Co-occurrence of Ochratoxin A and Citrinin in Cereals from Bulgarian Villages with a History of Balkan Endemic Nephropathy

Terry Vrabcheva,[†] Ewald Usleber,*[,‡] Richard Dietrich,[‡] and Erwin Märtlbauer[‡]

Mycotoxicological Laboratory, National Center for Hygiene, Medical Ecology and Nutrition, 15 Dimitar Nestorov Str., 1431 Sofia, Bulgaria, and Institute for Hygiene and Technology of Food of Animal Origin, University of Munich, Veterinärstrasse 13, 80539 Munich, Germany

Cereal samples were collected in 1998 from Bulgarian villages without [control village (C), $n = 20$] or with [endemic villages (E); E1, $n = 21$; E2, $n = 30$; E3, $n = 23$] a history of Balkan endemic nephropathy (BEN). Sampling included foods (wheat, corn) and feeds (barley, oats, wheat bran). Analysis of ochratoxin A and citrinin was done by enzyme immunoassays (EIA), with detection limits of 0.5 and 5 ng/g, respectively. Ochratoxin A-positive results were confirmed by HPLC after immunoaffinity chromatography. Highest toxin levels were found in wheat, wheat bran, and oats. For ochratoxin A, the percentages of positives were 35% (C), 29% (E1), 30% (E2), and 47% (E3), the mean/median values of positives were 1.5/1.3 ng/g (C), 11/1.6 ng/g (E1), 18/1.6 ng/g (E2), and 3.5/1.5 ng/g (E3). For citrinin, 5.0% (C), 14% (E1), 3.3% (E2), and 13% (E3) were positive, and the mean/median values were 6.1/6.1 ng/g (C), 180/83 ng/g (E1), 10/10 ng/g (E2), and 84/20 ng/g (E3). Highest concentrations of ochratoxin (maximum = 140 ng/g) and citrinin (maximum = 420 ng/g) were found in samples from endemic villages. Co-contamination with ochratoxin A and citrinin was found for one sample (14% of positives) from village C and for six samples (22% of positives) from villages E1−E3. Citrinin levels in these samples were 2−200 times higher than those of ochratoxin A.

Keywords: *Mycotoxin; ochratoxin A; citrinin; cereals; nephropathy; immunochemical assay*

INTRODUCTION

Balkan endemic nephropathy (BEN) is a noninflammatory bilateral kidney lesion that affects rural populations in several circumscribed areas of Bulgaria, Romania, and parts of former Yugoslavia (Krogh, 1992; Čeovič et al., 1992; Stoev, 1998). Although the disease was recognized in the mid-1950s and has been studied extensively since, its etiology and many epidemiological features remain obscure. A number of hypotheses have been investigated with respect to the involvement of various environmental factors, including heavy metals, molds, radiation, and organic matter, but none of these has gained satisfactory epidemiological support. The possibility that mycotoxins may be linked to this disease, however, is supported more and more strongly by evidence from studies within the laboratory and in human populations (Chernozemsky, 1991). The mycotoxin mostly frequently suspected to be responsible for BEN is ochratoxin A. In addition to BEN countries, ochratoxin A in the food chain and in human blood serum has been correlated with renal failure in humans in studies from North Africa (Zimmerli and Dick, 1995; Maaroufi et al., 1996).

Ochratoxin A is a nephrotoxic and carcinogenic mycotoxin produced by several *Aspergillus* and *Penicillium* species. The toxin frequently occurs as a contaminant in cereals worldwide, predominantly during storage. Ochratoxin A is known to cause mycotoxic porcine nephropathy (MPN), a disease resembling BEN, which is a major reason it is suspected to be implicated with BEN (Krogh, 1992; Stoev, 1998). However, no clear proof for this hypothesis has been presented so far.

Citrinin, another nephrotoxic mycotoxin produced by *Aspergillus*, *Penicillum*, and *Monascus* spp., has been given comparatively less consideration, although it is similar in toxicology and pathology to ochratoxin A and although both toxins may have additive or synergistic effects (Kanisawa, 1984; Hanika and Carlton, 1994; Föllmann et al., 1998). Citrinin is known as a contaminant in a variety of cereals (Abramson, 1997) and in rice fermented with *Monascus* spp. (Dietrich et al., 1999) and has been found together with ochratoxin A in feed grains (Krogh et al., 1973), but very few studies included parallel analyses of both toxins.

A range of analytical methods for ochratoxin A is available, including enzyme immunoassay (EIA) techniques (Märtlbauer and Terplan, 1988) and immunoaffinity chromatography/high-performance liquid chromatography (IAC/HPLC) (Van Egmond, 1991; Scudamore and MacDonald, 1998). Because ochratoxin A binds to serum proteins (Hagelberg et al., 1998) and is easily detected in blood serum, the average daily intake of this toxin may be calculated not only from analysis of food but from blood serum levels (Breitholtz et al., 1991). Numerous data on the daily intake of ochratoxin A are available from many countries (Hald, 1991; Krogh, 1992; Scott et al., 1998). However, although ochratoxin A has been the subject of intensive studies during the past 10 years in many European countries, data on its occurrence in cereals and in human blood serum from Bulgaria and other main BEN areas have not been

* Corresponding author (telephone +49 89 2180-2256; fax +49 89 2180-3793; e-mail E.Usleber@mh.vetmed.uni-muenchen.de).

[†] National Center for Hygiene, Medical Ecology and Nutrition.

[‡] University of Munich.

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obtained for almost 10 years now (Petkova-Bocharova and Castegnaro, 1985; Petkova-Bocharova et al., 1988, 1991).

If recent information about ochratoxin A from BEN areas has to be regarded as insufficient, even less is known about the occurrence of citrinin in cereals from Bulgarian BEN regions. Only one study using thin-layer chromatography for citrinin determination indicated that contamination frequency and contamination levels of beans and corn with citrinin and ochratoxin A were similar in the period of 1984−1990 (Petkova-Bocharova et al., 1991). The main reason for this limited information is the lack of suitable analytical routine methods for citrinin with sensitivities comparable to those achieved for ochratoxin A. Physicochemical detection methods described so far (Gimeno, 1984; Trantham and Wilson, 1984; Lepom, 1986; Dick et al., 1988; Scudamore et al., 1998) require a tedious sample extract cleanup with large volumes of halogenated solvents and suffer from problems such as loss of the toxin during solid-phase extraction, instable chromatographic behavior of citrinin, or poor sensitivity and recovery. EIAs for citrinin using polyclonal antiserum have been described (Abramson et al., 1995, 1996), but detection limits in cereals were ≥100 ng/g. With the availability of a highly sensitive monoclonal antibody-based EIA, routine screening for citrinin at the low nanogram per gram level became feasible only recently. The aim of this study was therefore to analyze cereal samples from rural villages of Bulgaria (with and without a history of BEN) for both ochratoxin A and citrinin.

EXPERIMENTAL PROCEDURES

Materials. Rabbit antiserum against ochratoxin A, the immunoglobulin fraction precipitated with 70% saturated ammonium sulfate and dialyzed against phosphate-buffered saline (PBS, phosphate buffer 0.01 mol/L, pH 7.2−7.3, containing 0.1 mol/L NaCl), and ochratoxin A−horseradish peroxidase conjugate were used as described earlier (Märtlbauer and Terplan, 1988). Details on the production of monoclonal antibodies against citrinin (clone 7C11) used in this study will be published elsewhere. Citrinin−glucose oxidase conjugate was prepared as described by Abramson et al. (1995). Rabbit anti-mouse IgG−horseradish peroxidase conjugate was purchased from DAKO A/S, Denmark. Ochratoxin A and citrinin were purchased from Sigma-Aldrich Vertriebs GmbH, Deisenhofen, Germany. Immunoaffinity chromatography columns (column capacity for ochratoxin A = 200 ng) were from R-Biopharm GmbH, Darmstadt, Germany (RIDA ochratoxin A column, R1303). Water was bidistilled. All solvents and reagents were of at least analytical grade, and solvents used for HPLC were chromatography grade.

Sample Collection. Samples were collected from three historically hyperendemic BEN villages (E1−E3) in the Vratza region of northwestern Bulgaria. A "control" village (C) was selected, which is in the nephropathy region, in which no cases of the disease had occurred in the past. Most samples were of local produce. The practice of food storage in some households may predispose to fungal spoilage, but in others the food was stored dry. Both cereals intended for use as food (corn, wheat) and feed (barley, wheat bran, and oats) were included in the sampling schedule. From each location, a 50−100 g sample was collected. Additionally, five samples of wheat were collected from the Sofia area (far away from BEN regions) as further controls. All samples were ground to a particle size of <1 mm and stored at −18 °C before analysis.

EIA Analysis of Ochratoxin A and Citrinin in Cereals. A sample extract preparation technique that was suitable for both ochratoxin A and citrinin was used. A test sample (2 g) was mixed with 5 mL of 1 M HCl in a 40 mL test tube and mixed on a magnetic stirrer at full speed. Then 10 mL of dichloromethane was added and stirred for another 15 min. The mixture was centrifuged (1500*g*, 15 min, 4 °C), and the upper aqueous layer was removed with a Pasteur pipet. The lower organic layer was extracted by magnetic stirring with 10 mL of 0.13 M aqueous $NaHCO_3$ solution (pH 8.3) for 15 min. The mixture was centrifuged again, and the upper layer was used for EIA analysis. For the ochratoxin A EIA, this extract was used directly. For the citrinin EIA, the extract was diluted at least 1:5 with PBS containing 10% methanol. Recovery of the sample extraction procedure was repeatedly checked by adding 25−100 μL of methanolic standard solution containing both ochratoxin A and citrinin to 2 g portions of toxin-negative samples of wheat, corn, barley, oats, and bran at least 30 min before extraction.

Competitive direct EIA for ochratoxin A was performed as described by Märtlbauer and Terplan (1988). In brief, the wells of microtiter plates were coated with 100 μL of rabbit antiserum against ochratoxin A (diluted 1:2000 with sodium bicarbonate buffer, 0.05 M, pH 9.6) overnight at ambient temperature. The liquid was removed and the plate made semidry. Ochratoxin A standard or sample extract solution (in 0.13 M $NaHCO_3$, 50 μL per well) and ochratoxin A−horseradish peroxidase conjugate (50 ng/mL in 0.13 M $NaHCO_3$ containing 1% Tween 20, 50 μL per well) were added and incubated for 2 h at ambient temperature. The plates were washed three times with wash solution (0.85% NaCl solution containing 0.25% Tween 20) and made semidry. Enzyme substrate/chromogen solution (100 μL/well) was added after the color reaction was stopped with 1 M H_2SO_4 (100 μL per well).

For competitive indirect EIA for citrinin, the wells of microtiter plates were coated with 100 μL of citrinin−glucose oxidase conjugate (diluted 1:4000 with sodium bicarbonate buffer, 0.05 M, pH 9.6) overnight at ambient temperature. The liquid was removed, and free protein binding sites were blocked by incubation with PBS containing 2% casein sodium salt for 30 min at ambient temperature. Citrinin standard or sample extract solution (in PBS containing 10% methanol, 50 μL per well) and monoclonal anti-citrinin antibody (15 ng/mL in PBS, 50 μL per well) were added and incubated for 1 h at ambient temperature. The plates were washed three times with wash solution (0.85% NaCl solution containing 0.25% Tween 20) and made semidry. Anti-mouse IgG−horseradish peroxidase conjugate (diluted 1:3000 with PBS containing 1% sodium caseinate, 100 μL per well) was added and incubated for another hour at ambient temperature. The plates were washed three times with wash solution (0.85% NaCl solution containing 0.25% Tween 20) and made semidry. Enzyme substrate/chromogen solution (100 μL/well) was added, and after 15 min the color reaction was stopped with 1 M H_2SO_4 (100 μL per well).

Absorbance was measured at 450 nm with a microtiter plate reader (400 AT, SLT, Crailsheim, Germany), and the results were evaluated using a competitive EIA software (Märtlbauer, 1993) as described earlier (Usleber et al., 1994). All standards and sample extracts were analyzed in quadruplicate. The EIA standard curve parameters detection limit and 50% binding inhibition level were recorded throughout the study. Analytical results obtained for cereal samples were evaluated using a SAS statistical software package.

HPLC Determination of Ochratoxin A. A test portion (5 g) was weighed in a 25 mL beaker and extracted with 25 mL of acetonitrile/water (60:40) on a magnetic stirrer for 30 min. The extract was filtered through a paper filter, and a portion was diluted 1:10 with PBS. An IAC column was conditioned with 5 mL of PBS containing 6% acetonitrile. Then 20 mL of diluted extract was added onto the column at a drop speed of ∼1−2 mL/min. The column was rinsed with 10 mL of PBS containing 6% acetonitrile, and then the gel matrix was dried by pressing air through the column. Ochratoxin A was eluted with 2 mL of methanol. The methanol was evaporated under reduced pressure in a rotary evaporator, and the residue was redissolved with 0.4 mL of methanol (corresponding to 0.4 g of sample). The recovery of this procedure was checked

Table 1. Characteristics of the Enzyme Immunoassays Used for Toxin Detection

test parameter	test system for ochratoxin A	test system for citrinin
mean 50% binding inhibition concn ($n = 40$)	250 ± 42 pg/mL	520 ± 110 ng/mL
mean detection limit ($n = 40$)	77 ± 31 pg/mL	170 ± 110 pg/mL
cross-reactions	ochratoxin B (2%)	none known
minimum sample extract dilution factor	5	25
mean detection limit cereals (ng/g)	0.5	5

by the addition of methanolic solutions of ochratoxin A to 5 g portions of test sample before extraction. Samples ($n = 33$) that were positive for ochratoxin A by EIA, plus six selected negative samples, were reanalyzed by HPLC after IAC extract cleanup.

The analytical equipment for HPLC consisted of a Shimadzu LC-10A system with a model 10AT pump, a model SIL 10A autosampler with a 50 μL sample loop, a CTO 10AC column oven set at 25 °C, and Class LC 10 chromatographic software. Ochratoxin A was detected with a Shimadzu RF 535 fluorescence detector set at $\lambda_{ex} = 330$ nm and $\lambda_{em} = 500$ nm. The analytical column was a LiChrocart 125 mm × 4 mm i.d., filled with LiChrosphere 100 RP18 material, 5 μm particle size (Merck), and protected by a 4 × 4 mm guard column filled with the same material. The mobile phase consisted of methanol/water (60:40) adjusted to an apparent pH of 2.3 with phosphoric acid. Injection volume for standard and extract solutions was 50 μL. Retention time for ochratoxin A was at 12.9 ± 0.1 min, and quantification was performed using peak area. The detection limit (corresponding to a peak area of ~1200−1500) for ochratoxin A standard solutions was at 400 pg/mL (20 pg injected).

RESULTS AND DISCUSSION

The overall test performance data of the EIAs for ochratoxin A and citrinin are summarized in Table 1. The detection limit for ochratoxin A in cereals (0.5 ng/g) was well below those levels (3−5 ng/g) that are under discussion within the European Union as tolerance levels. For citrinin, no specific regulations or recommendations exist, but because it has, at least qualitatively, similar toxic effects, a detection limit of 5 ng/g can be considered as sufficient. In any case the EIA method for citrinin used here is the most sensitive routine method for this toxin described so far. If more information about the potency of citrinin becomes available in the future, attempts to further improve the test sensitivity may be necessary.

In a concentration range from 2.5 to 10 ng/g, the overall mean recoveries of ochratoxin A from artificially contaminated cereals (Table 2) varied from about 60 to 75% for wheat, corn, and barley, whereas overall recoveries from oats (28%) and bran (51%) were lower. Recovery of citrinin (Table 3) was in general lower than that of ochratoxin A. However, method performance was still considered to be acceptable for this screening study, in particular because low recovery of citrinin from certain sample matrices seems to be a general problem for all methods, and similar or even lower recoveries have been reported in other studies (Trantham and Wilson, 1984; Wilson, 1991; Scudamore et al., 1998). However, it has to be kept in mind that the results presented for citrinin (and to a lesser extent for ochratoxin A) in naturally contaminated samples may represent a systematic underestimation of the true content of ~30−70%.

Table 2. Recovery of Ochratoxin A from Various Cereals (EIA Method)

toxin added, ng/g	toxin found: mean, ng/g	SD, ng/g	CV, %	recovery, %
	Wheat ($n = 3$)			
2.5	1.55	0.260	16.8	66
5	2.90	0.100	3.44	58
10	6.00	0.568	9.42	60
	Corn ($n = 3$)			
2.5	1.27	0.153	12.1	51
5	2.50	0.608	24.3	50
10	11.7	1.53	13.1	117
	Barley ($n = 3$)			
2.5	1.03	0.924	89.4	41
5	4.47	1.42	31.7	89
10	7.73	1.00	13.0	77
	Oats ($n = 2$)			
2.5	0.75	0.070	9.43	30
5	1.65	0.212	12.9	33
10	2.1	0.283	13.5	21
	Bran ($n = 2$)			
2.5	1.0	0.424	42.4	40
5	2.45	0.495	20.2	49
10	6.4	2.69	42.0	64

Table 3. Recovery of Citrinin from Various Cereals (EIA Method)

citrinin added, ng/g	citrinin found: mean, ng/g	SD, ng/g	CV, %	recovery, %
	Wheat ($n = 3$)			
10	3.83	0.757	19.8	38
20	11.5	0.751	6.55	57
40	19.1	2.15	11.3	48
	Corn ($n = 3$)			
10	3.33	1.54	46.1	33
20	6.30	3.15	50.0	32
40	15.2	3.45	22.7	38
	Barley ($n = 3$)			
10	4.27	0.577	13.5	43
20	12.1	4.01	33.2	61
40	27.5	7.86	28.6	69
	Oats ($n = 2$)			
10	4.25	1.91	44.9	43
20	5.6	0.424	5.58	28
40	6.27	2.17	34.7	16
	Bran ($n = 2$)			
10	4.45	1.63	36.5	45
20	8.65	0.071	0.82	43
40	11.3	1.20	10.7	28

The HPLC method used for confirmation of ochratoxin A had a somehow better performance in aspects of recovery from various cereals (Table 4), in particular for oats and bran. Due to the IAC cleanup used in the sample extract preparation procedure, highly purified extracts were obtained for chromatography, enabling a low detection limit of ~0.5 ng/g.

Table 5 summarizes the EIA results obtained for ochratoxin A and citrinin in cereals from the three endemic villages E1−E3 having a history of BEN compared with the control village. Interestingly, corn was found to be free of ochratoxin A and citrinin, whereas contamination of wheat and wheat bran was more frequent. For oats, a high contamination frequency with ochratoxin A was observed, but no citrinin was detected. For ochratoxin A, the overall percentages of positive samples were 35% (C), 29% (E1), 30% (E2), and

Table 4. Recovery of Ochratoxin A from Various Cereals (HPLC after IAC Cleanup)

ochratoxin A added, ng/g	ochratoxin A found			
	mean, ng/g	SD, ng/g	CV, %	recovery, %
	Wheat ($n = 1$)			
2.5	1.65			66
5	3.72			74
10	7.46			75
	Corn ($n = 2$)			
2.5	1.52	0.06	3.7	61
5	3.51	0.21	5.8	70
10	6.74	0.03	0.4	67
	Barley ($n = 1$)			
2.5	1.99			79
5	3.72			74
10	7.27			73
	Oats ($n = 1$)			
2.5	1.98			79
5	4.06			81
10	7.62			76
	Bran ($n = 1$)			
2.5	2.34			95
5	4.79			96
10	8.41			84

Table 5. Summarized Results for Mycotoxins in Cereal Samples Collected from Nonendemic and Endemic Nephropathy Villages

sample type	total no. of samples (no. of positive[a] samples) collected from				
	control village	endemic village 1	endemic village 2	endemic village 3	control samples, Sofia region
wheat	5 (2)	9 (3)	8 (0)	10 (5)	5 (1)
corn	5 (0)	5 (0)	8 (0)	5 (0)	0 (−)
barley	5 (0)	0 (−)	0 (−)	1 (0)	0 (−)
oats	0 (−)	2 (0)	5 (5)	2 (2)	0 (−)
wheat bran	5 (5)	5 (3)	9 (4)	5 (5)	0 (−)

[a] Ochratoxin A and/or citrinin.

47% (E3). For citrinin, the overall percentages of positives were 5.0% (C), 14% (E1), 3.3% (E2), and 13% (E3). Thus, no obvious difference in contamination frequency between endemic and nonendemic areas was found for ochratoxin A, whereas citrinin was slightly more frequent in villages E1 and E3. These results are in contrast to those reported by Petkova-Bocharova et al. (1991), who reported much higher incidences and levels of ochratoxin A and citrinin in corn and beans from endemic areas in Bulgaria in the period from 1984 to 1990. These authors also reported contamination frequencies for ochratoxin A and citrinin in corn from endemic/nonendemic areas of 42−97%/5−100% and of 27−44%/10−15%, respectively, whereas in our study none of 23 corn samples contained detectable amounts of either toxin.

The individual data for ochratoxin A and citrinin are given in Table 6. Agreement between EIA and HPLC for ochratoxin A in naturally contaminated samples was excellent ($r^2 = 0.988$). Furthermore, all six samples that were negative by EIA were also negative by HPLC. This shows that using EIA for screening purposes is a cost-effective and reliable analytical strategy. Work is in progress to develop a suitable IAC cleanup for citrinin, thus enabling the establishment of a reference HPLC method.

The mean/median EIA values for ochratoxin A-positive samples were 1.5/1.3 ng/g (C), 11/1.6 ng/g (E1), 18/1.6 ng/g (E2), and 3.5/1.5 ng/g (E3). The mean/median values for citrinin-positive samples were 6.1/6.1 ng/g (C),

Table 6. Positive Ochratoxin A and Citrinin Results for Cereal Samples from Nonendemic and Endemic Nephropathy Villages

sample type	result (ng/g, not corrected for recovery)		
	ochratoxin A		citrinin
	EIA	HPLC	
	Control Village (Nonendemic Area)		
wheat	0.65	nd[a]	<5
wheat	0.72	<0.5	<5
bran	3	2.6	6.1
bran	1.3	1.1	<5
bran	1.2	1.2	<5
bran	1.9	3.4	<5
bran	1.6	1.9	<5
	Endemic Village 1		
wheat	39	31	420
wheat	26	26	83
wheat	1.7	<0.5	<5
bran	1.5	1.3	36
bran	0.69	<0.5	<5
bran	0.54	0.58	<5
	Endemic Village 2		
bran	1.7	0.97	10
bran	1.6	1.1	<5
bran	0.99	0.91	<5
bran	0.5	nd	<5
oats	2.9	1.9	<5
oats	140	85	<5
oats	9.6	6.3	<5
oats	0.97	1.9	<5
oats	1.3	1.9	<5
	Endemic Village 3		
wheat	1.5	0.98	<5
wheat	0.83	<0.5	<5
wheat	<0.5	nd	20
wheat	0.94	<0.5	<5
wheat	19	5.7	<5
bran	1.1	1.4	5.9
bran	1.8	1.4	<5
bran	2.1	1.5	230
bran	1.2	1.1	<5
bran	0.97	1.5	<5
oats	6.1	2.3	<5
oats	2.9	0.89	<5
	Control Samples, Sofia Region		
wheat	1.1	1.3	<5

[a] nd, not determined.

180/83 ng/g (E1), 10/10 ng/g (E2), and 84/20 ng/g (E3). However, statistical evaluation (in particular, significance tests, both parametric and nonparametric) of the data was prohibited because of the limited sample size, high variances within each group, and heteroscedasticity both between endemic villages and between endemic and nonendemic villages. Therefore, mean or median toxin concentrations above are given for descriptive rather than statistical purposes.

However, a few observations are interesting to note. First, all samples with higher levels of ochratoxin A (>5 ng/g) or citrinin (>10 ng/g) were from the three endemic villages. Second, although the overall incidence of ochratoxin was higher than that of citrinin both in endemic and in nonendemic villages, the maximum levels found for citrinin exceeded those of ochratoxin A. Furthermore, it may be speculated that if a detection limit of 0.5 ng/g would have been achieved for citrinin, as was the case for ochratoxin, an increased frequency of citrinin-positive samples would have been observed. Third, all but one sample in which citrinin was detected were from endemic areas. Finally, except for one wheat sample, all citrinin-positive samples were also positive

for ochratoxin, but citrinin concentrations were 2−200 times higher than those of ochratoxin A. These results indicate the importance of citrinin analysis within a survey for nephrotoxic mycotoxins.

Maximum levels found for ochratoxin A (30−40 ng/g) and citrinin (420 ng/g) as found in one wheat sample are lower than those levels found by Petkova-Bocharova but still indicate a potential health risk. Daily consumption of 100 g of this product by a 75 kg person would result in an average daily ochratoxin A intake of 40−50 ng/kg of body weight, which is ∼3 times higher than the highest reported value for the acceptable daily intake of ochratoxin A (Kuiper-Goodman, 1991). Considering newer results indicating that citrinin is not only nephrotoxic but also possesses a genotoxic potential (Föllmann et al., 1998), the high concentrations of this toxin found in several samples could have an impact on human health which was underestimated in the past.

To our knowledge, feeds from Bulgaria have never been analyzed for citrinin, and data for ochratoxin are rare. Stoev (1998) reported that contamination frequency of feeds with ochratoxin A was 100% for samples ($n = 17$) from farms having problems with MPN and 0% for samples ($n = 5$) from farms without MPN. Our results did not indicate that there is a much higher incidence of ochratoxin A or citrinin in feeds from BEN villages than in those from the nonendemic village. However, 66% of all feed samples from endemic areas were positive for ochratoxin A and/or citrinin, with wheat bran being more affected than barley or oats, wereas only 17% of all food samples from BEN villages were positive for ochratoxin A or citrinin. Not all types of cereals were available in all villages; therefore, the selection and number of feed samples from endemic and nonendemic villages may look skewed. However, these samples represented what was actually used as feedingstuff in these villages and, therefore, represent the potential source for a carry-over into foods of animal origin. Because ochratoxin A enters the food chain when present in swine feed, wheat bran may be a major source of human ochratoxin A intake. Little is known about the carry-over of citrinin in swine tissue, but metabolites may be present in blood and kidney (Dunn and Friedman, 1994). Considering the high frequency of ochratoxin A and citrinin in feeds, more work is needed to clarify the impact of food of animal origin on the intake of both toxins.

In conclusion, the results of this study show that although statistical differentiation between endemic and nonendemic BEN regions was not feasible, there is some indication that both ochratoxin A and citrinin occur at higher levels in BEN regions. Because both toxins frequently co-occur in wheat and wheat bran, further work is needed to clarify whether ochratoxin A and citrinin could both contribute to the BEN syndrome. Data obtained for feeds (wheat bran, oats) indicate that further analyses of food of animal origin such as pork meat and blood serum are required to estimate the impact of ochratoxin A intake, and possibly that of citrinin, via the food chain.

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