirs and retailing markets. In pig farms, few biosecurity measures are implemented (Costard et al. 2009), leading to numerous food safety issues in pork meat (Rakotoharinome et al. 2012; Temmam et al. 2013). In particular, the misuse of hormones in pig farms has been increasingly reported during 2012 by local veterinary officers (personal communication, M. Rakotoharinome, Feb 15, 2012). Farmers and local animal health workers were suspected of treating pigs for promoting their growth and of using human progestins, especially MPA, as a chemical alternative method for the castration of sows that are then fattened before culling. Indeed, MPA may arrest the onset of farrowing, and induce post-lactational anoestrus in sows (van Leengoed et al. 2002). Because the use of synthetic hormones in pig husbandry is considered as a fraud under Malagasy regulations (Malagasy Ministry of Agriculture 2006), an exploratory study was carried out to confirm these suspicions and investigate the main substances concerned.

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Material and methods

Field data

The study considered 8 of the 22 regions of Madagascar where suspicions were reported by local field veterinarians: Bongolava, Vakinankaratra, Itasy and Haute Matsiatra in Central Madagascar; Betsiboka, Diana and Sofia in the Northern region; and Anosy in the Southern region. Samples were collected during October 2012 from two provincial abattoirs, that is, Tsiroanomandidy in Western Madagascar and Antsirabe city in Vakinankaratra province (Central Madagascar), and from four abattoirs in Antananarivo, the Malagasy capital city, where animals from all over the country were transported for slaughtering. Nineteen communes from 15 districts within these eight regions were consequently sampled. Only adult sows were sampled considering that chemical castration with progestagens was the most common hypothesis of the misuse of synthetic hormones in the field. Eighty kidney fat samples were collected after slaughtering by veterinary officers and stored at −80°C before testing. Samples were shipped in dry ice at −80°C from Madagascar to Nantes (France). Samples were initially pooled by district and analysed by group (n = 21). One pool and three individual samples for which results were not validated by the laboratory’s internal quality control were discarded from the final analysis. Fifty samples among the first 10 positive pools were tested individually.

Laboratory methods

Sample preparation

Kidney fat samples were extracted, purified and analysed in LABERCA (Oniris, Nantes, France) under ISO 17025:2005 standards. The reference steroids, MPA and MPA-d₃, were, respectively, purchased from Steraloids, Inc., Ltd (London, UK) and RIKILT (Wageningen, The Netherlands). Most of the reagents and solvents were of analytical grade quality and provided by VWR International (Pessac, France). The SPE (C18 and silica) columns were from UCT (Bristol, PA, USA). Target progestagen’s analytes were first extracted by using accelerated solvent extraction on 4 g of filtered kidney fat. Organic solvent was evaporated to dryness before successive purification steps on SPE on C18 (2 g/10 mL) and silica (1 g/6 mL) cartridges. After evaporation to dryness, the dry residue was reconstituted by 50 µL of H₂O/ACN mixture before injection (10 µL).

Detection and identification of gestagens

Data were acquired on a triple quadrupole (Agilent 6410) operating in the positive ESI mode coupled to an LC system (Agilent 1200). The method used was validated according to the 2002/657/EC decision (Antignac et al. 2002) and decision limit (CCα) was calculated at 0.5 µg kg⁻¹. Table 1 details the chemical constituents targeted for screening, namely, flugestone acetate, norgestomet, delmadinone acetate, altrenogest, clomadinone acetate, melegestrol acetate and MPA and their respective CCα. Data acquisition was based on a targeted approach using SRM for the acquisition of four specific signals for each analyte. MPA transitions were 387.3 > 285.3, 387.3 > 123.2, 387.3 > 97.1 and 387.3 > 327.3. MPA-d₃ transition was 390.3 > 288.3. Before quantification of the MPA in kidney fat samples, the chemical residue, when detected, was identified according to the 2002/657/EC decision criteria (relative retention time and transition relative intensities) (European Commission 2002). Matrix calibration curves were used for MPA quantification (5 points, range 0–50 µg kg⁻¹).

Results

Method performance

As can be seen from the ion chromatograms shown in Figure 1, the specificity and sensitivity of the analytical method was fit-for-purpose. The multi-dimensional MS technology used for this study is nowadays recognised as the gold standard for target residue analysis (De Brabander et al. 2007; Noppe et al. 2008; Le Bizec et al. 2009). The use of a labelled internal standard (MPA-d₃) provided an accurate quantification, as the recovery was automatically taken into account.

Main observations

The screening of the 80 kidney fat samples did not reveal residues of progestagens other than MPA. Out of the 19 communes where animals were raised, synthetic hormone residues were detected in pork carcasses originating from 14
communes (73.6%). Figure 2 shows the communes where MPA-positive samples were detected: (a) Ambilobe; (b) Antsohihy and Bealanana; (c) Tsaratanana; (d) Ankadinondry-Sakay, Maroharona, Tsiroanomandidy Fihaonana, Tsinjoarivo and Miandrarivo; (e) Imerintsiatosika; (f) Ambatolampy, Ambohibary and Antsirabe; (g) Ambohimahasoa; and (h) Betroka. MPA-positive samples were detected in 10 out of 15 districts (66.7%) and in all eight surveyed regions except one (87.5%), namely, Diana region in Northern Madagascar. Out of the 50 individual samples selected among the first 10 positive pools, 10 fat kidney samples (20%) were tested positive for MPA contamination, according to the criteria described in the 2002/657/EC regulation (Table 1).

Table 2 indicates the number of pools of samples found to be contaminated within different ranges of levels of progestin MPA. Concentrations of MPA in pools are distributed between 0.5 µg kg\(^{-1}\) and 139 µg kg\(^{-1}\). For individual samples, concentrations of progestin MPA ranged from 0.5 µg kg\(^{-1}\) (for the less concentrated sample) to 187 µg kg\(^{-1}\) (for the most concentrated one).

Discussion

Our results highlighted a significant contamination of pork products by synthetic hormone residues, which was highly prevalent considering that such residues are not likely to occur according to Malagasy regulation, and compared also with the published literature (Heaton et al. 1996). The wide distribution observed in the Malagasy provinces, mainly in central and northern regions, confirmed a non-controlled extension of this fraud and the seriousness of the issue.

The multi-residue detection method showed that only MPA was present in our samples, confirming the large-scale misuse of one medicinal product only, and excluded other synthetic progestagen substances at this time. Previous studies reported the spread of the MPA contamination through the food chain by the supply of MPA-contaminated liquid feed to pig farms by the compound feed industry (Tielen 2003). However, because animal feed supply in Malagasy pig farms has been commonly based on crop and local raw materials, and little on compound feed, the hypothesis of a MPA contamination of pork by animal feed was discarded, especially because the MPA concentrations in specimens were high.

Investigations by Malagasy veterinary services found that farmers and animal health workers purchased syringes of progestagens (Confiance™, equivalent to Depo-Provera® 150 mg mL\(^{-1}\) Sterile Suspension for Injection, Pfizer (New York, NY, USA)). These low-price progestins were made easily available in private local dispensaries and in basic health centres (public sector) thanks to the efforts of international development agencies in the framework of the national family planning and reproductive

Figure 1. (colour online). SRM chromatograms for MPA and MPA\(_{d3}\) in MRPL spiked (left), blank (middle) and non-compliant (right) kidney fat sample.

Note: MPA, medroxyprogesterone acetate; MRPL, minimum required performance limit.
health programmes throughout the country (Office of Inspector General 2011). Without any control, farmers then had the opportunity to use MPA in pigs easily.

Consequently, Malagasy Veterinary and Public Health Services should henceforth advocate for a better control of human drugs in the pharmaceutical retailing system and a better concern by developing agencies and the medical sector for possible side effects (Pfizer Canada Inc. 2011). In particular, significant risk factors for osteoporosis largely exist in the Malagasy poor populations (e.g., alcohol abuse, chronic use of drugs, low body mass index or eating disorder), and, consequently, involuntary but regular ingestion of MPA in food by women might increase bone disorders in human population (Matson et al. 1997; Clark et al. 2004). MPA accumulation in organs over time was not demonstrated, but menstrual irregularity and possible immediate clinical effect (anaphylactic responses) of MPA residues in food should be investigated. Finally, epidemiological surveys about reproductive and bone disorders in human population, especially in female teenagers, should be urgently promoted.

Because official routine inspection of pig carcasses at abattoirs by veterinary services is ineffective to rapidly detect both hormones and antimicrobial drug residues, surveillance programmes using rapid tests should be promoted to better estimate the risk for consumers. However,
the analytical strategy needs to be re-thought within the framework of subsequent risk management programmes with the competent authority according to local constraints, for example, the financial aspects, the availability of MS techniques and technical capacity in laboratories. LC-MS-MS was the method of choice in our situation for detecting multi-residues at low concentrations under a risk assessment approach. Although no previous information was available about either the field situation or the expected concentration in the analysed matrix, the LC-MS-MS technique was suitable for a large screening of multiple residues, including both progestins and androgens. But, because only one component, that is, MPA, was detected in Madagascar with relatively high concentrations, ELISA methods, whose detection limits are of the order of 0.5 µg kg\(^{-1}\) for the screening of MPA in kidney fat, may be appropriate for veterinary inspection and could be put in place in Madagascar without a large investment (Chifang et al. 2006; Peng et al. 2008). GC-MS/MS (Smets et al. 1997) or high performance thin layer chromatography (HPTLC) methods may also be an alternative for MPA monitoring (De Brabander & Van Hoof 1990). In addition, for on-farm control, MPA, because of its lipophilic properties, could not be detected in urine but on the contrary very well in faeces (Hamoir et al. 1998; Impens et al. 2002, 2003). HPTLC and ELISA could be used to detect MPA in faeces, but LC-MS-MS could be used as a reference method (gold standard). However, faeces constitute a complex matrix to analyse and need many steps for purification. Fat tissues sampled by subcutaneous biopsies of superficial fat would be more appropriate to investigate MPA contamination in live animals.

However, African swine fever and other epizootic outbreaks have dramatically affected the Malagasy pig sector since 1998 (Ravaomanana et al. 2011) and led to the collapse of large-scale commercial farms. Resource-poor smallholder pig farms are now predominant (Randriamaparany et al. 2005; Ravaomanana et al. 2011). Biosecurity measures in these small-scale pig farms are poorly implemented (Costard et al. 2009; Madec et al. 2010), and pig farmers have poor knowledge about veterinary drugs (Rakotoharinome et al. 2013). Thus, public awareness campaigns are urgently needed to alert pig farmers and professionals about the negative consequences for public health of such misuse of synthetic hormones in animal. They should also clarify the content of official regulations, that is, prohibition of synthetic hormones in livestock farming, sanctions and control measures to implement. Veterinary universities and training programmes for technicians in animal production should also disseminate relevant up-to-date information to students and young professionals to improve their basic knowledge about veterinary drug use in livestock production and about drugs residues in animal products.

This first study confirmed the presence of synthetic hormones in pork kidney fat produced in Madagascar whatever the production area was, and MPA is the only progestagen that has been isolated. It showed the need for a national monitoring and information system dedicated to the livestock sector and animal products to better protect Malagasy consumers.

Acknowledgements
The authors gratefully thank the Service de Coopération et d’Action Culturelle from the French Embassy in Madagascar, the Regional Council of La Réunion (French overseas territory), the European Regional development Funds and the French government for their financial support through the QualiREG research network in Indian Ocean (www.qualireg.org). Thanks also to Mrs. Ravaomanana Fleurette, Mrs. Rabibisoa Lalao Francine and Mr. Rabenariavhyh René for their technical assistance and for collecting biological samples in abattoirs.

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