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Ochratoxin A in the morning and afternoon portions of urine from Coimbra and Valencian populations ☆

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ABSTRACT

The widespread contamination of foodstuffs and beverages by mycotoxins, such as ochratoxin A (OTA), has made the monitoring of human contamination levels essential.

By using a sensitive, accurate and speedy method that combines extraction with 5% $NaHCO_3$, immunoaffinity column clean-up and HPLC with fluorescence detection, the human exposure to OTA through urine analysis can be monitored. This method is less invasive than blood monitoring and has the potential to be a good marker of human exposure. The limit of quantification of the method was 0.007 ng/mL of urine, with recoveries of OTA, from urine samples spiked at levels between 0.02 and 0.1 ng/mL, higher than 91% with RSD lower than 15.5%.

This study evaluated OTA contamination levels in human urine sample fractions, collected in the morning and afternoon, in two populations, one from Coimbra city, in Portugal, and another from the Valencian community, in Spain. In the Coimbra population, 60 samples from 30 healthy individuals were analyzed, levels of OTA in 13 morning samples and 14 afternoon samples having been detected, with concentrations ranging from 0.011 to 0.208 and 0.008 to 0.11 ng/mL respectively. In the Valencia population, 62 samples from 31 healthy individuals were analyzed, with OTA being detected in 25 morning samples and 26 afternoon samples. The concentrations varied between 0.007 and 0.124 ng/mL in the morning samples, and 0.008 and 0.089 ng/mL in the afternoon samples. Significant differences were found between the morning levels of OTA from both populations ($P = 0.033$). For afternoon samples, significant differences were not found, P value = 0.163.

1. Introduction

Ochratoxin A (OTA) is a secondary fungal metabolite produced by several *Aspergillus* and *Penicillium* species, mainly *Aspergillus alutaceus* in warmer and tropical climates, and *Penicillium verrucosum* in temperate climates (Moreno Guillamont et al., 2005). Due to the ubiquitous nature of these fungal species, OTA is one of the most prevalent human contaminants in the food chain. Its presence, in the human body, is due to the ingestion of small quantities present in a variety of foodstuffs and beverages. Namely, in Portugal and Spain, due to high consumption of cereals and cereal products, spices, rice,

☆ *Ethical statement*: The collection of human urine was according to Declaration of Helsinki and it was based only in the evaluation of OTA concentration in the urine of different groups of populations. This research project does not involve any human risks for the participating subjects, since no ingestion or administration was given to the subjects. Each individual involved in the study also given a signed authorization allowing the collection of urine for the study of the evaluation of OTA levels. The subjects were volunteers, and were asked to complete a rapid questionnaire about age, clinical history, and occupation.

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dried fruits, coffee, milk, beer and wine (Bascarán et al., 2007; González et al., 2006; González-Osnaya et al., 2007, 2008; Burdaspal and Legarda, 2007; Blesa et al., 2006; Pena et al., 2005; Juan et al., 2006, 2007, 2008; Lino et al., 2006; Visconti et al., 2000; Skaug et al., 2001) this fact is also verified. This mycotoxin is also detected in meat products due to transmission into muscle, kidney, liver and blood from animals fed with natural contaminants (Moreno Guillamont et al., 2005).

Toxicological studies have demonstrated OTA's nephrotoxic, hepatotoxic, immunotoxic, teratogenic and carcinogenic activity towards several animal species, having been classified by the International Agency for Research on Cancer (IARC) as a possible carcinogen to humans (Group 2B) (IARC, 1993). This mycotoxin is also suspected of being involved in the etiology of Balkan endemic nephropathy and is associated with the occurrence of urinary tract tumors (Pfohl-Leszkowicz et al., 2002).

Human exposure to OTA has clearly been demonstrated in studies performed in blood, urine and breast milk samples (Dinis et al., 2007; Lino et al., 2008; Pena et al., 2006; Skaug et al., 2001). This exposure is further enhanced due to OTA's unusually long serum half-life, as a result of its ability to bind to plasma protein, its enterohepatic circulation and its re-absorption from urine (Studer-Rohr et al., 2000).

OTA excretion from the human body is mainly done by elimination through urine (Li et al., 1997). However, the concentration levels of this mycotoxin in urine are very low, therefore a sensitive and accurate method of detection is required to monitor OTA contamination. Due to recent developments in analytical methodology, urinary monitoring is now more feasible, and has the advantage of being less invasive than blood monitoring, having the potential to be a good marker of human exposure to this mycotoxin (Gilbert et al., 2001). However, data in the frequency and concentration of OTA in human urine are still scarce.

Only a few studies were carried out in Europe (Domijan et al., 2003; Petkova-Bocharova et al., 2003; Pascale and Visconti, 2000; Pena et al., 2006; Fazekas et al., 2005; Gilbert et al., 2001), Africa (Jonsyn-Ellis, 1999, 2000) and Asia (Castegnaro et al., 1990) reporting the presence of OTA in urine human samples. These studies have shown that OTA occurs in urine in different mean concentrations, oscillating between 0.027 ng/mL in Portugal (Pena et al., 2006) and 2.39 ng/mL in Croatia (Domijan et al., 2003).

The most widely employed analytical methodology for OTA quantification is liquid chromatography (LC) with fluorescence or mass spectrometry detection coupled with immunoaffinity column (IAC) clean-up. Since OTA has good fluorescence properties, it can be accurately and sensitively determined with fluorescence detection.

The present study is the first approach to follow-up OTA levels and frequency of detection in urine samples collected in the morning and afternoon, from samples of healthy inhabitants from two cities, Coimbra (Portugal) and Valencia (Spain), in order to evaluate the differences between OTA levels in the more concentrated morning urine samples and the afternoon urine samples, and to evaluate the populations' contamination.

2. Experimental

2.1. Solvents and materials

Acetonitrile, toluene and methanol (HPLC grade) were purchased from Carlo Erba (Milan, Italy). Acetic acid, hydrochloric acid, sodium sulfate anhydrous, sodium hydroxide, sodium hydrogen carbonate, sodium chloride, potassium chloride, potassium dihydrogen-phosphate and anhydrous disodium hydrogen-phosphate were obtained from Merck (Darmstadt, Germany). OTA and boron trifluoride 14% methanolic solution were purchased from Sigma Chemicals Co. (St. Louis, USA).

OTA stock solution (250 μg/mL) was prepared by dissolution of the solid standard in 4 mL toluene–acetic acid (99:1), and stored at −20 °C. An intermediate standard solution (10 μg/mL) was prepared diluting 1 mL of the stock standard solution with 25 mL toluene–acetic acid (99:1). The actual concentration of the OTA was calculated using an ultraviolet light spectrophotometer set at 333 nm ($\varepsilon = 5440$) (Entwisle et al., 2000).

For the fortification assays, a standard solution (1 μg/mL) was prepared in toluene–acetic acid (99:1). To derive the calibration curve, a standard solution was prepared evaporating 100 μL of the intermediate standard solution until dryness, under a gentle stream of nitrogen. The residue was then diluted in 10 mL of mobile phase, a mixture consisting of acetonitrile–water–acetic acid (49.5:49.5:1 v/v/v). This working standard solution was then diluted accordingly to prepare the external calibration curve (1, 2, 5, 10 ng/mL).

The phosphate buffer saline (PBS) solution was prepared from potassium chloride (0.2 g), potassium dihydrogen-phosphate (0.2 g), anhydrous disodium hydrogen-phosphate (1.2 g) and sodium chloride (8 g), added to distilled water (900 mL). After dissolution, the pH was adjusted to 7.4, with 0.1 M HCl or 0.1 M NaOH as appropriate. The solution was made to 1 L.

The water used was purified by distillation and passage through a Milli Q system (Millipore, Bedford, MA). All chromatographic solvents and water were filtered through a 0.20 μm membrane filter (∅ 50 mm Schleicher & Schuell, Germany) under vacuum and degassed for 15 min in an ultrasonic bath.

Clean-up was performed using IACs OchraTest™ (Vicam/Watertown, USA). A vortex mixer Retsh (Haan, Germany) and an ultrasonic bath Sonorex RK 100 (Berlin, Germany) were also used.

Amber glassware was used to prevent light deterioration of the mycotoxin. Decontamination of the glassware was performed using a sodium hypochlorite solution. It was then acid-washed by immersing the glassware in a solution of 4 mL/L H_2SO_4, followed by washing to neutral pH by rinsing with distilled water.

2.2. Apparatus and chromatographic conditions

The HPLC system consisted of a Gilson pump, model 307 (Gilson Medical Electronics, Villiers-le-Bel France), a Rheodyne injector, model 7125 (Cotati, CA, USA) and a Perkin-Elmer spectrofluorimeter, model LS45 (Perkin-Elmer, Beaconsfield, UK), operated at an excitation wavelength of 333 nm and an emission wavelength of 477 nm. The spectral bandwidth was 10 nm for both excitation and emission. The results were recorded on a Hewlett-Packard integrator, model 3390 A (Hewlett-Packard, Philadelphia, USA).

A C_{18} Nucleosil 5 μm 120 Å (4.0 × 30 mm i.d.) guard column and a C_{18} Nucleosil 5 μm 100 Å (4.6 × 250 mm i.d.) column were used.

The mobile phase consisted of a mixture of acetonitrile–water–acetic acid (49.5:49.5:1 v/v/v) maintained at a flow rate of 1 mL/min, and at room temperature.

For the fluorescence detection of OTA, the emission and excitation wavelengths were optimized by obtaining the spectrum of OTA standard solution in HPLC mobile phase. At an excitation wavelength of 333 nm, the maximum emission wavelength was found to occur at 477 nm.

Isocratic analysis under the conditions described above allowed the elution of OTA with good resolution.

2.3. Extraction and clean-up

Extraction and clean-up were performed according to the method developed by Pena et al. (2006). Briefly, human urine diluted with a solution of $NaHCO_3$ and filtered was cleaned up through the OchraTest™ IAC, and eluted with methanol. The eluted extract, after evaporation to dryness under a gentle stream of nitrogen at 50 °C, was dissolved in 120 μL of the HPLC mobile phase, instead of 150 μL of the original method in order to improve the LOQ. Finally, 50 μL was injected into the HPLC system.

2.4. Sampling

In the city of Coimbra were collected 30 morning urine samples and 30 afternoon urine samples, from 30 healthy resident individuals. From the 30 participants involved, 57% ($n = 17$) were female, aged 15–55 years old, and 43% ($n = 13$) were male, aged 22–67 years old.

The samples from the Valencian community were collected from 31 healthy individuals, 31 being from the morning portion of urine and 31 from the afternoon portion. Of the participants involved, 68% ($n = 21$) were female, aged 24–53, and 32% ($n = 10$) were male, aged between 18 and 51.

Urine samples were collected between September 2005 and February 2006 in Coimbra, and in April 2005 at Valencia.

After collection, the samples were stored at −20 °C until analysis.

All volunteers were asked to fill a rapid questionnaire about age, gender, height and weight. They were clearly informed and gave their written permission.

2.5. Chemical confirmation of OTA by methyl ester formation

In order to confirm the presence of OTA in the positive samples, a chemical confirmation was performed using the Castegnaro et al. (1990) method. This method involves the formation of an OTA methyl ester using boron trifluoride. In this procedure, extracts from the positive samples were evaporated to dryness and 150 μL of boron trifluoride 14% methanolic solution was added. The mixture was then left at 60 °C for 10 min, after which it was evaporated under a gentle stream of nitrogen. The residue was then dissolved in 150 μL of mobile phase and 50 μL was injected into the HPLC system.

2.6. Statistical analysis

Database management and statistical analysis were performed using SPSS (Statistical Package for the Social Science), Microsoft version 10.0. The Pearson chi-square test was used to test for significant differences between groups. $P \leqslant 0.05$ (two-tailed) was considered to be statistically significant. For statistical analysis, if the concentration was below the limit of quantification (LOQ) it was set to 50% of that limit when the mean and SD were calculated.

3. Results

The calibration curve was obtained using the linear least-squares regression procedure of the peak area versus the concentration. The linearity using LC–FD for OTA, in the working standard solutions at three determinations of four concentration levels, between 1 and 10 ng/mL (1.0, 2.0, 5.0 and 10.0 ng/mL), was good as shown by the correlation coefficient (r^2) determined, 0.998.

The proposed analytical method was optimized in order to obtain good accuracy and precision. The accuracy of the method was evaluated by analyzing spiked OTA-free sample, at three different levels, 0.02, 0.05 and 0.1 ng/mL, with mean recoveries ranging from 91.9% to 96.7%. The intra-day repeatability varied between 4.8% and 7.9%, and the inter-day repeatability between 12.5% and 15.4% for the above-mentioned fortification levels (Table 1).

The LOQ of OTA in urine was 0.007 ng/mL, determined at a signal-to-noise ratio of approximately 10:1.

Table 1
Accuracy and inter- and intra-assay validation results ($n = 3$)

Fortification level (ng/mL)	Recovery mean (%)	RSD intra-day[a] (%)	RSD inter-day[b] (%)
0.02	91.9	7.9	15.4
0.05	91.9	5.1	12.5
0.1	96.7	4.8	15.2

[a] Repeatability based on three replicates at 0.02, 0.05 and 0.1 ng/mL within the same day.

[b] Three days' repeatability based on the analysis at 0.02, 0.05 and 0.1 ng/mL day.

Fig. 1 shows representative LC spectrofluorimetric chromatograms obtained from an OTA standard solution, from one fortified urine sample, one positive morning sample and one afternoon sample.

Using the above-described methodology, 60 samples of human urine, from 30 healthy individuals living in the Portuguese city of Coimbra, were analyzed. Two samples were collected from each individual in the same day, one in the morning and one in the afternoon. OTA was detected in 13 of the morning samples (43.3%), with concentrations ranging from 0.011 to 0.208 ng/mL, and in 14 of the afternoon samples (46.7%), with concentrations ranging from 0.008 to 0.11 ng/mL. The mean concentrations and standard deviation in the morning and afternoon samples were 0.019 ± 0.041 and 0.018 ± 0.027 ng/mL, respectively. Significant differences were found between the morning and afternoon OTA levels, with $P = 0.000$ (Table 2).

Comparing the data by gender, OTA was detected in 53.8% of the male morning samples and in 61.5% of the afternoon samples, with concentrations ranging from 0.015 to 0.208 ng/mL in the morning samples, and 0.008 and 0.110 ng/mL in the afternoon. The mean concentration and standard deviation were 0.026 ± 0.056 and 0.023 ± 0.031 ng/mL for the morning and afternoon samples, respectively. In the female samples, OTA was detected in 35.3% of both the morning and afternoon samples, with concentrations ranging from 0.011 to 0.093 ng/mL in the morning, and 0.014 to 0.079 ng/mL in the afternoon. The mean concentrations and standard deviation were 0.013 ± 0.025 ng/mL for the morning female samples and 0.014 ± 0.024 ng/mL for the afternoon samples. Significant differences were not found between females and males in the morning samples ($P = 0.168$) or in the afternoon samples, with a P value of 0.279 (Table 3).

The same method and conditions were used to analyze the 62 samples of human urine collected from 31 healthy individuals living in the Spanish city of Valencia. OTA was present in 25 (80.6%) of the morning samples and 26 of the afternoon samples (83.9%), in concentrations ranging from 0.007 to 0.124 ng/mL in the morning and 0.008 to 0.089 ng/mL in the afternoon. The mean concentrations and standard deviation were 0.032 ± 0.031 and 0.028 ± 0.019 ng/mL in the morning and afternoon samples, respectively. Significant differences were found between the morning and afternoon OTA levels, with $P = 0.002$ (Table 2).

Comparing the frequency of detection and OTA levels by gender, it was observed that, in the female urine samples, OTA was present in 80.9% of the morning samples, and in 85.7% of the afternoon samples, with concentrations varying from 0.007 to 0.114 ng/mL and from 0.008 to 0.075 ng/mL in the morning and afternoon samples, respectively. The mean concentration and standard deviation for the morning samples and afternoon samples were 0.033 ± 0.031 and 0.027 ± 0.021 ng/mL, respectively.

In the male urine samples, OTA was detected in 80% of both the morning and afternoon samples, with concentrations ranging from 0.01 to 0.124 ng/mL in the morning and 0.007 to 0.089 ng/mL in the afternoon. The mean

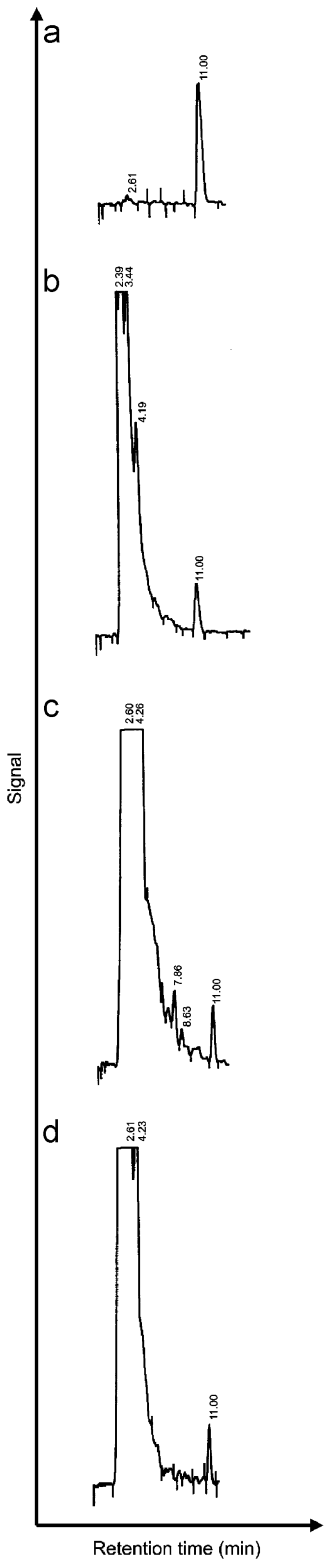

Fig. 1. Chromatograms of ochratoxin A (OTA) standard solution (a) (5 ng/mL), a fortified sample (b) (0.05 ng/mL), a morning positive sample (c) (0.015 ng/mL of urine) and an afternoon positive sample (d) (0.017 ng/mL of urine). Both the morning and afternoon positive samples are from the same individual.

concentration and standard deviation were 0.029 ± 0.036 and 0.031 ± 0.028 ng/mL for the same samples, respectively. Moreover, the mean concentration of morning female samples was higher than that of male samples, 0.033 versus 0.029 ng/mL, without significant differences ($P = 0.466$). An opposite situation was observed for the afternoon samples, 0.031 ng/mL for male samples versus 0.027 ng/mL for female samples. Significant differences were not found ($P = 0.134$) (Table 3).

When comparing the results from both cities it was shown that the Spanish city of Valencia had a higher frequency of detection and a higher OTA mean concentration, in both the morning and afternoon samples. However, significant differences were only found in the morning samples, $P = 0.033$. In the afternoon samples, no differences were found ($P = 0.163$).

4. Discussion

This study revealed that the frequency of detection in the Coimbra population is higher than in the Sierra Leone population, about 24–25% (Jonsyn-Ellis, 1999, 2000), similar to that from India, 40% (Castegnaro et al., 1990), and lower than Italy (Pascale and Visconti, 2000), Hungary (Fazekas et al., 2005), the UK (Gilbert et al., 2001), Bulgaria (Petkova-Bocharova et al., 2003) and Croatia (Domijan et al., 2003) populations, with 58%, 60%, 92%, 87–92% and 94%, respectively. A similar situation was observed in studies conducted in the Coimbra population in 2005, with 70% (Pena et al., 2006). The Valencian population revealed frequencies of detection similar to those found in the Belia Izvor population at Bulgaria, 87.3%, and lower than Gorno Peshtene at Bulgaria (Petkova-Bocharova et al., 2003), 92%, and in Croatia, 94% (Domijan et al., 2003) (Table 4).

Comparing our results, in terms of contamination levels determined, with other studies from different populations, the concentrations found in the Coimbra population, 0.019 ± 0.041 ng/mL, are higher than mean concentrations in Hungary, 0.013 ng/mL (Fazekas et al., 2005), lower than those found in the Coimbra population in 2005, 0.027 ± 0.004 ng/mL (Pena et al., 2006), in Bulgaria populations, 0.0508 ± 0.044 and 0.168 ± 0.111 ng/mL (Petkova-Bocharova et al., 2003) and in Croatia, 2.39 ± 1.29 ng/mL (Domijan et al., 2003). Regarding the results from the Valencian community, 0.03 ± 0.031 ng/mL, it is observed that they are similar to those found in the Coimbra population in 2005 (Pena et al., 2006), lower than those from different populations in Europe (Petkova-Bocharova et al., 2003; Domijan et al., 2003) and higher than the Hungarian population (Fazekas et al., 2005) (Table 4).

Regarding the OTA ranges in urine for both populations, 0.008–0.208 ng/mL in Coimbra and 0.007–0.124 ng/mL in Valencia, they are similar to those found in some European countries such as Italy (Pascale and Visconti, 2000) and the UK (Gilbert et al., 2001). However, for most countries a great variation in the range of OTA levels is observed (Table 4).

Previous studies about OTA serum levels in healthy populations or nephropathic patients (Lino et al., 2008; Jimenez et al., 1998; Dinis et al., 2007) clearly show the exposure of Portuguese and Spanish populations to this mycotoxin. This is corroborated with the presence of OTA in Spanish and Portuguese foodstuffs such as cereals and cereal products, rice, dried fruits, milk and wine (Bascarán et al., 2007; González et al., 2006; González-Osnaya et al., 2007, 2008; Burdaspal and Legarda, 2007; Blesa et al., 2006; Pena et al., 2005; Juan et al., 2006, 2007, 2008).

Regional differences in OTA levels in biological fluids in different populations may be related to different situations. Factors such as climatic conditions of the different areas, Coimbra and Valencia, and probably the ingestion of

Table 2
Mean, standard deviation, range (ng/mL) and frequency (%) of OTA in morning (M) and afternoon (A) urine samples from Coimbra and Valencia

Samples	Frequency (%)	Range (ng/mL)	Mean ± RSD (ng/mL)	*P* value
Coimbra M[a,b]	43.3	0.011–0.208	0.019 ± 0.041	0.000[a]
Coimbra A[a,c]	46.7	0.008–0.110	0.018 ± 0.027	0.002[d]
Valencia M[d,b]	80.6	0.007–0.124	0.032 ± 0.031	0.033[b]
Valencia A[d,c]	83.9	0.008–0.089	0.028 ± 0.019	0.163[c]

[a] Coimbra: morning versus afternoon.
[b] Morning: Coimbra versus Valencia.
[c] Afternoon: Coimbra versus Valencia.
[d] Valencia: morning versus afternoon.

Table 3
Mean, standard deviation, range (ng/mL) and frequency (%) of OTA in female (W) and male (M) morning (MOR) and afternoon (AFT) samples from Coimbra and Valencia

Gendre-city	Frequency (%)	Range (ng/mL)	Mean ± RSD (ng/mL)	*P* value
M-Coimbra-MOR[a]	53.8	0.015–0.021	0.026 ± 0.056	0.168[a]
W-Coimbra-MOR[a]	35.3	0.011–0.093	0.013 ± 0.025	
M-Valencia-MOR[b]	80.0	0.01–0.124	0.029 ± 0.036	0.466[b]
W-Valencia-MOR[b]	80.9	0.007–0.114	0.033 ± 0.031	
M-Coimbra-AFT[c]	61.5	0.008–0.110	0.023 ± 0.031	0.279[c]
W-Coimbra-AFT[c]	35.3	0.014–0.079	0.014 ± 0.024	
M-Valencia-AFT[d]	80.0	0.007–0.089	0.031 ± 0.028	0.134[d]
W-Valencia-AFT[d]	85.7	0.008–0.075	0.027 ± 0.021	

Table 4
Ochratoxin A in human urine samples from different countries

Country	% Detection	Range (ng/mL)	Mean (ng/mL)	Reference
Bulgaria				Petkova-Bocharova et al. (2003)
Gorno Peshtene ($n = 25$)	92	0.010–0.33	0.0508 ± 0.044	
Beli Izvor ($n = 55$)	87.3	0.010–1.910	0.168 ± 0.111	
Croatia—eastern ($n = 35$)	94	0.99–5.22	2.39 ± 1.29	Domijan et al. (2003)
Italy ($n = 38$)	58	0.012–0.046	–	Pascale and Visconti (2000)
India ($n = 152$)	40	0.005–0.03	–	Castegnaro et al. (1990)
Hungary ($n = 88$)	61	0.0006–0.65	–	Fazekas et al. (2005)
UK ($n = 50$)	92	0.01–0.058	0.013	Gilbert et al. (2001)
Sierra Leone				Jonsyn-Ellis (2000)
Dry season ($n = 244$)	25.8	0.07–148.0		
Rainy season ($n = 190$)	24.7	0.6–72.2		
Sierra Leone ($n = 54$)	24	0.3–26.6		Jonsyn-Ellis (1999)
Portugal—Coimbra ($n = 60$)	70	0.021–0.105	0.027 ± 0.004	Pena et al. (2006)
Present study				
Portugal—Coimbra ($n = 30$)	43.3	0.011–0.208	0.019 ± 0.041	
Spain—Valencia ($n = 31$)	80.6	0.007–0.124	0.03 ± 0.031	

item foods with deficient procedures of conservation have great importance. Different consumption habits, consumption of alcoholic beverages, such as beer and wine, and regional food intake patterns may contribute to the difference in OTA concentrations (Lino et al., 2008). Regional differences have also been observed in different countries, as previously described (Table 4). The climate may also be responsible for fluctuations in human exposure, because the consumed food may be contaminated at different levels under different climatic conditions (Jimenez et al., 1998). Climatic factors such as temperature, humidity, O_2 versus CO_2 ratio as well as social conditions and behavior as methods of food preservation, and environmental pollution are all accumulating factors that enhance proliferation of toxigenic fungi (Wafa et al., 1998).

In spite of a study in the UK that concluded that OTA in urine is a better indicator of its consumption than OTA in plasma (Gilbert et al., 2001), Studer-Rohr et al. (2000) showed that in Swiss human volunteers dosed with tritiated OTA, only 42–54% of the radioactivity that appeared in urine corresponded to unchanged OTA, showing that further work needs to be done to verify if urine concentrations are a short- or a long-term indicator of exposure to this mycotoxin.

5. Conclusions

This study showed that there are significant differences between OTA urine levels in morning and afternoon samples from both analyzed populations. It was also demonstrated that frequency of detection and mean concentration are higher in the Valencian community than in the Coimbra population.

It was also observed that between genders, significant differences were not found, in both morning and afternoon samples, in the same analyzed population.

In addition, the data of this study demonstrated that both populations are less exposed to OTA than the generality of the European countries.

The widespread contamination of the world population, due to ingestion of OTA-contaminated commodities, is a reality. Therefore it is necessary to continue to monitor OTA levels, in order to evaluate the exposure of the populations to this toxin.

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