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Ochratoxin A in Blood from Slaughter Pigs in Sweden: Use in Evaluation of Toxin Content of Consumed Feed

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Samples of pig blood, intended for ochratoxin A analysis, were collected from pigs of 279 randomly selected herds. The samples were obtained at nine different slaughterhouses from different areas of Sweden. Pigs from 47 herds (16.8% of the total) exhibited ochratoxin A in amounts of ≥2 ng of ochratoxin A per ml of blood. One sample each from a single pig per herd identified herds contaminated with ochratoxin A in amounts exceeding three times the detection limit of the method (3 × 2 ng of ochratoxin A per ml of blood = 6 ng of ochratoxin A per ml of blood). There was a good agreement between ochratoxin A concentrations in the blood from different pigs within the same herd (correlation coefficient = 0.80). The ochratoxin A concentration in pig blood was used as an estimate of the ochratoxin A content of the consumed feed. This method showed that feed from grain produced on-farm contained higher concentrations of ochratoxin A than commercial feed preparations. No geographical variation of ochratoxin A occurrence within Sweden was detected.

The occurrence of ochratoxin A in pig feed can be surveyed by analyzing the toxin in the blood of pigs consuming the feed. A strategy for the analysis and evaluation of such data was recently published (1). This paper presents the first results obtained with the new sampling technique. The results given here must therefore be considered not only as values of ochratoxin A in pig blood, but also as a measure of ochratoxin A contamination of consumed feed.

To develop an understanding of factors responsible for ochratoxin A production, the occurrence of the toxin in the blood of pigs consuming a particular feed lot was related to different feed parameters.

MATERIALS AND METHODS

Collection of samples. Blood samples were collected from slaughter pigs of 279 herds in areas covered by nine slaughterhouses situated in different parts of Sweden from May to August 1978. The feed used during that period contained grain produced in 1977. Each herd was represented by two pigs. No anticoagulant was added to the blood samples. The samples were frozen and transported to the laboratory where they were kept frozen until analysis.

Analysis of samples. Blood samples of 2.5 ml each were analyzed for ochratoxin A by a spectrofluorometric procedure (1). The method involves extraction with chloroform and a mixture of HCl-$MgCl_2$ and, after washing with water, cleavage with carboxypeptidase A in tris(hydroxymethyl)aminomethane-sulfuric acid buffer. The fluorescence of the buffer solution is measured before and after incubation for 2 h at 37°C with carboxypeptidase A.

The decrease of fluorescence intensity at 380 nm in a 450-nm emission is proportional to the concentration of ochratoxin A. The detection limit is 2 ng of ochratoxin A per ml of blood.

Blood from one pig per herd was analyzed for ochratoxin A. If the toxin was encountered, blood from the other pig of the same herd was analyzed. An equal number of second samples, within each slaughterhouse area, from herds where the first sample was negative for ochratoxin A, were also analyzed.

Statistical evaluation. The herds analyzed were classified by feed type and herd size into 12 groups. The three different feed types used were feed based on grain produced on-farm, commercially prepared feed, and a combination of the two. The four herd categories used were ≤200, 200 to 500, 500 to 1,000, and ≥1,000 slaughter pigs produced per annum.

For examining variation in the results, a statistical model of risk was used. The model, expressing an assumption that feed type and herd size influenced independently the risk of ochratoxin A occurrence, was $P_{ij} = a_i b_j$, where P_{ij} is the risk probability of ochratoxin A being detected in a randomly chosen blood sample from feed type group i (i = 1, 2, 3) and herd size group j (j = 1, 2, 3, 4), and a_i and b_j are multiplicative risk factors expressing the effects of feed type and herd size, respectively.

The risk factors were estimated by the statistical method of maximum likelihood.

RESULTS AND DISCUSSION

From blood samples of 279 randomly selected pig herds, 47 herds were found to contain ≥2 ng of ochratoxin A per ml of blood (Table 1). Blood from 14 herds contained ≥10 ng of ochratoxin A

per ml of blood. The highest value found was 280 ng/ml.

Ochratoxin A concentrations in blood from pigs of the same herd. Blood samples from two pigs per herd were taken to enable a study of the heterogeneity of toxin distribution between animals of a herd. After analysis of the first sample, the second sample from every herd with a first positive sample (≥2 ng/ml) was analyzed. An equal number of second samples from herds with initial negative samples from each slaughterhouse area were analyzed.

Results from 77 herds in which at least one sample was found free of ochratoxin A (<2 ng of ochratoxin A per ml of blood) showed that low amounts of ochratoxin A were rather frequently encountered in one sample from a herd, whereas the other sample was free from toxin. The probability of a value exceeding twice the detection limit (4 ng of ochratoxin A per ml of blood) for the second sample, given a nondetected value for the first sample, was estimated to be as low as 0.03.

A total of 30 herds were found in which both samples were positive. For this data a rather high correlation (0.80) was found between the concentrations of ochratoxin A in samples from the same herd.

The conclusion drawn from these results was that a single sample from a herd is almost as good as two samples whether the herd is contaminated with ochratoxin A or not.

Estimated occurrence of ochratoxin A in feed. From the values of the ochratoxin A concentrations in the blood of pigs, the amount of ochratoxin A in the feed given to the pigs can be calculated. The relationship between the concentration of ochratoxin A in plasma (nanograms per milliliter) and the concentration of ochratoxin A in feed (nanograms per gram) is 1.5 (1). The corresponding relationship between plasma and blood is 2.3 (3). Thus, the concentration of ochratoxin A in feed (nanograms per gram) = 1.5 × the concentration of ochratoxin A in the blood (nanograms per milliliter).

From the data in Table 1 it was calculated that 16.8% of the herds had been given feed containing ≥3 ng of ochratoxin A per g of feed. Of these herds 6.5% were given feed containing more than 10 ng/g.

Parameters affecting the presence of ochratoxin A in feed. Questionnaires were sent to all owners of the herds examined. The following data were collected: (i) herd size (measured as the number of pigs annually delivered for slaughter); (ii) feed used (feed produced on-farm, commercially prepared feed, or a mixture of the two); (iii) cereals used, including variants; and (iv) conservation procedures used for cereals produced on-farm (no drying, warm-air drying, cold-air drying, and acid treatment). Out of 279 questionnaires sent, 239 answers were received (85.7%) (Table 2). The following considerations were based on the results from the herds about which answers were received.

Influences of feed type and herd size on ochratoxin A occurrence were investigated with a multiplicative model of risk. Calculations were made on three different data sets corresponding to three levels of ochratoxin A contamination (≥2, ≥5, and ≥10 ng of ochratoxin A per ml of blood). The multiplicative model was statistically tested and found to be adequate.

The estimated risk factors are shown in Table 3. The risk factors indicated that ochratoxin A was more abundant in feed containing grains produced on-farm than in commercially prepared whole feed. The risk factors for feed types varied more at high levels of ochratoxin A con-

TABLE 1. *Ochratoxin A concentrations in blood from slaughter pigs in Sweden*[a]

Slaughterhouse	Locality in Sweden	Total no. of herds	No. of herds with ochratoxin A	Concn in positive herds (ng of ochratoxin A per ml of blood)
A	Southern	52	6	2, 4, 15, 54, 74, 109
B	Southern	53	4	2, 4, 6, 12
C	Middle	51	6	2, 2, 8, 10, 12, 25
D	Middle	44	10	2, 2, 2, 2, 2, 3, 3, 3, 4, 9
E	Middle	20	4	2, 5, 26, 33
F	Middle	14	5	2, 2, 4, 14, 17
G	Northern	20	5	2, 2, 3, 5, 6
H	Northern	19	4	2, 3, 44, 187
I	Northern	6	3	2, 3, 3
Total		279	47	

[a] The data represent the average concentrations of ochratoxin A in the blood from two pigs per herd.

TABLE 2. *Ochratoxin A-positive herds grouped by grain type and herd size*

Grain type	Ochratoxin A-positive herds/total herds[a] for herd size:[b]				
	≤200	200–500	500–1,000	≥1,000	Total
On-farm	9/32	4/20	3/10	0/8	16/70
Commercial	2/17	4/23	3/29	8/67	17/136
On-farm and commercial	2/5	2/7	3/7	2/14	9/33
Total	13/54	10/50	9/46	10/89	42/239

[a] The data represent the number of herds in which the average concentration of ochratoxin A in the blood of two pigs was ≥2 ng/ml.

[b] The herd size is represented by the number of pigs delivered annually for slaughter.

TABLE 3. *Estimated risk effects with respect to grain type and herd size*

Parameters	Risk factors[a] at the following levels (ng of ochratoxin A per ml of blood) of contamination		
	≥2	≥5	≥10
Grain type			
On-farm	0.32	0.58	0.75
Commercial	0.22	0.14	0.05
On-farm and commercial	0.46	0.28	0.20
Herd size[b]			
≤200	0.79	0.32	0.16
200–500	0.68	0.24	0.23
500–1,000	0.71	0.34	0.38
≥1,000	0.43	0.11	0

[a] The risk factors at three levels are calculated from $P_{ij} = a_i b_j$; $\Sigma a_i = 1$.

[b] The herd size is represented by the number of pigs delivered annually for slaughter.

tamination. The difference between feeds was statistically significant (5%) at 10 ng and almost significant at 5 ng. The relationship between the tendency for increasing differences in risk factors between feed based on grain produced on-farm and commercial feed and the level of positive ochratoxin A contamination suggests that feed contaminated with ochratoxin A will exhibit a heavy contamination within a single herd. However, a commercial feed blending appears to reduce ochratoxin A levels.

No significant difference between herd sizes could be detected. The dependence on geographical locality was complex, and no conclusions could be drawn. The other questionnaire parameters, cereal variants and conservation procedures used, could not be tested because of variability in the responses.

The results obtained in this study show that a blood sampling method is useful in studying parameters affecting the feed content of ochratoxin A. The method provides a procedure for monitoring the feed quality on a continuous basis. Specific questions, such as cereal variants, could also be studied through the selection of herds before sampling.

Public health aspects of the occurrence of ochratoxin A in pig blood. The 279 herds represented here together annually deliver about 187,000 pigs for slaughter, amounting to about 6% of the total number of pigs slaughtered annually in Sweden. The average content of ochratoxin A extrapolated to all pigs belonging to the herds investigated is about 3 ng of ochratoxin A per ml of blood.

From the known concentration of ochratoxin A in the blood it is possible to calculate the concentration in the kidney (3), the liver, fat, and muscle (2). In Denmark all macroscopically changed kidneys must be analyzed for ochratoxin A. If the concentration of the toxin exceeds 10 ng/g in the kidney the whole carcass of that pig is condemned (Landbrugsministeriets instruks af 27 September 1974 om udøvelse af kødkontrol §23, pkt. 6. Aendring av instruks af 14 August 1978 [The Agricultural Board's instructions about the exercising of meat control, 24 September 1974, §23, point 6; change in the instructions, 14 August 1978.]). An ochratoxin A concentration of 10 ng/g in the kidney corresponds to 50 ng/ml in the blood. If this limit were used for the Swedish pigs represented in this investigation, 0.7% would be rejected. This investigation has not taken macroscopically changed kidneys into consideration. The occurrence of altered kidneys is only an indication of established ochratoxicosis but is not strictly related to the presence of the toxin in the animal.

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LITERATURE CITED

1. **Hult, K., E. Hökby, U. Hägglund, S. Gatenbeck, L. Rutqvist, and G. Sellyey.** 1979. Ochratoxin A in pig blood: method of analysis and use as a tool for feed studies. Appl. Environ. Microbiol. **38**:772–776.
2. **Krogh, P., N. H. Axelsen, F. Elling, N. Gyrd-Hansen, B. Hald, J. Hyldgaard-Jensen, A. E. Larsen, A. Madsen, H. P. Mortensen, T. Møller, O. K. Petersen, U. Ravnskov, M. Rostgaard, and O. Aalund.** 1974. Experimental porcine nephropathy. Acta Pathol. Microbiol. Scand. Sect. A Suppl. 245.
3. **Rutqvist, L., N-E. Björklund, K. Hult, E. Hökby, and B. Carlsson.** 1978. Ochratoxin A as the cause of spontaneous nephropathy in fattening pigs. Appl. Environ. Microbiol. **36**:920–925.