# Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11

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# Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11 [1]

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**ABSTRACT:** Genetically modified corn has been approved as an animal feed in several countries, but information about the fate of genetically modified DNA and protein in vivo is insufficient. Genetically modified corn Bt11 is developed by inserting a recombinant DNA sequence encoding insecticidal Cry1Ab protein from *Bacillus thuringiensis* subsp. *kurstaki*. We examined the presence of corn intrinsic and recombinant *cry1Ab* gene by PCR, and the Cry1Ab protein by immunological tests in the gastrointestinal contents of five genetically modified corn Bt11-fed and five nongenetically modified corn-fed pigs. Fragments of corn zein (242 bp), invertase (226 bp) and of ribulose-1,5-bisphosphate carboxylase/oxygenase genes (1,028 bp) were detected in the gastrointestinal contents of both Bt11 and nongenetically modified corn-fed pigs. Fragments of recombinant *cry1Ab* gene (110 bp and 437 bp) were detected in the gastrointestinal contents of the Bt11-fed pigs but not in the control pigs. Neither corn intrinsic nor *cry1Ab* gene fragments were detected in the peripheral blood by PCR. The gastrointestinal contents were positive for Cry1Ab protein by ELISA, immunochromatography, and immunoblot; however, these methods did not work for blood and precluded conclusions about any potential absorption of the protein. These results suggest that ingested corn DNA and Cry1Ab protein were not totally degraded in the gastrointestinal tract, as shown by their presence in a form detectable by PCR or immunological tests.

Key Words: Feed, Maize, Recombinant DNA, Pigs

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## Introduction

Genetically modified (**GM**) corn has been approved as an animal feed in several countries; however, there is growing anxiety in European countries and in Japan about the safety assessment of foreign genes and proteins expressed in plants by recombinant DNA (**rDNA**) technology (Hino, 2002). For the evaluation of GM feed safety, compositional and nutritional properties and animal performance of GM crops as feed have been examined (FAO/WHO report, 2000). Further information on the fate of rDNA after ingestion is additional concern. Genetically modified Bt11 corn (**Bt11**) is developed by inserting rDNA sequence encoding insecticidal Cry1Ab protein from *Bacillus thuringiensis* subsp. *kurstaki*. Polymerase chain reaction for rDNA has been used to differentiate GM and non-GM corn (Matsuoka et al., 2000), but information on the fate of the rDNA and its products (Cry1Ab protein) in the gastrointestinal (**GI**) tract after ingestion of GM feed is limited. The objective of the present study was to determine if the *cry1Ab* gene and Cry1Ab protein, as well as corn-intrinsic genes, could be detected in GI tract contents and peripheral blood of pigs fed Bt11 and non-Bt isoline corn.

## Materials and Methods

### *Diet and Animals*

*Diet.* Genetically modified Bt11 (N58-D1 Lot No. 2608611, Novartis Seed, Inc., Greensboro, NC) and non-

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**Table 1**. Components of diets (%, as fed)

| Item | Control | Bt-fed |
|---|---|---|
| Non-Bt isoline | 60.0 | — |
| Bt11 maize | — | 60.0 |
| Milo | 16.11 | 15.30 |
| Soybean meal (>45% CP) | 19.6 | 20.4 |
| Fishmeal (>60% CP) | 2.5 | 2.5 |
| Calcium carbonate | 0.65 | 0.65 |
| Dicalcium phosphate | 0.44 | 0.44 |
| Salt | 0.35 | 0.35 |
| Vitamin B premix[a] | 0.15 | 0.15 |
| Vitamin ADE premix[b] | 0.10 | 0.10 |
| Mineral premix[c] | 0.10 | 0.10 |
| Total | 100.0 | 100.0 |

[a]Vitamin B premix provided 1.5 mg of thiamine nitrate, 1.5 mg of riboflavin, 0.75 mg of pyridoxine chloride, 16.35 mg of calcium pantothenate, and 9 mg of nicotinamide per kilogram of the final diet.

[b]Vitamin ADE premix provided 10,000 IU of Vitamin A, 2,000 IU of Vitamin $D_3$, and 10 mg of DL-$\alpha$-tocopherol acetate per 1 kg of the final diet.

[c]Mineral premix provided 50 mg of manganese, 50 mg of iron, 10 mg of copper, 60 mg of zinc, and 1 mg of iodine per kilogram of the final diet.

Bt isoline corn were purchased from Novartis Seed, Inc. Corn meal was prepared using a hammer mill attached to a 5-mm sieve. All of the feeds contained 60% of Bt11 or nonBt corn kernels, and each diet was designed to satisfy the requirements in "The Japanese Feeding Standard for Pigs" (Agriculture, Forestry and Fisheries Research Council Secretariat, MAFF, Tokyo, Japan) (Table 1).

*Animals.* Ten castrated pigs of approximately 40 kg of BW (Large White/Duroc cross) were housed individually in pens, and an indigestible marker of 0.1% chromic oxide (as-fed basis) was included in their diets for 1 wk to check digestibility before experiment. The pigs were randomly allotted to experimental or control groups of five pigs each and allowed ad libitum access to diets containing Bt11 or non-Bt corn for 4 wk. The animals were monitored for performance. Ten milliliters of peripheral blood was obtained from the vena cava in evacuated tubes with EDTA (for DNA extraction) or without EDTA (for serum) in the last week of the experiment. At necropsy, after 28 d of performance experiment, the stomach, duodenal, ileal, cecal, and rectal contents of all pigs were collected in plastic bags, placed on ice, and stored at –20°C until analysis. This experiment was done according to the guidelines for animal experiments of the National Institute of Livestock and Grassland Science (Tsukuba, Ibaraki, Japan).

## *Polymerase Chain Reaction and Sequencing*

*Deoxynucleic Acid Extraction.* Deoxynucleic acid from gastrointestinal contents was extracted using a kit (QIAamp DNA stool mini kit, Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol with slight modifications. Because corn kernels were visible in the GI contents, the samples (200 mg) were homogenized before extraction at 5,000 rpm (1.9 cm net travel distance, compressed figure-8 travel pattern; Mini BeadBeater, BioSpec Products, Inc., Bartlesville, OK) for 2 min in 2-mL tubes with 20 zirconium beads (3 mm in diameter, 1.45 g) and 1.4 mL of buffer (ASL buffer, included in the kit). From peripheral blood, DNA was extracted from the buffy coat using a kit (DNeasy tissue kit, Qiagen GmbH) according to the manufacturer's protocol. For the positive stool control and spike trials, DNA was extracted from a 1:1 mixture of rectal contents and corn powder. For the feed control, DNA was extracted from each diet containing Bt11 and non-Bt corn. The DNA content was measured by UV absorption at 260 nm, then its quality was checked from the 260/280 nm and 260/230 nm UV absorption followed by electrophoresis at 100 V on 0.8% agarose gel supplemented with 0.5 μL/mL of ethidium bromide (TaKaRa Bio Inc., Otsu, Japan) and photographed with a CCD camera under UV irradiation at 312 nm.

*Oligonucleotide Primers.* Five pairs of oligonucleotide primers were used to detect the intrinsic corn genes and the recombinant *cry1Ab* (Table 2): IVR for the corn invertase gene (226 bp) (Hurst et al., 1999), rbcL for the chroloplast encoding of ribulose-1,5-bisphosphate carboxylase/oxygenase (**RUBISCO**) large subunit (1,028 bp) (Doebley et al., 1990) and ZEn1-5′-ZE02 (242 bp), which was designed to detect shorter sequence within corn specific *Ze1* targeted by ZE01-ZE02 (Chowdhury et al., 2003a). Bt11 1-5′-cryIA 1-3′ (110 bp) and IVO1-CR01 (437 bp) were designed to amplify the rDNA between the 35S promoter region of the cauliflower mosaic virus and the synthetic *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki* (Matsuoka et al., 2000, 2001) (Table 2). Primers were synthesized at a laboratory (Hokkaido System Science Co. Ltd., Sapporo, Japan), diluted to a final concentration of 50 μ*M* and stored at –20°C until use.

*Polymerase Chain Reaction Conditions.* The PCR was done using a kit (AmpliTaq Gold, Applied Biosystems, Foster City, CA) in a thermal cycler (iCycler, Bio-Rad Laboratories, Hercules, CA). The reaction volume of 25 μL contained a 25-ng sample of DNA, 2.5 μL of PCR buffer II, 200 μ*M* of each dNTP (dATP, dCTP, dTTP, and dGTP), 1.5 m*M* $MgCl_2$, 0.025 U/μL of DNA polymerase, and 0.5 μ*M* of each primer. The PCR cycle was as follows: preincubation at 95°C for 10 min, and then 45 cycles consisting of denaturation at 95°C for 30 s; annealing at 63°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 7 min (Matsuoka et al., 2000; 2001). The negative control consisted of no template DNA and no primer, which is an official standard method in Japan for detection of GM crops. The PCR amplicons were electrophoresed at 100 V on 3% agarose gel supplemented with 0.5 μL/mL of ethidium bromide (TaKaRa Bio Inc., Otsu, Japan). The gel was photographed by an imager (Molecular Imager FX system, Bio-Rad Laboratories, Inc.). Each PCR was done three times. Samples with positive results at least

**Table 2**. PCR primers, sequences, and specificity

| Name | Sequence | Specificity | Source[a] | Amplicon | Reference |
|---|---|---|---|---|---|
| ZEn1-5′ | 5-TTG GGT ACC ATG AAC CCA T-3′ | *ZE1*/sense | Maize | 242 bp | Chowdhury et al. (2003b) |
| ZE02 | 5-GTC GCA GTG ACA TTG TGG CAT-3′ | *ZE1*/antisense | Maize | | |
| IVR1 | 5-CCG CTG TAT CAC AAG GGC TGG TAC C-3′ | Invertase gene/sense | Maize | 226 bp | Hurst et al. (1999) |
| IVR2 | 5-GGA GCC CGT GTA GAG CAT GAV GAT C-3′ | Invertase gene/antisense | Maize | | |
| rbcLF | 5-ATG TCA CCA CAA ACA GAG AGA CTA AAG C-3′ | RUBISCO/sense | Maize | 1,028 bp | Doebley et al. (1990) |
| rbcLR | 5′-AAA GTT ATT TCG CGT TCC CCT TCT AAC T-3′ | RUBISCO/antisense | Maize | | |
| Bt11 1-5′ | 5-CCA TTT TTC AGC TAG GAA GTT C-3′ | *adh1-1S*/sense | Bt11 | 110 bp | Matsuoka et al. (2001) |
| cryIA 1-3′ | 5-TCG TTG ATG TTK GGG TTG TTG TCC-3′ | *cry1Ab*/antisense | Maize | | |
| IV01 | 5-GGT ACA GTA CAC ACA CAT GTA T-3′ | *adh1-1S*/sense | Bt11 | 437 bp | Matsuoka et al. (2000) |
| CR01 | 5-GAT GTT TGG GTT GTT GTC CAT-3′ | *cry1Ab*/antisense | Maize | | |

[a]RUBISCO = ribulose-1,5-bisphosphate carboxylase/oxygenase.

twice were considered positive (Chowdhury et al., 2003a).

*Sequencing.* The PCR amplicons derived from Bt11 1-5′-cryIA 1-3′, IV01-CR01 and ZEn1-5′-ZE02 were sequenced through cloning in a PCR2.1-TOPO vector at a laboratory (Hokkaido System Science, Co. Ltd., Sapporo, Japan). The DNA sequences were compared with the registered sequence of *cry1Ab* (Matsuoka et al., 2000) and zein (*Ze1*) (EMBL accession No. X07535).

### *Cry1Ab Protein Detection*

*ELISA.* An ELISA kit was used for the detection according to the manufacturer's protocol (Cry1Ab/Cry1Ac plate kit, Envirologix Inc., Portland, ME). Twenty milligrams of GI samples in microcentrifuge tubes was mixed with 500 μL of Cry1Ab extraction buffer and agitated for a minimum of 1 min. The centrifuged supernatants were further diluted to 1:11 with the extraction buffer. Plates were read at 450 nm in a microplate reader (Thermo-Max, Molecular Devices, Sunnyvale, CA). The amount of Cry1Ab protein present in the rectal contents was converted into parts per billion (ng/g), and Cry1Ab protein digestibility was estimated from the digestibility assay. Each sample was assayed in duplicate.

*Immunochromatography.* A kit (Trait Bt1 Corn Grain Test, Strategic Diagnostic Inc., Newark, NJ) was used according to the manufacturer's instructions, with minor modifications. In brief, 500 mg of each GI sample (stomach, duodenal, ileal, cecal, or rectal contents) and 1 mL of sample buffer were added in plastic cups and agitated until they were homogenized. The test strips were inserted into the cups and left until the control line was developed. Standard positive and negative controls for Cry1Ab protein (Agdia Inc., Elkhart, IN) served as controls.

*Immunoblot.* Twenty microliters of sera (1:80 dilution) or 20 μL of stomach or rectal content extracts was supplemented with 2% SDS loading buffer and electrophoresed in 5 to 20% gradient polyacrylamide gels at 20 mA, and transferred to a 0.45-μm pore size nitrocellulose membrane. The membranes were blocked (Block Ace, Dainihon Pharmaceutical, Osaka, Japan) overnight at 4°C, washed, and incubated with 1:5,000 dilution of rabbit anti-Cry1Ab serum (raised with recombinant Cry1Ab in an *Escherichia coli* system) for 30 min at room temperature (Chowdhury et al., 2003b). After washing, the membranes were incubated with 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Sigma, St. Louis, MO) for 30 min at room temperature and developed with a substrate system (BCIP/NBT phosphatase, Kirkegaard & Perry Lab., Gaithersburg, MD).

## Results and Discussion

### *Animal Performance*

During the experiment, pigs in both groups were in good health. Mean food intake was 2.20 kg/d in both groups. Mean growth rate was 1.03 kg/d in the Bt-fed group, whereas it was 1.02 kg/d in the non-Bt-fed group. Feed efficiency was 0.47 in both groups. There was no difference in general health and growth rate of the pigs between both groups.

### *Detection of DNA Fragments*

Most of the DNA extracted from GI contents showed a smear pattern on agarose gel electrophoresis, suggesting degradation, whereas extracted DNA from the blood buffy coat was more than 10.0 kp in size (data not shown). The primer pairs detected corn DNA fragments in the GI contents of Bt11- and non-Bt-fed pigs (Figure 1). The stomach, duodenal, and cecal samples were positive for the RUBISCO gene in all 10 pigs, ileal samples were positive for seven pigs, and rectal samples for five pigs (Table 3). The invertase gene was detected in the stomach and ileum of all 10 pigs, the duodenum and cecum of eight pigs and the rectum of seven pigs. The *Ze1* was detected in the stomach in seven of 10 pigs, and in three of 10 pigs in the duodenum, ileum, cecum, and rectum. The detection of RUBISCO, invertase and zein genes by PCR indicated that the ingested corn-derived DNA was partially degraded in the

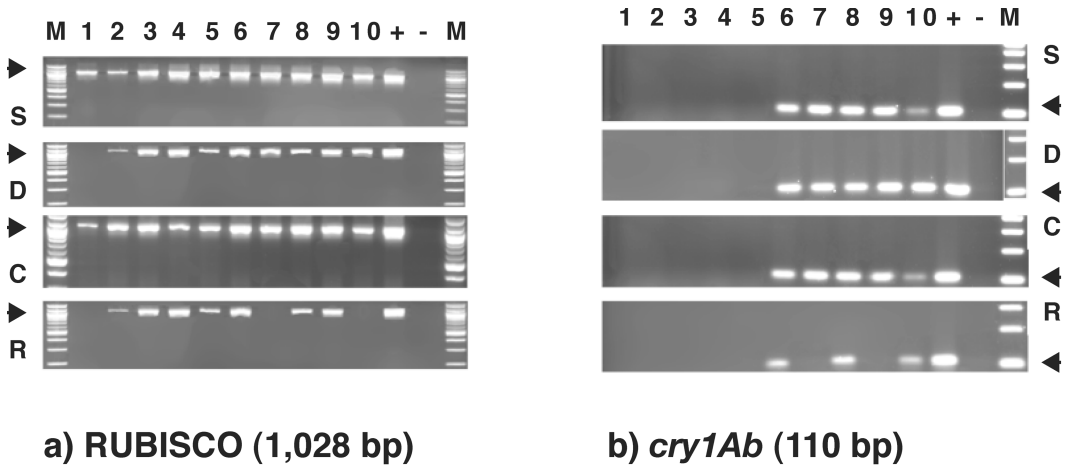


**Figure 1**. The PCR products from the gastrointestinal contents of pigs fed Bt11- and non-Bt corn are shown in this figure. a) The rbcL (1,028 bp) for chloroplast DNA of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) (left), b) Bt11 1-5′-cryIA 1-3′ (110 bp) for *cry1Ab* (right). Arrows indicate the expected length of PCR products. Lanes 1 to 5 = non-Bt-fed pigs (control); lanes 6 to 10 = Bt11 fed pigs; + = positive (Bt11), and − = negative control (no DNA). Additional coding: M = marker, S = stomach contents, D = duodenal contents, C = cecal contents, and R = rectal contents.

GI tract. Although the expected length of the rbcLF-rbcLR is comparatively large (1,028 bp), it detected RUBISCO as frequently as IVR1-IVR2. This might be due to multiple copies of the chloroplast gene of RUBISCO in the corn genome. Invertase gene fragment has been detected from the corn grits and corn products by IVR1-IVR2 (Hurst et al., 1999).

The primer pair Bt11 1-5′-cryIA 1-3′ (110 bp) detected *cry1Ab* in all of the stomach, duodenal, ileal, and cecal contents, and in the rectal contents in three of five pigs; in addition, IV01-CR01 (437 bp) detected *cry1Ab* in the stomach contents of five pigs, in the duodenal, ileal and cecal contents of three of five pigs, and in the rectal contents of one pig (Table 3). In contrast, none of the control pigs exhibited a positive result by these two primer sets. The IVO1-CR01 (437 bp) exhibited a weaker signal than Bt11 1-5′-cryIA 1-3′ (110 bp) did.

The use of primer with a shorter expected length increased the chance of detection of plant and corn DNA from chickens and cows (Einspanier et al., 2001). The use of two or more primer sets increased the reliability of detection by PCR. The primer sets for *cry1Ab* were designed to amplify DNA sequence from two or three different exogenous organisms to prevent false positive reaction in the present results (Matsuoka et al., 2000). The sequenced amplicons from GI contents showed 100% homology when compared with the targeted DNA sequence of the *Ze1* (EMBL accession No. X07535) and *cry1Ab* sequenced from Bt11 corn (Matsuoka et al., 2000). Thus, corn DNA and GM DNA were considered not totally degraded but rather present in a form detectable by PCR in the gastrointestinal tract. We obtained similar results in a pig performance study using GM StarLink CBH351 corn and its non-GM isoline (Chowdhury et al., 2003a). While in an in vitro study, fragments of corn chromosomal DNA and the *cry1Ab* gene (1,914 bp) remained detectable after 30 min of incubation in silage effluent and for at least 60 min in ovine saliva, whereas the same target sequence was not detectable after a 1-min incubation in rumen fluid (Duggan et al., 2000). Similar in vitro results were found in Japan (S. Tajima, M. Mitsumori, and M. Kurihara, personal communication). These results suggested the difference of experimental results between in vitro and in vivo.

We detected neither corn nor *cry1Ab* gene fragments in the peripheral blood by PCR (data not shown). On the other hand, fragments of ubiquitous plant chloroplast gene (199 bp) have been detected in blood lymphocytes of cows and tissues of chickens, whereas *cry1Ab* rDNA fragments were not detected (Einspanier et al., 2001). Further examination on the fate of feed-derived DNA is needed.

### *Detection of Cry1Ab Protein*

With ELISA, the amount of Cry1Ab protein in the Bt11 diet was 600 ng/g, and that in the rectal contents was 300 ± 140 ng/g (mean ± SD). The digestibility of Cry1Ab protein was estimated to be 92% by comparison with indigestible chromic oxide. The Cry1Ab protein was variously detected in the contents of stomach, duodenum, ileum, cecum and rectum (Table 4). None of the GI contents of the control pigs showed positive reaction to Cry1Ab. With immunochromatography, a similar reaction was observed (Figure 2, Table 4). With immunoblot, a band of a 65-kDa mass was detected in stomach contents of three of five pigs and rectal contents of four of five pigs, but the reaction was weak (data not shown). This suggested Cry1Ab protein of 130 kDa was cleaved into half sized 65-kDa protein and still existed in the GI tracts. These results suggested that although dietary protein was mostly degraded, antigenicity of

**Table 3.** Detection of DNA fragments of corn gene from gastrointestinal contents of pigs by PCR[a]

| | Maize intrinsic gene | | | | | | | | | | | | *cry1Ab* gene | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ZEn1-5′-ZE02 (142 bp) | | | | IVR1-IVR2 (226 bp) | | | | rbcLF-rbcLR (1,028 bp) | | | | Bt11 1-5′-cryIA 1-3′ (110bp) | | | | IVO1-CRO1 (437 bp) | | | | | | | |
| Pigs[b] | S | D | I | C | R | S | D | I | C | R | S | D | I | C | R | Pigs | S | D | I | C | R | S | D | I | C | R |
| E1 | ++ | ++ | ++ | − − − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | +− − | E1 | ++ | ++ | ++ | ++ | +− − | ++ | ++ | ++ | +− − | +− |
| E2 | ++ | −− − | ++ | − − − | −− − | ++ | ++ | ++ | +− − | −− − | ++ | ++ | ++ | ++ | −− − | E2 | ++ | +− − | ++ | ++ | +− − | ++ | +− − | ++ | ++ | −− − |
| E3 | ++ | +− − | −− − | − − − | +− − | ++ | +− − | +− − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | E3 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | −− − |
| E4 | ++ | ++ | +− − | − − − | +− − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | +− − | E4 | ++ | ++ | ++ | ++ | +− − | ++ | ++ | ++ | ++ | −− − |
| E5 | +− − | +− − | +− − | − − − | +− − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | −− − | ++ | ++ | E5 | ++ | +− − | ++ | ++ | +− − | ++ | +− − | −− − | +− − | −− − |
| C1 | ++ | −− − | −− − | − − − | −− − | ++ | ++ | ++ | +− − | ++ | ++ | ++ | ++ | ++ | −− − | | | | | | | | | | | |
| C2 | −− − | −− − | ++ | − − − | −− − | ++ | ++ | ++ | ++ | −− − | ++ | ++ | ++ | ++ | ++ | | | | | | | | | | | |
| C3 | +− − | ++ | +− − | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | +− − | ++ | ++ | | | | | | | | | | | |
| C4 | ++ | −− − | −− − | +− − | +− − | ++ | ++ | ++ | ++ | +− − | ++ | ++ | +− − | ++ | +− − | | | | | | | | | | | |
| C5 | ++ | −− − | +− − | +− − | +− − | ++ | +− − | ++ | ++ | −− − | ++ | ++ | ++ | ++ | +− − | | | | | | | | | | | |

[a]Results are presented from each of three PCR. Gastrointestinal segments are denoted by: S = stomach contents, D = duodenal contents, I = ileal contents, C = cecal contents, R = rectal contents.
[b]Coding of pig identification is: E1 through E5 = Bt11-fed pigs and C1 through C5 = non-Bt fed control pigs. For pigs with three (+++) or two (++−) results were judged as positive for detection of the gene. Pigs with one (+− −) or no (− − −) positive results were not considered to be positive. None of the samples in non-Bt fed control pigs was positive for *cry1Ab*.

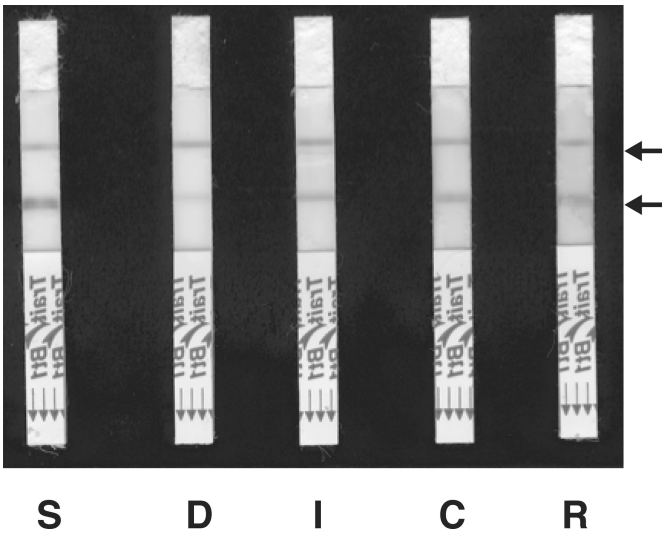


**Figure 2**. Immunochromatography for the Cry1Ab protein in the gastrointestinal contents of Bt11 corn-fed pigs. Additional coding: S = stomach contents, D = duodenal contents, I = ileal contents, C = cecal contents, and R = rectal contents. The arrow indicates control line; the arrowhead indicates test line. Test line shows the presence of Cry1Ab protein. Pigs fed non-Bt showed no positive reaction.

the Cry1Ab was still retained in the GI tracts. Similar results were observed using these three immunoassays in a calf performance experiment by feeding Bt11 corn (Chowdhury et al., 2003b). However, because the ELISA or immunochromatography kits and immunoblot did not work for blood samples, the present trial could not determine whether Cry1Ab was absorbed into the blood. The Cry1Ab protein in GI tracts is not harmful to the mammalian gut epithelial cells because of the lack of Cry protein receptors (Sacchi et al., 1986; Hofmann et al., 1988; Kuiper et al., 2001).

Accidental or mechanical spread of feeds into the soil may artificially introduce GM into environment. Fecal excretion of fragments of the *cry1Ab* gene and Cry1Ab protein into the soil may be additional concern. However, *B. thuringiensis* and Cry proteins are ubiquitous in the Japanese soil (Mizuki et al., 1999). The Bt toxin released in the root or from biomass of Bt corn is not toxic to earthworms, nematodes, protozoa, bacteria, or fungi experimentally (Saxena and Stotzky, 2001). In field studies, no detrimental effects have been found on the beneficial insects fed on GM corn (Pilcher et al., 1997; Betz et al., 2000).

## Implications

The present results suggest that no difference in general health and growth rate is detected between Bt11 corn-fed and control pigs. Whether intrinsic genes or recombinant sequence, corn-derived deoxyribonucleic acid is largely degraded in the gastrointestinal tract;

**Table 4**. The Cry1Ab protein in the gastrointestinal contents of Bt11 corn-fed pigs by ELISA and immunochromatography[a]

| | Pig No. | | | | |
|---|---|---|---|---|---|
| Organ | 1 | 2 | 3 | 4 | 5 |
| Stomach | ±/+ | –/+ | +/+ | +/+ | +/+ |
| Duodenum | ±/+ | –/± | –/– | ±/+ | –/± |
| Ileum | ±/± | +/+ | +/± | ±/+ | –/± |
| Cecum | +/+ | +/+ | +/+ | +/+ | +/+ |
| Rectum | +/+ | +/+ | ±/– | +/+ | +/+ |

[a]Data show results for both assays as ELISA results/immunochromatography results. Coding used is + (>200 ng/g by ELISA or positive in immunochromatography), ± (50 to 200 ng/g by ELISA or weak in immunochromatography) and – (<50 ng/g by ELISA or negative in immunochromatography).

however, fragmented corn deoxyribonucleic acid is still detectable by polymerase chain reaction. Most of the recombinant Cry1Ab protein is also degraded in the gastrointestinal tract, but antigenecity of the protein is retained. Such Cry1Ab protein is not harmful to the mammalian gut epithelial cells because of the lack of Cry protein receptors. Scant amounts of fragmented *cry1Ab* deoxyribonucleic acid and degraded Cry1Ab protein can be excreted, of which the environmental effect may be negligible.

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