


Control for Stilbene Residues in Cattle in the Korça Region			Healthcare
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Abstract			
<p>In the period from 2013 to 2014, 160 random samples of urine from slaughtered and live cattle collected from different locations in Korca region in Albania were analyzed for the presence of the stilbene derivatives diethylstilbestrol (DES) and hexestrol (HEX). For detection of these substances is used ELISA test (MaxSignal) as fast screening method. Detection limit of ELISA test is respectively 0.15ng/ml for diethylstilbestrol and 0.25ng/ml for hexerol residues. From 160 urine samples of live cattle was detected positive with residues of diethylstilbestrol (DES) 6 samples or about 3.8 % of tested samples. The residues of hexestrol (HEX) were detected in 2 out 160 or 1.2% of total samples. The incidence of diethylstilbestrol (DES) in urine samples collected from cattle in slaughterhouses was identified in value 3.4% (2/58). There were not detected residues of hexestrol (HEX) in urine samples from slaughtered cattle.</p>			

1. Introduction

Natural hormones are produced by bodily organs in cattle and are transported into the bloodstream to affect sites regulating physiological responses such as growth, development and reproduction. Synthetic hormones cause the mimic actions in live animals (Arts et al., 1991). Diethylstilboestrol (DES) and hexoestrol as stilbenes are synthetic estrogen hormones were used in cattle production in the 1950s in USA and other developed countries (Raun, 2002). Stilbenes are broken down in different metabolic ways after ingestion and become non-functional. Usually cattle are treated by frequent injections or other ways of administration. There are many studies reporting DES as carcinogenic substance for humans (Bernstein et al., 1990). In 1987, The International Agency for Research on Cancer listed diethylstilboestrol in carcinogenic substances (Carlstrom, 1996). Codex Alimentarius Commission, as international food standard body, does not set safe level for DES in food (FDA, 2009). Stilbenes including DES are not allowed to be used in breeding of cattle for meat production. But stilbenes, including DES, are growth promoters of muscle and fat in cattle. Control of stilbenes in live animals and food is remaining not easy process. Many companies have developed analytical methods and have produced screening kits and equipments for detection of stilbenes and derivates in biological liquids as urine, blood as well direct from muscles or other tissues (Arts et al., 1991). Some method have been developed to determine residual stilbenes such as diethylstilbestrol (DES), dienestrol (DIS) and hexestrol (HS) in animal tissues using solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). The procedures for extraction, cleanup on an LC-Si solid phase extraction cartridge and derivatization of stilbenes were established and optimized in many laboratories. In some national monitoring programs are widely used screening tests as ELISA for qualitative and quantitative evaluation of stilbene residues in urine, blood and animal tissues. In this study we have used ELISA test as screening method for detection of stilbene residues in urine samples.

The study aim was focused on detection of diethylstilbestrol and hexestrol in urine samples collected from different farms and slaughterhouses in Korca region in Albania.

2. Materials and method

As study material are used urine samples are collected from live cattle in different locations from farms and slaughterhouses in region of Korca in Albania. The urine samples are collected from farms and slaughterhouses in Devoll, in Pogradec and in Kolonja districts. All samples are taken in aseptically way and are kept and transported in 4° C to laboratory. The chosen procedure was ELISA essay as confirmatory test. The advantage of this test consists on analyzing a large numbers of samples in relatively short period of time. Diethylstilbestrol and hexestrol (HEX) residues were determined by ELISA test as a screening system, which is simple, rapid, sensitive and cost-effective compared with traditional methods. MaxSignal™ ELISA Test Kit enables to detect diethylstilbestrol and hexestrol in animal matrices in response to customer concerns about food safety. The method is based on a competitive colorimetric ELISA assay. The substance of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target substance. If the residue is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is binding with the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample. There are used three different antibodies to detect respectively diethylstilbestrol, hexerol residues in urine samples collected from farms and slaughterhouses in Korca region. Samples were refrigerated at 2-4°C for no more than 1-2 days. For long period of store samples have been kept in freezing at -20°C. Frozen samples were thawed at room temps (20–25°C) or in a refrigerator before use. Detection limit of this kit for Diethylstilbestrol in urine is 0,15pp/g. MaxSignal™ Diethylstilbestrol (DES) ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). The kit is stored at 2-8°C. The shelf life is 12 months when the kit is properly stored. The test was performed according to instruction for use.

3. Results and discussion

During the period 2013-2014 there was collected urine samples from farms and slaughterhouses in Korca. All samples are analyzed for stilbene residues using ELISA kit “MaxSignal™”.

Table 1. Results of analytical control by performing ELISA test for stilbene residues (diethylstilbestrol and hexerol) in 160 urine samples collected from slaughtered and live cattle in region of Korca from 2013-2014.

Urine samples	No. samples	No. of positive samples (stilbene residues)	No. of positive samples (diethylstilbestrol)	No. of positive samples (hexerol)
Farms	102	6/102	4/102	2/102
Slaughterhouses	58	2/58	2/58	0/58
Total	160	8/160 (5%)	6/160 (3.8%)	2/160 (1.2%)

This study carried from 2013-2014 confirmed that there are evident use of stilbenes in cattle in region of Korca in Albania. From 160 urine samples taken from cattle in region of Korca are identified positive with the stilbene residues 5% or 8 out 160.

Results for detection of diethylstilbestrol showed the incidence 3.8% or 6 out 160 urine samples collected from farms and slaughterhouses. Check with ELISA test confirmed the positive results for hexestrol residues in 1, 2% (2/160) of urine samples.

Feeding cattle with DES is practical because of the ease of administration, no undesirable side-effects, and the potential to withdraw the compound and because feeding allows the accurate administration of a constant dosage (Scippo et al., 1994). The physiological concentrations of this hormone are increased and blood levels changed according to dosage (Yang et al., 2009).

The administration of DES to laboratory animals during shown that this synthetic oestrogen is responsible in female mice for the following pathological entities: structural malformations of the oviduct, uterus, cervix, and vagina. It is reported that use of DES cause salpingitis, paraovarian cysts and vaginal adenocarcinoma (Scippo et al., 1993).

In addition, to these anatomic and tissue-based malformations, infertility and sterility were observed in adult mice of both sexes. Studies have established reference values for these substances in plasma and urine in values 0.15 ppb/ml (Chilt & Stephany, 1996). Some studies demonstrated that detection of DES is not easy because diethylstilbestrol concentration in the plasma after treatment is decreased (Newbold et al., 2000).

On the one hand, it has also been shown that DES modifies function of the male genital tract by interfering with effects of androgens on the normal Mullerian ducts. So, administration of DES to male mice during the perinatal period results in the appearance of testicular tumors during adulthood (Newbold et al. 2000).

Authors mentioned that tumor incidence is increased in the second and third generations of those males who were originally injected with diethylstilbestrol.

4. Conclusions

It is concluded that the use of stilbene derivatives in cattle in Albania is increased. Of 160 samples investigated, only 8 (5%) were indicated as stilbene positive. The presence of DES was confirmed in 6 samples or 3.8%. Stilbene residues in live cattle and beef expose the Albanian consumers and are remaining possible risk. Breeding of cattle in Korca region is associated with use of banned substances as diethylstilbestrol and hexerol derivates. Study results confirmed that there is illegal treatment of cattle with stilbenes.

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