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Assessing the transfer of genetically modified DNA from feed to animal tissues

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Abstract

In Europe, public and scientific concerns about the environmental and food safety of GM (Genetically Modified) crops overshadow the potential benefits offered by crop biotechnology to improve food quality. One of the concerns regarding the use of GM food in human and animal nutrition is the effect that newly introduced sequences may have on the organism. In this paper, we assess the potential transfer of diet-derived DNA to animal tissues after consumption of GM plants. Blood, spleen, liver, kidney and muscle tissues from piglets fed for 35 days with diets containing either GM (MON810) or a conventional maize were investigated for the presence of plant DNA. Only fragments of specific maize genes (*Zein, Sh-2*) could be detected with different frequencies in all the examined tissues except muscle. A small fragment of the *Cry*1A(b) transgene was detected in blood, liver, spleen and kidney of the animals raised with the transgenic feed. The intact *Cry*1A(b) gene or its minimal functional unit were never detected. Statistical analysis of the results showed no difference in recovery of positives for the presence of plant DNA between animals raised with the transgenic feed and animals raised with the conventional feed, indicating that DNA transfer may occur independently from the source and the type of the gene. From the data obtained, we consider it unlikely that the occurrence of genetic transfer associated with GM plants is higher than that from conventional plants.

Introduction

The release of GM crops into the environment and their placing on the market has raised several issues concerning the safety of these new products (Conner et al., 2003). Among the issues most discussed, potential pleiotropic effects linked to the introduction of a new nucleotide sequence in the host plant genome are of particular concern. The organism is exposed every day to variable amounts of DNA from ingested food. DNA is a stable molecule that can survive extreme environmental conditions. In living organisms, ingested DNA, as part of digested feed/food components, is broken down in the gut into small fragments by the mechanical processes of mastication, acid hydrolysis and gastro-intestinal enzymatic activity involving DNase I and DNase II (Beever & Kemp, 2000). Naked DNA appears to be more susceptible to degradation than DNA contained in food matrices where it is somehow protected from enzymatic digestion (Hohlweg & Doerfler, 2001).

Studies employing oral administration of naked bacteriophage M13 and vector DNA to mice showed that DNA persists in a fragmented

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form in different sectors of the gut and was found in cells of the intestinal wall, peripheral white blood cells, spleen and liver (Schubbert et al., 1994, 1997).

In situ hybridisation on mouse tissues demonstrated that DNA from ingested food was found in several tissues of foetuses and newborn offsprings of female fed with M13 DNA but they were never found to be transgenic for the foreign DNA, suggesting a transplacental transmission mechanism (Schubbert et al., 1998). Fragments of up to 1600 bp of the rubisco (ribulose-1,5-biphosphate carboxylase) gene were found in the intestinal digesta of mice fed with soybean leaves. A small fragment (180 bp) of the plant gene was also detected in spleen and liver (Hohlweg & Doerfler, 2001). After intramuscular injection of a vector harbouring the green fluorescent protein (GFP) gene, the expression of fluorescent protein was observed locally in muscle tissue and a 769 bp fragment of the GFP gene was detected in mouse organs and digesta. Mice were monitored for eight generations and authors reported that germline transfer of orally administered foreign DNA is an unlikely event (Hohlweg & Doerfler, 2001).

Other studies employing farm animals fed with conventional and transgenic feed showed that only DNA fragments of about 200 bp from a chloroplast gene was detected in milk, rarely in blood cells but not in the organs of cows (Einspanier et al., 2001; Phipps et al., 2003). Plant DNA was also found in organs of broilers, but sequences belonging to the transgene were not identified. Further experiments showed that recombinant DNA was detected in gut contents of pigs fed with Bt-maize (Reuter & Aulrich, 2002). Attempts to detect the transgene in pig tissues were unsuccessful even though they were positive for the presence of short sequences of the rubisco gene.

Recently, studies on the survival of transgenic plant DNA in human gut performed on ileostomists volunteers showed that a 180 bp fragment from the *EPSPS* (5-enolpyruvylshikimate-3-phosphate synthase) transgene was recovered in every ileum digesta at different rates, depending on the individual (Netherwood et al., 2004). The full length *EPSPS* gene was also detected occasionally in the digesta. The transgene fraction was completely degraded in the large intestine since it was not detected in the faeces. Large gene fragments were detected only in digesta (Schubbert et al., 1994; Duggan et al., 2003; Netherwood et al., 2004). Currently, to the best of our knowledge, only small amplicons used for the identification of GMO events have been reported in DNA traceability studies in animal tissues (Reuter & Aulrich, 2002; Duggan et al., 2003; Netherwood et al., 2004).

Literature data confirms that plant and microbial DNA fragments remaining in intestinal digesta could be absorbed through the intestinal mucosa either directly by epithelial cells or by antigen-presenting cells of the immune system. Damage in the intestinal epithelial surface could allow the diffusion of DNA and other macromolecules (Beever & Kemp, 2000). Uptake may occur via specialised M-cells of the lining of the gastrointestinal tract, which actively sample gut contents as part of the process of protecting the body from infection (Nicoletti, 2000). *In vitro* studies with rat and human intestine have shown that the absorption of small oligonucleotides is consistent with a transcellular mechanism (Wu-Pong et al., 1999).

In the present study we trace the fate of food-derived DNA in tissues of piglets raised on conventional or transgenic feed and show that small fragments belonging to three target plant genes can be detected in different animal tissues. The data obtained will help to conduct a safety assessment with regard to the transfer of genetic information following food consumption and to evaluate if the use of GM plants in livestock feed presents a higher risk than conventional plant use.

Materials and methods

Animals, growing conditions and feed

Sixteen piglets of the hybrid (Large White × Landrace), forty-days-old and with average weight of 9 kg, were utilised for the feeding studies. The animals were fed for 35 days until they reached 35 kg of average weight in flat-deck cages in rooms with controlled climate and natural daylight, at the Cerzoo (Centro Ricerche per la Zootecnia e l'Ambiente), Piacenza, Italy, during 2002. All experiments were performed in compliance with relevant laws and institutional guidelines. The experiment was approved by the Italian Ministry of Health

(Notification No. CZ/99/020/MONS/BTSU). The housing was cleaned and disinfected with non toxic solutions before introducing the piglets. The room remained empty for at least 7 days before the arrival of the animals. Test and control animals were housed in separate rooms. The animals had free access to drinking water and were fed *ad libitum*. A pre-experimental observation period of 10 days was done. The piglets were divided into two groups of 8 animals fed either with transgenic feed (test group) or conventional feed (control group). The test group consumed a diet containing 50% Bt-maize (MON810) grown in experimental farms in Italy (Authorization No. 35791 of December 4, 1998 of the Ministero per le Politiche Agricole e Forestali, Italy). The seeds were supplied by Monsanto Agricoltura Italia S.p.A. (Lodi, Italy). The control group was fed the same diet but using the near-isogenic non GM maize of MON810. The remaining 50% of the diet was a typical basal meal containing 20.7% soybean meal, 2% soybean oil, 7% barley meal , 8% barley flake, 3% fish meal , 5% dehydrated whey, 0.5% L-lysine, 0.1% DL methionine, 0.3% limestone, 2.7% dicalcium phosphate, 0.2% sodium chloride, 0.5% minerals. The transgenic maize hybrid MON810 is genetically engineered to resist the European corn borer (ECB), *Ostrinia nubilalis*. This line was developed by introducing the *Cry*1A(b) gene from *Bacillus thuringiensis* (*Bt*), under the control of the CaMV35S promoter and NOS terminator, into the maize cultivar Hi-II by particle acceleration transformation (Armstrong et al., 1995). The presence of the hsp70 intron of maize fused to the coding region guaranteed high levels of expression of the *Cry*1A(b) gene.

Sampling procedure

During the sampling procedure special care was taken to avoid accidental contamination from the environment or from the surgical tools. The working place and the tools were accurately cleaned with a 5% hypochlorite solution rinsed in sterile water. Disposable gloves were changed between each organ sampling. The day before study termination, animals were immobilized and the blood samples collected via jugular vein puncture. Before inserting the needle, the surface was cleaned and the first mL of blood was discarded to avoid contamination from residues of feed. The tube was replaced with a sterile one and an average of 8–9 mL of blood for each animal was then collected. The samples were immediately stored at −20°C. Termination was done first for the control animals and then for the test animals. On the day of termination, the organs (liver, spleen, kidney and thigh muscle) were removed, placed in clean nylon bags and then transferred to the sampling laboratory. Slaughter and sampling rooms were distinct to avoid possible contamination.

The outmost layer of tissue from each organ was removed to sample the inner part: this was done to avoid possible residues of feed on the surface of the organs could contaminate the sample. The samples were immediately stored at −20°C in sterile tubes.

DNA isolation, PCR and Southern blot analysis

Genomic DNA was isolated from blood and all collected organs for analysis of fragments of the *Zein*, *Sh*-2 and *Cry*1A(b) genes by PCR and Southern blotting. DNA isolation was done following the Wizard® Genomic DNA Purification procedure (Promega, Madison, USA), except for muscle tissues which were processed with the Wizard® Magnetic DNA Purification System for Food (Promega, Madison, USA). PCR (Polymerase Chain Reaction) reactions were assembled in a hood that was UV sterilized before and after each procedure to avoid contamination. The reaction mixtures contained 50 ng genomic DNA, 10 pmoles of each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1 Unit HotStarTaq Polymerase (Qiagen GmbH, Hilden, Germany), 1 × PCR buffer, in a 30 μL final volume. Table 1 lists the primers employed in PCR reactions, the target genes and the expected PCR products size. Primers were designed on Genbank Accession No. M23537, United States Patent Application No. 5689052 (Brown et al., 1997), Genbank Accession No. M81603 and Genbank Accession No. M17704 to amplify fragments of the maize *Zein* gene, *Cry*1A(b) transgene, ADP glucose pyrophosphorylase (*Sh-2*) gene and swine growth hormone (*Sgh*) gene, respectively. PCR cycles were: 95°C for 15 min, followed by 40 cycles consisting of 1 min at 95°C, 1 min at the annealing temperature indicated in Table 1, 1 min at 72°C, and a final step of 10 min at 72°C. PCR products were detected by electrophoresis in 2% (w/v) agarose gel. Southern

Table 1. Primers used in PCR reactions to detect four target sequences. The sequence of sense and antisense primers for each target gene, the annealing temperature (Ta) for each primer pairs and the expected PCR product size are reported

Target sequence	Primer sequence (5′ → 3′ orientation)	Ta	Expected product size
Maize *Zein* gene	GCT TGC ATT GTT CGC TCT C CTA GAA TGC AGC ACC AAC AAA G	57°C	439 bp
*Cry*1A(b) transgene	CCA AAT CTA CGC TGA GAG CTT CCG GAA GCT ACC GTC GAA GTT CTC CAG G	63°C	519 bp
Maize *Sh*-2 gene	TTG TAA TCT TGA GTG GCG ATC AGC TAG TGT CAG ATT CTA CAG CAA AAT AG	55°C	533 bp
Pig *Sgh* gene	GGG TGG TGG AGA GGG GTG AAT T TCA GTT TAC ACT CAC CTG ATA GCA TCT	60°C	108 bp

blots were performed according to standard procedure (Sambrook et al., 1989). The PCR specific products were cloned into the pGEM-T Easy® Vector System (Promega, Madison, USA) and sequenced, prior to be used as probes. The probes were labelled with dCTP 5′-[α^{32}P] through the Ready-To-Go® DNA Labelling Beads (Amersham Biosciences, Little Chalfont, UK). The probe for *Zein* gene was a PCR product of 439 bp identical to the sequence on which the primers were designed.

Statistical analysis

The frequency sample is represented by a total of 9 observations: for each organ three samples were taken, one DNA isolation was done on each sample, three PCR reactions were run for each DNA isolation and each gene analysed. The χ^2 test was employed to verify homogeneity between the 8 animals of each group. Since no individual heterogeneity was detected ($p > 0.05$), the frequencies of the eight animals from each group were pooled in a single sample of 72 data. The χ^2 test for independency for the 2×2 contingency tables was performed on these samples: two types of results (positive, negative) per two levels (conventional vs. transgenic, comparison between pairs of target genes, comparison between pairs of organs). The comparisons were performed with the Yates correction for 2×2 tables. The χ^2 test was replaced by the exact method of Fisher when at least one expected frequency was below 5. The independency test was repeated on each organ and for each gene. All the statistical analysis was performed with the analytical procedure of the contingency tables of the SPSS 11.0.1 software (SPSS Inc., 2003).

Results

DNA traceability

The traceability study with the PCR technique was carried out on blood, spleen, liver, kidney and thigh muscle. Investigation of gut content and tissues was not performed since sampling of intestinal contents was considered to represent an additional risk in contamination of the surrounding organs and detection of exogenous DNA in the faeces is already well documented in the literature (Schubbert et al., 1994; Hoelweg & Doerfler, 2001; Reuter & Aulrich, 2002; Duggan et al., 2003; Netherwood et al., 2004). Identification of DNA fragments in the intestinal wall may have constituted a proof of the passage of nucleic acid but it is almost impossible to thoroughly clean the samples from the intestinal content and the risk of false positives due to contamination was too high. It was therefore decided to focus on the presence of DNA in the main organs that were sampled with extreme care, as described in materials and methods. Because the presence or absence in animal tissues of DNA derived from feed was assumed to be a rare event, an experimental scheme was designed that could give consistent and reproducible results. Samples of the different tissues from sixteen pigs were labelled with anonymous tags in order to perform a blind test. The identity of each sample was revealed only at the end of the experimentation to avoid influencing results. Three independent DNA isolations were performed on each organ and tested with PCR for the presence of three target gene fragments (*Zein*, *Cry*1A(b), *Sh-2*), as described below. PCR tests were performed in triplicate. One out of three PCR

repetitions for each gene was confirmed by hybridisation with a gene specific probe.

The samples of each independent DNA isolation (sixteen samples for each organ) were processed all together to reduce variability of yield and quality. To exclude the presence of inhibitors in PCR reactions, DNA quality was confirmed by amplification of a 108 bp fragment of the swine growth hormone (*Sgh*) gene from all the genomic DNA preparations (Figure 1a). The genes chosen for detection were selected in order to highlight differences in PCR amplification and tissue specificity. *Zein* represents a maize multicopy gene (Woo et al., 2001) used as positive control to identify maize DNA. *Cry*1A(b) is the synthetic single copy gene inserted into the maize line MON810 (Armstrong et al., 1995). The maize single copy gene *Shrunken-2* (*Sh-2*, ADP glucose pyrophosphorylase) (Shaw et al., 1992) was used as control to compare the behaviour of a single copy gene with *Cry*1A(b). To confirm that *Cry*1A(b) and *Sh-2* are single copy genes, Southern blot analysis on Bt-maize genomic DNA was performed. The hybridisation pattern obtained (data not shown) indicated that both genes are present as a single copy, as reported in the literature. Experiments were focused on the amplification of about 500 bp gene fragments of all three genes analysed since preliminary experiments showed that neither the complete 3500 bp *Cry*1(A)b sequence nor the 1800 bp region of the minimal functional unit were not detectable. Fragments of this size were difficult to amplify even on raw material such as animal feed.

As shown in Figure 1b, *Zein* primer pairs often generate multiple band patterns in which the specific probe recognises a single signal (Figure 1c). This multiple band pattern occurs because *Zein* primers share partial homology with other *Zein* genes which have different sizes. Data for *Zein* detection were collected by considering all those signals that have the specific size and hybridise with the probe. *Sh*-2 and *Cry*1A(b) primer pairs give single band patterns (Figure 1b) that are easily distinguishable and are confirmed by hybridisation with the proper specific probe (Figure 1c).

Figure 2 summarises the results of this study. All the experimental variables such as, tissues, independent DNA isolations, target genes, number and grouping of the animals and PCR replicates are organised to give an order to the experimental data. This kind of disposition was also used to generate the input data for the statistical analysis. The figure allows a comprehensive view of the experimentation, focusing attention on the grey cells which represent the positive hits. The positive samples appear to be distributed randomly with an apparent clustering by tissue, that is, samples from blood had the major number of hits, followed by

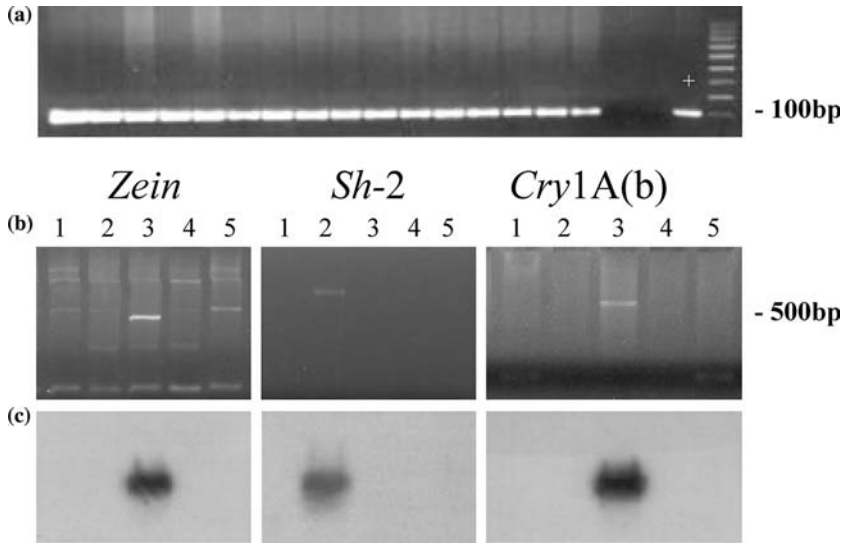

Figure 1. PCR reactions (a) for the *Sgh* gene, PCR reactions (b) and Southern blots (c) for three target genes (*Zein*, *Sh*-2, *Cry*1A(b)). (a) Electrophoretic separation on agarose of PCR products from a complete set (16 individuals) of liver samples. + indicates the PCR positive control. (b) Electrophoretic separation on agarose of PCR products from liver samples of five individuals, for each target gene. (c) Southern hybridisation with gene specific probe showing the positive samples.

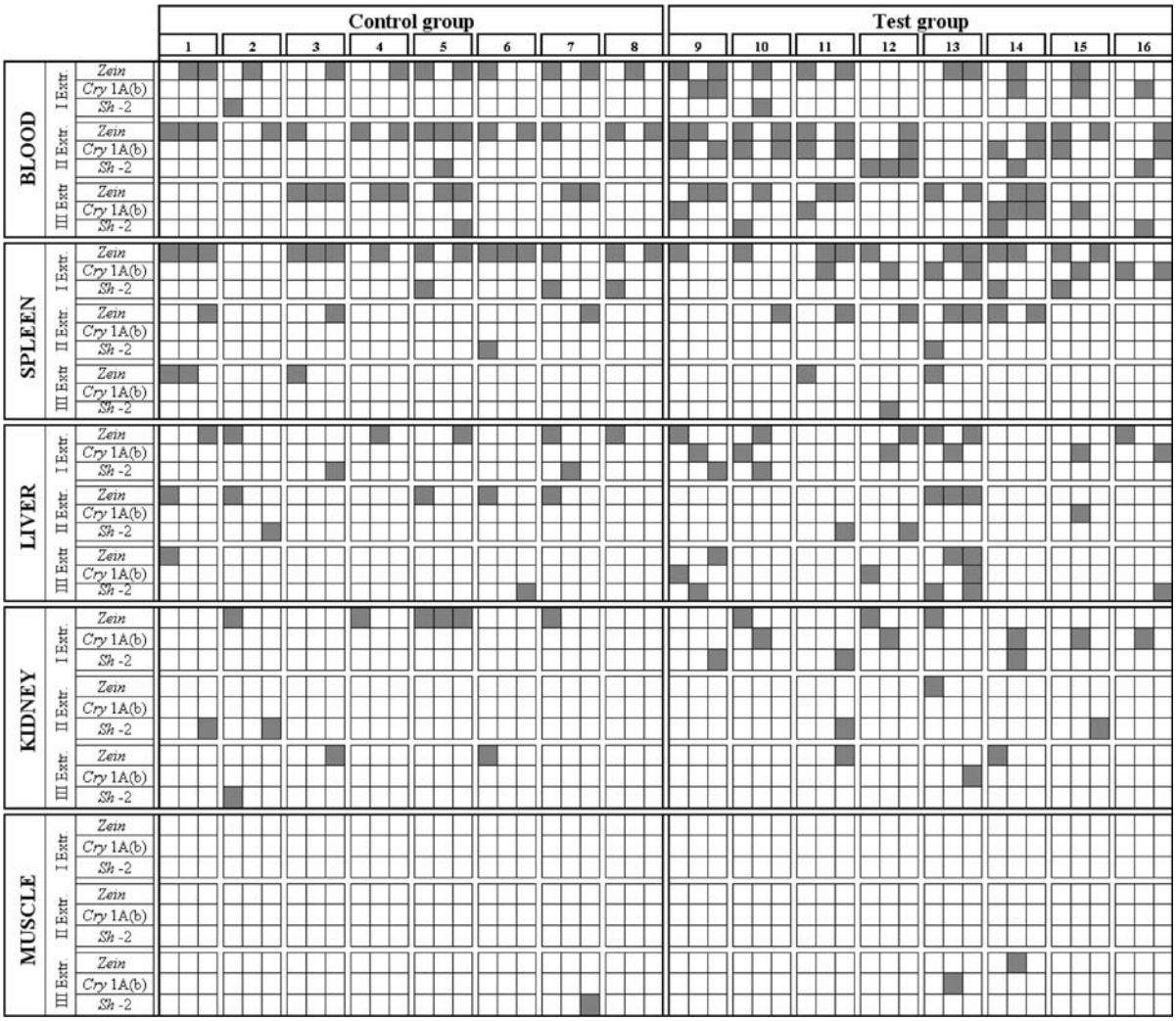

Figure 2. Representation of the results obtained from PCR reactions performed on five tissues, three independent DNA isolations (I, II, III Extr.), three target genes and sixteen individuals (1–8 from the control group, 9–16 from the test group). The basic unit of this scheme is a square of 3 × 3 cells. The three top horizontal cells represent the three PCR replicates for the detection of *Zein*, the middle cells the replicates for the detection of *Cry*1A(b) and the bottom cells the replicates for the detection of *Sh*-2. A blank cell means a negative result in the PCR reaction while a grey cell means a positive result.

spleen, liver, kidney and muscle, with the last one having only three hits in total. It can also be assessed that independent DNA isolations show different pattern of results (compare isolation I and II from spleen samples). PCR results for a specific gene fragment are often not consistent either in the three replicates or across the three DNA isolations (see *Zein* in blood for the animal number 1). If we would define as positive animal the individual for which a specific target gene is positive in each of the three PCR replicates in every DNA isolation (and subsequently in every tissue) then we can assess that none of the examined animal is positive for the presence of feed derived DNA in the tissues. Nevertheless, performing the experiments with a single PCR test on a single DNA isolation per sample, should not be considered significant. The low reproducibility of the detection events may be explained as low concentration of foreign plant molecules. In order to be detected, the experimental conditions for a specific target must be reproduced perfectly. For instance, a PCR reaction that does not work at top efficiency in the first cycles may compromise the result. The low reproducibility may also be explained by free unstable foreign DNA. This

would also account for differences in detection frequency between independent DNA isolations.

We summarised the recovery of positive hits as the percentage of total positive samples for a specific target gene in the control group (animals raised with conventional feed) and in the test group (animals raised with transgenic feed) in Figure 3, considering each positive sample as an independent event. Tissues from the control group were all negative for the presence of a 519 bp fragment of the *Cry*1A(b) gene that was instead detected in tissues from the test group. The other two target genes were detected in all the tissues. The *Zein* fragment showed higher detection frequencies compared to *Cry*1A(b) and *Sh-2*. The number of positives for *Zein* is comparable between control and test groups while for *Sh*-2 fragment there are some variations, as can be seen in Figure 3. The tissue with the higher number of hits, calculated as total positives for the three target genes on 432 observations (3 genes × 3 PCR repetitions × 3 DNA isolations × 16 individuals) was blood (22.7%), followed by spleen (13.0%), liver (10.6%), kidney (6.7%) and muscle (0.7%).

Detection frequency

We define the term 'detection frequency' as the number of positive hits in the different comparison levels considered. The differences in detection frequencies of the three target genes (*Zein*, *Cry*1A(b) and *Sh-2*) in five different organs (liver, spleen, kidney, muscle and blood) were investigated with a statistical approach in order to determine if data distribution in the matrix of Figure 2 and the grouping generated in Figure 3 are significant. We considered each positive sample as an independent event and we applied a χ^2 test to evaluate a comparison between different set of data (e.g. transgenic vs. conventional, single copy gene *vs*. multi copy gene, detection of a specific gene in a tissue vs. another tissue, etc).

First, analysis of the frequency distribution of the three target genes in the tissues did not reveal any significant difference between animals of the control and test group (Table 2), except for the detection frequency of a *Cry*1A(b) fragment in blood, spleen, liver and kidney, since no positives were found in the control group. The χ^2 test was not significant for muscle. DNA transfer was therefore detected in both groups with the same frequency indicating that genetic modification of a plant does not increase the passage of genetic information from the feed to the organism. In Table 3, the detection frequency of the target genes in each tissue were compared to each other within single feeding groups to reveal a possible effect of the copy number. In the control group only the comparison *Zein* vs. *Sh*-2 was possible. The difference in detection frequency between the *Zein* fragment and the single copy *Sh*-2 fragment was significant in blood and spleen for both test and control groups. This difference was not significant for liver and kidney and this could be due to the effect of the low number of positives hits found in these organs; blood and spleen were in fact the tissues with the highest number of hits.

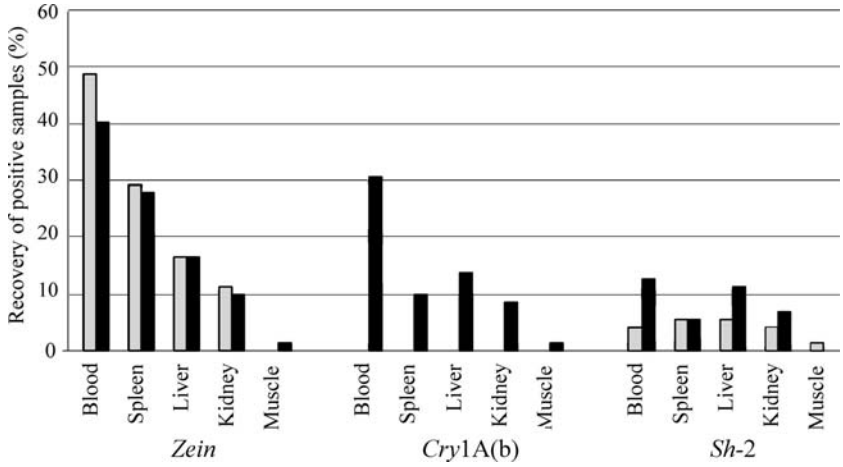

Figure 3. Recovery of positives samples (%) of the transgene and plant gene fragments in five tissues of animals from the control (■) and test (■) groups. The percentage of positives for each tissue is calculated as number of positives over 72 observations (3 PCR repetitions × 3 independent DNA isolations × 8 individuals).

Table 2. Probabilities of χ^2 values obtained by comparing the detection frequencies of the three target genes in five tissues between animals belonging to the control and test groups

Gene	Tissue				
	Blood	Spleen	Liver	Kidney	Muscle
Zein	0.703	1.000	1.000	0.778	1.000
*Cry*1A(b)	0.000*	0.013*	0.001*	0.028*	1.000
Sh-2	0.129	1.000	0.367	0.719	1.000

*Data with significant value at $p < 0.05$.

Comparison between *Sh-2* and *Cry*1A(b) revealed that they have the same detection frequency, except for blood where there is a high number of positives hits for the short sequence of *Cry*1A(b). This suggests that these genes behave in the same manner, as would be expected since they are both single copy genes. On the other hand, comparison between *Zein* and *Cry*1A(b) revealed that frequency detection is not significantly different, except in the spleen.

The differences in frequency detection of single genes between the five organs is reported in Table 4. The aim was to point out detection frequencies linked to tissue specificity. The differences between organs in *Sh-2* detection frequencies were non significant (data not shown). Significant differences among organs were observed for *Zein* detection: blood was the tissue with the highest number of positive hits followed by spleen, liver, kidney and muscle. In the test group, *Cry*1A(b) behaved like *Sh-2* except in blood where detection was significantly higher compared with the other organs.

Discussion

The goal of our study was to assess the persistence of feed-derived DNA fragments in animal tissues and to validate the methods employed for the investigation. Indeed, our results agree with most of the literature which reports the detection in animal tissues of DNA derived from feed and support the hypothesis that DNA fragmented by the digestive process may persist in the gut and be taken up by the organism through the gastrointestinal apparatus.

The present experiments represent a more natural scenario than the one from the experiments of Schubbert and Hohlweg (Schubbert et al., 1994, 1997, 1998; Hohlweg & Doerfler et al., 2001) where bacteriophage and vector DNA, directly fed to mice, were detected in blood, liver and spleen. In this paper, we showed how short fragments of plant DNA ingested as a part of feed can be detected in animal tissues from young piglets (35 kg of weight). Also, we found substantial differences between our results and those reported by Reuter and Aulrich (2002) where neither Bt fragments nor maize-specific DNA sequences were detected in tissues of relatively old pigs (108 kg of weight). Moreover, they found only short plant DNA fragments (200 bp) and at a high frequency in different muscle tissues and in ovary with much less positives in blood. In our case, blood was the tissue with the highest number of positives, followed by organs rich in blood vessels and involved in filtration, such as

Table 3. Probabilities of χ^2 values obtained by comparing the detection frequencies of the three target genes against each other, in the tissue examined, inside the control or the test group

Feeding group	Comparison	Tissue				
		Blood	Spleen	Liver	Kidney	Muscle
Control	*Zein* vs. *Sh*-2	0.000*	0.000*	0.061	0.208	1.000
Test	*Zein* vs. *Sh*-2	0.000*	0.000*	0.470	1.000	1.000
	Zein vs. *Cry*1A(b)	0.296	0.010*	0.817	1.000	1.000
	Sh-2 vs. *Cry*1A(b)	0.014*	0.532	0.801	1.000	1.000

*Data with significant value at $p < 0.05$.

Table 4. Probabilities of χ^2 values of the detection frequency among the five tissues for *Zein* and *Cry*1A(b) gene fragments in the two feeding groups

	Tissue	Blood	Spleen	Liver	Kidney
		Zein			
Control group	Blood	–			
	Spleen	0.026*	–		
	Liver	0.000*	0.112	–	
	Kidney	0.000*	0.012*	0.471	–
	Muscle	0.000*	0.000*	0.000*	0.006*
		Zein			
Test group	Blood	–	–		
	Spleen	0.159	–		
	Liver	0.003*	0.160	–	
	Kidney	0.000*	0.004*	0.207	–
	Muscle	0.000*	0.000*	0.002*	0.116
		*Cry*1A(b)			
	Blood	–			
	Spleen	0.003*	–		
	Liver	0.028*	0.607	–	
	Kidney	0.001*	1.000	0.427	–
	Muscle	0.000*	0.063	0.009*	0.116

*Data with significant value at $p < 0.05$

liver and kidney. The relatively high recovery of plant DNA in spleen suggests that in young animals the immune and the lymphatic systems may play important roles in nucleotide sequences absorption.

From the data presented, we can conclude that blood is the main tissue involved in the uptake of short DNA fragments since it collects macromolecules directly absorbed by the intestinal epithelium and the cells of the immune system. DNA molecules may be transported in the organism via blood circulation. Progressive decrease in DNA detection moving towards peripheral positions exclude the possibility that organs like liver, kidney and spleen elicit an accumulation effect. This consideration, together with the lower detection frequency and low reproducibility found in organs, may account for the transitory nature of DNA fragments that are progressively degraded or diluted before they reach peripheral positions. These are the reasons why, in respect to our results, we consider the DNA transfer to muscle tissues to be an unlikely event. In fact, only three cases of foreign DNA presence over 432 tests were detected in muscle. Similar results were obtained by Jennings et al. (2003) on muscle tissues from broilers.

In this paper we reported the detection of short fragments (about 500 bp) of recombinant and maize DNA. *Zein* fragments were the most frequently detected sequences and no significant differences were observed between samples derived from animals fed with transgenic and conventional feed. The same result was observed for *Sh-2*, though with a lower number of positives, as expected for a single copy gene compared to *Zein*. Detection frequency of a 519 bp fragment from *Cry*1A(b) was higher than *Sh-2* in blood samples, but was not statistically different from *Zein*, except in the spleen. This discrepancy may be due either to an underestimation of the *Zein* detection frequency, or to the casual distribution of the positive hits in sets of independent DNA isolations as reported in the previous section.

In conclusion, we have shown that small feed-derived DNA fragments could be detected in animal tissues and this can occur for all types of sequences independently of the source. We hypothesize that foreign DNA uptake may be a natural process as well as absorption of small peptides or microbial agents whose effects are widely known. On the contrary severe health risks associated with nucleic acids have never been

shown and naked DNA is much less resistant to the organism defences. Moreover, we conclude that the risk of DNA transfer from food containing genetically modified plants to an organism is not higher than DNA transfer from food containing the corresponding conventional plant. The risk that a transgene is transferred to an organism is not higher than the risk for the transmission of other plant genes and therefore consumption of GMO should not raise concerns about safety in animal production as well as, based on physiological similarities, in human nutrition.

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