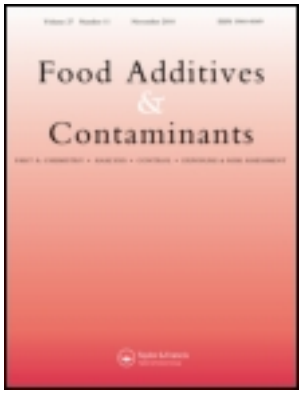

Food Additives & Contaminants: Part A
Publication details, including instructions for authors and subscription information:
http://tandfprod.literatumonline.com/loi/tfac20

Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products

L.A.P. Hoogenboom [a], J.G. Bokhorst [b], M.D. Northolt [b], L.P.L. van de Vijver [b], N.J.G. Broex [a], D.J. Mevius [c], J.A.C. Meijs [d] & J. Van der Roest [a]

[a] RIKILT Institute of Food Safety, Wageningen, the Netherlands

[b] Louis Bolk Institute, Driebergen, the Netherlands

[c] Central Veterinary Institute, Lelystad, the Netherlands

[d] Biologica, Utrecht, the Netherlands

Available online: 26 Sep 2008

To cite this article: L.A.P. Hoogenboom, J.G. Bokhorst, M.D. Northolt, L.P.L. van de Vijver, N.J.G. Broex, D.J. Mevius, J.A.C. Meijs & J. Van der Roest (2008): Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products, Food Additives & Contaminants: Part A, 25:10, 1195-1207

To link to this article: http://dx.doi.org/10.1080/02652030802014930

Food Additives and Contaminants
Vol. 25, No. 10, October 2008, 1195–1207

Taylor & Francis
Taylor & Francis Group

Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products

L.A.P. Hoogenboom[a]*, J.G. Bokhorst[b], M.D. Northolt[b], L.P.L. van de Vijver[b], N.J.G. Broex[a], D.J. Mevius[c], J.A.C. Meijs[d] and J. Van der Roest[a]

[a]*RIKILT Institute of Food Safety, Wageningen, the Netherlands;* [b]*Louis Bolk Institute, Driebergen, the Netherlands;* [c]*Central Veterinary Institute, Lelystad, the Netherlands;* [d]*Biologica, Utrecht, the Netherlands*

(*Received 29 August 2007; final version received 18 February 2008*)

Organic products were analysed for the presence of contaminants, microorganisms and antibiotic resistance and compared with those from conventional products. No differences were observed in the *Fusarium* toxins deoxynivalenol and zearalenone in organic and conventional wheat, during both a dry period and a very wet period which promoted the production of these toxins. Nitrate levels in head lettuce produced organically in the open field were much lower than those in conventional products. In iceberg lettuce and head lettuce from the greenhouse, no differences were detected. Organically produced carrots contained higher nitrate levels than conventional products. Both organic and conventional products contained no residues of non-polar pesticides above the legal limits, although some were detected in conventional lettuce. Organic products contained no elevated levels of heavy metals. *Salmonella* was detected in 30% of pig faeces samples obtained from 30 organic farms, similar to the incidence at conventional farms. At farms that switched to organic production more then 6 years ago no *Salmonella* was detected, with the exception of one stable with young pigs recently purchased from another farm. No *Salmonella* was detected in faeces at the nine farms with organic broilers, and at one out of ten farms with laying hens. This is comparable with conventional farms where the incidence for *Salmonella* lies around 10%. *Campylobacter* was detected in faeces at all organic broiler farms, being much higher than at conventional farms. One of the most remarkable results was the fact that faeces from organic pigs and broilers showed a much lower incidence of antibiotic resistant bacteria, except for *Campylobacter* in broilers. It is concluded that the organic products investigated scored as equally well as conventional products with regard to food safety and at the same time show some promising features with respect to antibiotic resistance.

Keywords: organic; lettuce; carrots; pigs; broilers; antibiotic resistance; *Salmonella*; *Campylobacter*

Introduction

Organic production differs from conventional production in a number of ways. The use of synthetic pesticides is, for example, prohibited, the use of antibiotics is restricted and under strict control, only animal manure is used for fertilization, and animals are allowed to forage outside. Theoretically, this may result in a different composition of the products, both in terms of natural ingredients, but also with regard to contaminants and microorganisms. For example, it has been suggested that wheat may contain higher levels of mycotoxins, since no fungicides are used during production. On the other hand, the mechanical treatment of the soil against weeds, which is not so common in conventional practice, and the much poorer fertilization may create less advantageous conditions for the survival of moulds during the winter. Foraging outside might expose the animals to higher levels of environmental contaminants, which end up in the edible products. This has, for example, been demonstrated in the case of organic eggs, which may contain elevated levels of dioxins and dioxin-like PCBs (Traag et al. 2002; Schoeters and Hoogenboom 2006; Van Overmeire et al. 2006; Kijlstra et al. 2007). The use of animal manure may also result in increased levels of heavy metals in organically produced vegetables and the presence of dangerous microorganisms like *Salmonella* and *Escherichia coli* O157. The restricted use of antibiotics might be expected to result in a decreased antibiotic resistance, especially against the newer generation of antibiotics.

A number of studies have focused on potential differences between organic and conventional products, but these are still limited (Woese et al. 1997; Soil Association 2001; Magkos et al. 2006). The aim of the present study was first to examine the food safety aspects of organic products and secondly to look for potential differences with conventional products, focusing on a limited number of contaminants and microorganisms. A major issue in this type of studies is

*Corresponding author. Email: ron.hoogenboom@wur.nl

ISSN 0265–203X print/ISSN 1464–5122 online

DOI: 10.1080/02652030802014930
http://www.informaworld.com

the sampling of the products, since various factors may influence the results. It was decided to focus on Dutch products and to sample these as much as possible at the farms, thus allowing a check on the source. Furthermore, it allowed an analysis of factors that might contribute to the occurrence or levels of certain contaminants or microorganisms. Certain conventional products were sampled at stores. In other cases, data were compared with data from existing monitoring programmes. The study included the most important organic vegetable products in the Netherlands, being lettuce, wheat, carrots and potatoes. In addition, eggs, broilers, pigs and cows were studied. Investigated were factors such as mycotoxins, nitrate, heavy metals, pesticides and *E. coli* O157 in vegetables, and *Salmonella*, *E. coli* O157, antibiotic-resistant bacteria, heavy metals and veterinary drugs in animals or their products. As such the study contributes to the essential and, fortunately, growing amount of information about the food safety of organically produced food products.

Materials and methods

Sampling of products

Vegetables

Samples of 1 kg organic or conventional wheat were collected in August 2003 and August/September 2004 at processing plants. Due to limited availability, this comprised in 2003 nine samples conventional winter wheat and nine samples organic summer wheat, and in 2004 fourteen samples conventional winter wheat, 17 samples conventional summer wheat and 22 samples organic summer wheat. Samples came from several provinces in the Netherlands, with the exception of the organic summer wheat in 2004 that came primarily from the centre of the Netherlands, Flevoland.

Organic lettuce was collected in 2003 and 2004 at three distribution centres and at small farms. Samples came from various locations in the Netherlands. Conventional lettuce was sampled at super markets. Each sample contained three to eight pieces of lettuce. The lettuce produced in the field was sampled in 2003 in September/October, and in 2004 in June/July. Lettuce from the greenhouse was sampled in January until March in 2004. In each case similar numbers of samples were taken from both types of production.

Organic carrots were sampled in 2003 at farms shortly after the harvest (October–November). In addition, organic and conventional carrots were sampled at farms (15 organic, four conventional), distribution centres (four conventional) and shops (seven conventional) in March–April 2004. Although spread all over the Netherlands, most farms were located in Flevoland. Each sample contained 40 carrots.

Organic potatoes were to a large extent sampled at the same farms as the carrots in October–November 2004 shortly after the harvest. Each sample contained 40 potatoes.

Animal products

In the case of pigs, 31 organic pig farms were visited, ten in December 2003–January 2004 and 21 in April–May 2005. For analysis on *Salmonella* and *E. coli* O157, at each farm faeces sampled from twelve pigs, shortly before slaughter, were pooled into a combined sample. On farms with more stables, two or three pooled samples were taken. To study the incidence of antibiotic-resistant bacteria, faecal samples were taken from five pigs per farm. All samples were immediately sent to the laboratories. Pig kidneys and diaphragm from 20 animals of 20 different organic farms were sampled at the slaughterhouse.

Samples of bovine faeces were collected at ten organic farms according to routine procedures of the Food and Consumer Products Safety Authority. The ten farms were visited in October–November 2003. One farm with a positive result on *E. coli* O157 was revisited in June 2004. Each pooled sample consisted of faeces from twelve cows. The number of samples per farm was two for fewer than 40 cows, three for 40–59 cows, four for 60–199 cows and five for more than 200 cows. Samples were put in polystyrene boxes and shipped immediately to the laboratory. Ten bovine kidneys from ten organic farms were sampled at the slaughterhouse.

To examine *Salmonella* in faeces of laying hens and broilers,, respectively, ten (April–May 2004) and nine (April–June 2005) organic farms were visited and five pooled samples, each from twelve fresh droppings, were collected and sent immediately to the laboratory, according to the protocol of the Product Boards for Livestock, Meat and Eggs (PVE). For antibiotic-resistant bacteria in broilers, five samples of one fresh dropping per farm were collected and send to the laboratory, according to standard protocols. At ten organic farms with eggs, 30 fresh eggs per farm were collected and mixed at the laboratory into one pooled sample per farm.

Analysis of samples

For chemical analysis, lettuces, carrots and potatoes were frozen in liquid nitrogen and homogenized. Wheat was milled with a sieve of 1 mm. For microbiological examination samples were not pre-treated.

Mycotoxins in wheat

The 71 wheat samples were analysed for mycotoxins using an LC-MS/MS multi-method, including

aflatoxin B_1, fumonisins B_1 and B_2, ochratoxin A, zearalenone, deoxynivalenol, T-2 and HT-2 toxins. Zearalenone was also examined with an HPLC method and fluorescence detection. In short, from each sample two portions of 10 g were taken. One portion was extracted with water and the other portion with a mixture of acetonitrile/water (84 : 16 v/v). Both portions were extracted by shaking intensively for at least 3 h. After filtering from each extract 250 µl were mixed and injected on a LC-MS-MS system in multiple reaction monitoring (MRM) mode. To diminish matrix influences, the analytical method was performed by a standard addition protocol. The analysis of mycotoxins was performed on a Waters Ultima LC-MS/MS system in the MRM-mode. A water (5 mM formic acid)/acetonitrile (5 mM formic acid) gradient was run at a flow of 0.20 ml min^{-1} on a Phenomenex Synergy HydroRP column (150 × 2 mm, 5 µm). All mycotoxins were analysed under ESI+ conditions with the exception of zearalenone which was analysed under ESI– conditions. Reference substances (>95% purity) were obtained from Biopure Reference Substanzen GmbH (Tulln, Austria). Reference materials (Test materials) were obtained from FAPAS (Central Science Laboratory, York, UK). Detection limits were 0.1 mg kg^{-1} for deoxynivalenol and T-2 toxin, 0.1 mg kg^{-1} for HT-2 and fumonisins B_1 and B_2, 5 µg kg^{-1} for aflatoxin B_1, and 50 µg kg^{-1} for ochratoxin A and zearalenone.

Heavy metals and arsenic

Samples were treated with 70% HNO_3 in a microwave oven (MARS 5). An aliquot was subsequently analysed in an electrothermal atomic absorption spectrophotometer (ETAAS, SIMAA 6100) for cadmium, lead and arsenic by atomic adsorption at 228.8, 283.3 and 193.7 nm. Concentration measurements were determined from a working curve (linear) after calibrating the instrument with standards of known concentration.

Another aliquot was analysed for mercury in a Flow Injection Mercury System, FIMS 100 (Perkin-Elmer, Groningen, the Netherlands). In short, the mercury in the digested samples was reduced to the elemental state by tin(II) chloride solution and liberated from solution in a closed vessel system, cold vapour technique (CV AAS). The mercury vapour passed through a cell positioned in the light path of an atomic absorption spectrometer (FIMS). Its absorbance is measured at a wavelength of 253.7 nm. The absorbance signal is a function of the mercury concentration in the samples.

Nitrate

Samples of lettuce, carrots and potatoes were analysed according to the SKALAR method. In short, any debris (e.g. soil) from the sample was removed (without water) and a representative part of the sample was heated with water for 10–15 min in a boiling water bath. After cooling, the volume was fixed to a known amount and the samples filtered. The method procedure for the determination of nitrate is based on the hydrazinium reduction method (Skalar Methods, cat. no. 455–311). In short, the nitrate in the sample was reduced to nitrite with hydrazinium sulphate and the nitrite was determined by diazotizing with sulphanilamide and coupling with naphthylethylenediamine dihydrochloride to form a highly coloured azo dye. This dye was measured at 540 nm in a continuous flow detector (Skalar, Breda, the Netherlands).

Pesticides

Pesticides were analysed by a multi-method based on that of Anastassiades et al. (2003). Samples were extracted by a modified Quechers method (Díez et al. 2006). After addition of magnesium sulphate and salt, the mixture was centrifuged and an aliquot of the acetonitrile was purified with primary–secondary amine. After cleaning by dispersive solid-phase extraction the extracts were analysed on an Agilent Technologies 6890N GC (Waldbronn, Germany) equipped with an ATAS Optic 3 PTV injector (Veldhoven, the Netherlands) and a LECO Pegasus III TOF-MS (St Joseph, MI, USA). A Restek RTX-CL Pesticides column (30 m × 0.25 mm, 0.25 µm, Bellefonte, PA, USA) was employed with helium as carrier gas and a linear programmed temperature programme from 40°C to 280°C with 10°C min^{-1}. All analytes were measured in EI+ mode. Reference substances were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The method quantifies a number of selected pesticides and detects a large number of others qualitatively. If detected, these pesticides are quantified by injection of standards. LOQs vary between 0.01 and 0.5 mg kg^{-1}. The method is able to detect over 300 pesticides, including the major organophosphates, organochlorines and pyrethroids.

Veterinary drugs

Eggs, kidneys and meat were investigated for the presence of antibiotics (macrolides, aminoglycosides, sulphonamides, tetracyclines, quinolones, colistin) using microbial methods (Nouws et al. 1999; Pikkemaat, Mulder et al. 2007). Test systems consisted of a series of agar plates, each one specific for a group of residues and optimized for a particular matrix (Pikkemaat, Oostra-van Dijk et al. 2007). Methods were validated to conform to 657/2002/EC. Detection limits (CCß) were below the European Commission maximum residue limits, except for valnemulin,

cloxacillin and dicloxacillin in kidney, and valnemulin, cefapirin, cefalexin, kanamycin, cloxacillin and dicloxacillin in meat (one to four times the MRL).

Eggs were analysed for residues of toltrazuril and its metabolite ponazuril by LC-UV according to the method described by Mulder et al. (2005). In brief, homogenized whole egg was extracted with ethyl acetate. The solvent was evaporated and the residue dissolved in 0.02 M trisodium phosphate buffer. Purification was carried out by solid-phase extraction on an Oasis® HLB cartridge. LC-UV analysis was performed on a Symmetry® C18 150 × 3.0 mm column with a methanol/water/acetic acid gradient and ultraviolet light detection at 245 nm. The detection limit was 25 μg kg^{-1} for toltrazuril and 10 μg kg^{-1} for ponazuril.

Livers from broilers were analysed for residues of nitrofuran metabolites by LC-MS/MS based on the method of Hoogenboom et al. (1991) as modified by Cooper et al. (2005). In brief, homogenized liver samples were treated overnight with 0.1 M hydrochloric acid in the presence of 2-nitrobenzaldehyde. The samples were extracted twice with ethyl acetate. After evaporation, the residue was dissolved in mobile phase. LC-MS/MS analysis was performed on a Symmetry® C18 150 × 3.0 mm column with a acetonitrile/water/acetic acid gradient and detection with positive ESI in multiple reaction monitoring mode. The detection limit for furazolidone and furaltadone metabolites was 0.5 μg kg^{-1} and for nitrofurazone and nitrofurantoin 1.0 μg kg^{-1}. Quantification was performed by isotope dilution.

Salmonella and Escherichia coli O157

Lettuce was examined for *Salmonella* using two methods. An aliquot of 25 g of sample (outer leaves) was incubated in 225 ml buffered peptone water (BPW) for 18 h at 37°C. This pre-enrichment broth was subcultured in two selective enrichment media: Rappaport Vassiliadis soya broth (RVS) and Müller-Kaufmann tetrathionate novobiocin broth (MKTTn). After 24-h incubation at, respectively, 41.5 and 37°C, aliquots of the selective media were streaked at both XLD and BGA selective media. After 24-h incubation at 37°C the plates were evaluated for the presence of specific *Salmonella* colonies. Suspected colonies were confirmed using biochemical and serological methods. The VIDAS screenings method, based on an Enzyme Linked Fluorescent Assay, was started from the selective enrichment broths after 6–8 h. VIDAS-negative samples do not require confirmation and can be considered '*Salmonella* not detected'. VIDAS-positive samples were further treated as described above.

Lettuce was examined for *E. coli* O157 as follows. An aliquot of 25 g of sample (outer leaves) was incubated in 225 ml modified tryptone soy bouillon at 41.5°C. After both 6 and 18–24 h an immunomagnetic separation was carried out with Dynabeads® anti-*E. coli* O157. Aliquots were plated on two selective plates (CT-SMAC and Chromagar O157^{TM}). After 18–24-h incubation at 37°C the plates were evaluated for the presence of specific colonies. Suspected colonies were confirmed based on indole-production and agglutination with specific antiserum. Positive isolates were also confirmed by the RIVM (Bilthoven, the Netherlands).

Faeces samples were examined for *Salmonella* according to both the VIDAS or ISO 6579:2002 methods, as described for lettuce. From February 2004 faeces samples, after enrichment in BPW, were also inoculated in modified semi-solid Rappaport Vassiliadis medium (MSRV) with 0.01 g novobiocin l^{-1}. After incubation for 24 and 48 h at 41.5°C, plates were evaluated for specific growth areas, if necessary inoculated at selective growth media XLD and BGA, and further treated as described above. *E. coli* O157 in faeces was examined as described above for lettuce.

Antibiotic-resistant bacteria

The isolation of *E. coli*, *Enterococcus faecium*, and *Campylobacter* spp. was started directly upon arrival of the samples at CVI-Lelystad by making a 1 : 10 w/v suspension of the faeces in buffered peptone water with 20% glycerol, from which on the same day selective media were inoculated. Subsequently, the suspensions were stored at −20°C. *E. coli* and *E. faecium* were isolated on, respectively, MacConkey agar and Slanetz and Bartley agar by inoculating 50 μl of a serial dilution series of the 1 : 10 diluted sample with a spiral plater (*E. faecium*) or by direct inoculation of the plates with sterile swabs (*E. coli*). The MacConkey plates were incubated overnight aerobically at 37°C and the Slanetz Bartley plates during 2 days aerobically at 42°C. From each sample, an *E. coli* colony with the typical morphology was subcultured on heart infusion agar with 5% sheep blood and a colony typical of *E. faecium* on Columbia agar with 5% sheep blood. Both were incubated overnight at 37°C. Subsequently the strains were suspended in buffered peptone water with 20% glycerol and stored at −80°C. All *E. coli* isolates were identified biochemically by the indole reaction. The species identification of *E. faecium* was performed with a polymerase chain reaction (PCR) described by Dutka-Malen et al. (1995). For the isolation of *Campylobacter* spp. CCDA-agar was used with 32 μg ml^{-1} cefoperazone and 10 μg ml^{-1} amphotericine B to suppress the growth of Gram-negative bacteria and moulds. Plates were inoculated directly upon arrival of the samples with a swab. The CCDA-plates were incubated for 48 h in micro-aerophilic conditions at 42°C. Typical colonies were streaked on HIS plates and again incubated for 48 h

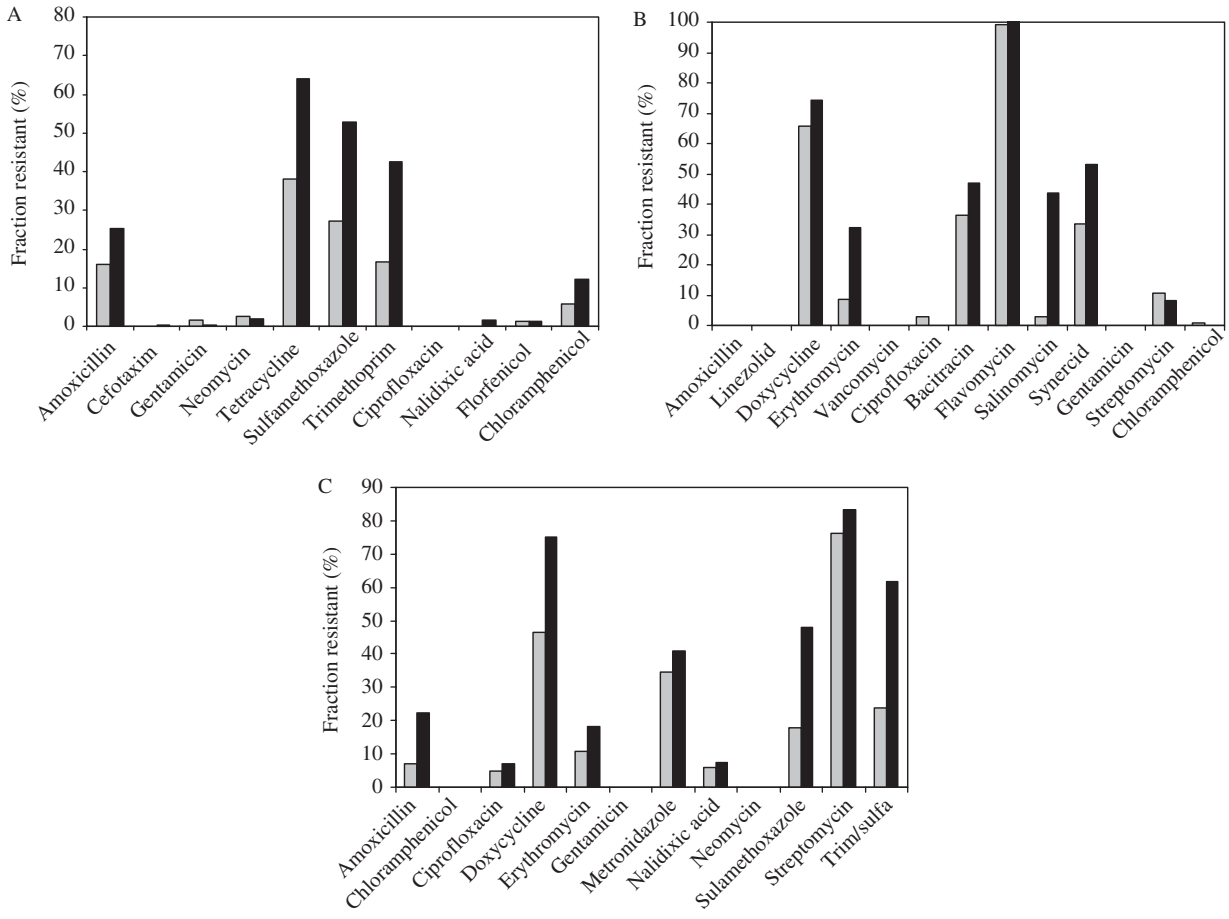

Figure 1. Incidence of antibiotic resistant *E. coli* (a), *E. faecium* (b) and *Campylobacter* (c) in faeces of organic (gray bars) and conventional (black bars) pigs. Five and one faeces samples were collected at each organic and conventional farm, respectively. *E. coli* was isolated from 155 and 296 samples, *E. faecium* from 105 and 110 samples, and *Campylobacter* from 84 and 198 samples, respectively.

micro-aerophilically at 37°C. The isolates were all identified as *Campylobacter coli* by PCR.

The susceptibility of the isolates was determined quantitatively using a broth micro-dilution method according to CLSI guidelines (M31-A2 and M100-S16), with cation-adjusted Mueller Hinton broth. Microtitre plates with dehydrated dilution series of a panel of antibiotics (Figures 1 and 2) were provided by Trek Diagnostic Systems (Basingstoke, UK). For *Campylobacter* spp. the plates, after inoculation with 50 µl per well of a 200-fold diluted 0.5 McFarland suspension in NaCl 0.85% solution, were incubated micro-aerophilic in a shaking incubator at 37°C for 24 h. ATCC strains *E. coli* 25922, *E. faecalis* 29212 and *C. jejuni* ATCC 33560 were tested daily to control the quality of the results.

The minimum inhibitory concentrations (MICs) were defined as the lowest antibiotic concentration at which, visually bacterial growth was observed. Strains with MICs higher than the MIC breakpoints defined by CLSI were classified as resistant. For those antibiotics (e.g. neomycin and florfenicol for *E. coli*, the former growth promoters for the enterococci) and bacterial species (*Campylobacter* ssp.), for which CLSI has not defined clinical MICs, breakpoints used in the Dutch Resistance Monitoring Programme were used (MARAN 2004). Subsequently resistance proportions were calculated for each individual antibiotic tested. Moreover, proportions of fully susceptible isolates and resistant to one, two, three, four and more than four different antibiotic classes were calculated.

Results and discussion

The present study focused on a limited number of contaminants and microorganisms in organic products and organic production systems. Selection was based on a report of the Expertise Centre for Agriculture in the Netherlands, and on information on other projects already running in this area. Contaminants already studied in ongoing studies, like dioxins in eggs

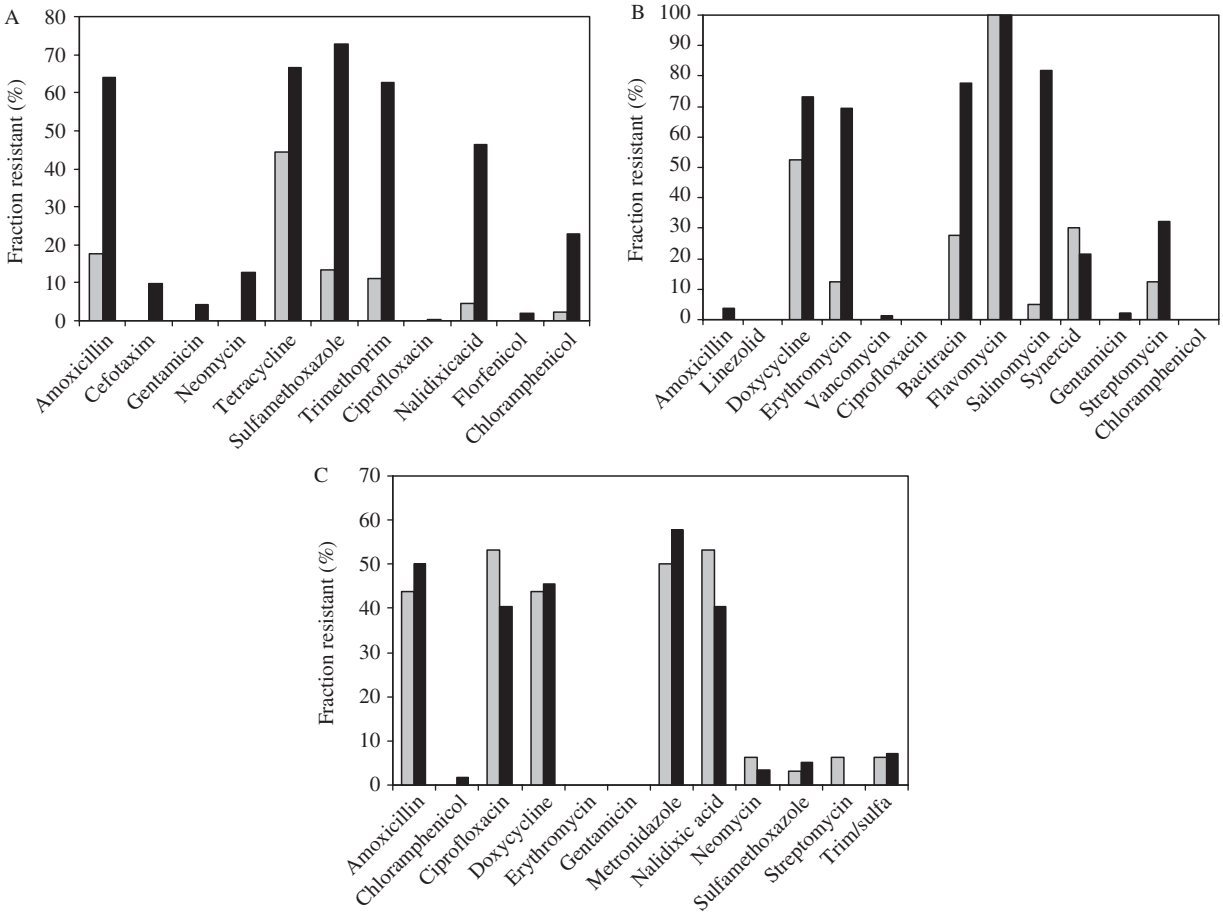

Figure 2. Incidence of antibiotic resistant *E. coli* (a), *E. faecium* (b) and *Campylobacter* (c) in faeces of organic (gray bars) and conventional (black bars) broilers. Five and one faeces samples were collected at each organic and conventional farm, respectively. *E. coli* was isolated from 45 and 300 samples, *E. faecium* from 45 and 180 samples, and *Campylobacter* from 32 and 57 samples, respectively.

(Kijlstra et al. 2007), were not included in this study. Results were subsequently compared with those from conventional agriculture, partly coming from this project and partly from other monitoring programmes in the Netherlands. In addition a comparison was made with results from other studies.

A very important issue is the sampling strategy and in particular the site of sampling; the farm or the shop/supermarket (Magkos et al. 2006). Samples taken at a shop give information on the products actually bought by the consumer. However, in this study the choice was made to collect data as much as possible at the farm, thus allowing the control on the origin of the product and a survey on the actual farming conditions. This would also allow an investigation on the potential causes for undesirable contaminants or microorganisms. It should be stressed that organic farming in the Netherlands is a growing market and that most of the organic farms switched to organic practices only recently. In cases where sampling at the farms was not possible, due to logistic or hygienic reasons, samples were taken at distribution centres and slaughterhouses. At the time of this study there were about 500 farms involved in the organic production of vegetables, potatoes and grains. In the present study 24, 35 and 21 farms were sampled for lettuce, carrots and potatoes, respectively. Results will be described and discussed for each type of product investigated.

Wheat (pesticides, mycotoxins, heavy metals)

Wheat was collected in 2003 and 2004. Twelve of the 20 organic samples came from farms that switched to organic less than five years ago. The organic wheat covered races like Melon, Lavett and Pasteur; the conventional wheat was not inventoried. The yield of organic wheat varied between 4300 and 7900 kg hectare^{-1}. Eighteen pooled samples of organic wheat were investigated for non-polar pesticides but none of the more than 300 different compounds could be detected.

The levels of the seven examined mycotoxins in wheat were below detection limits in the conventional and organic wheat samples, with the exception of the

Table 1. Levels of DON and ZON (median and range) in organic and conventional wheat sampled in 2003 and 2004.

Sample date	Production type		DON ($mg\,kg^{-1}$)	ZON ($mg\,kg^{-1}$)	Number of samples
2003	Organic	Summer	<0.1 (<0.1–0.2)	<0.05	9
	Conventional	Winter	0.3 (<0.1–1.0)	<0.05	9
2004, until 24 August	Organic	Summer	0.2 (<0.1–1.8)	<0.05 (<0.05–0.12)	7
	Conventional	Summer	0.2 (<0.1–1.5)	<0.05 (<0.05–0.13)	11
	Conventional	Winter	<0.1 (<0.1–0.4)	<0.05	14
2004, after 24 August	Organic	Summer	1.7 (<0.1–11.0)	2.6 (<0.05–5.7)	15
	Conventional	Summer	2.0 (0.5–6.3)	0.7 (<0.05–5.2)	6

Note: No significant differences between conventional and organic (Student's *t*-test, two-sided).

Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON) (Table 1). In most of the samples collected in 2003 levels of DON were below the action limit of $0.1\,mg\,kg^{-1}$, with the exception of six conventional products showing levels between 0.1 and $1\,mg\,kg^{-1}$. Of the samples collected in 2004 before the end of August, nine out of 14 samples (64%) of conventional winter wheat and eight out of eleven samples (73%) of conventional summer wheat, contained DON levels above the action limit (range = 0.1–$1.5\,mg\,kg^{-1}$) as compared with five out of seven (71%) samples organic summer wheat (range = 0.1–$1.8\,mg\,kg^{-1}$). However, after a period with heavy rain fall by the end of August 2004, DON was detected in all samples at levels up also to $11\,mg\,kg^{-1}$. In these samples, zearalenone was also increased with levels up to $5.7\,mg\,kg^{-1}$. Overall there was no significant difference between organic and conventional wheat. This is contradictory to studies by Malmauret et al. (2002) who did find higher levels of the *Fusarium* toxins DON, nivalenol, and zearalenone in organic wheat and barley. However, Döll et al. (2002), Bernhoft et al. (2003), Schneweis et al. (2005), Rossi et al. (2006) and Pussemier et al. (2006) also observed lower average levels in organic cereals. Czerwiecki et al. (2002), analysing various cereals in Poland in two subsequent years for ochratoxin A, observed higher frequencies of contamination of organic cereals in one year, but the opposite in the next year with some very high levels in some conventional wheat. In general, there is a tendency for organic cereals to contain lower mycotoxins levels but differences appear to be rather small (Magkos et al. 2006) and factors like climate may play a much more crucial role than the type of production.

Lead, mercury and arsenic levels in most samples were below the detection limits of, respectively, 0.1, 0.01 and $0.1\,mg\,kg^{-1}$. In four organic wheat samples mercury levels just above the LOD were detected, i.e. levels of 0.013, 0.011, 0.016 and $0.016\,mg\,kg^{-1}$. Cadmium could be detected in most samples but the highest level of $0.077\,mg\,kg^{-1}$ was well below the European Union limit of $0.2\,mg\,kg^{-1}$. There was no difference in cadmium levels between organic and conventional wheat (data not shown). This is contrary to Rossi et al. (2006), who found slightly lower cadmium but higher lead levels in organic wheat, although not at levels exceeding the European Union limits.

Lettuce (microorganisms, pesticides, nitrate, heavy metals)

Organic lettuce was sampled at three distribution centres, responsible for 70% of the organic lettuce. Half of the farms produced organically for two decades or more, the other half for less than 10 years. Conventional lettuce was obtained from local stores. None of the samples tested positive for *Salmonella* or *E. coli* O157, two types of microorganisms that might be introduced through the use of animal dung. This shows that this is not a common problem but cannot exclude the presence of these pathogenic microorganisms in a small fraction of the products. To minimize the potential risk of contamination, several countries like the USA and Canada do not allow the use of non-composted manure.

Ten pooled samples of organic and conventional lettuce were examined for non-polar pesticides but none of the organic products tested positive. Two pooled samples of conventional lettuce contained residues of the permitted pesticide vinclozolin and one of these in addition malathion and piperonyl-butoxide. Levels were below the residue limits.

In both organic and conventional iceberg lettuce produced outside, nitrate levels were below the limits, with a range of 370–$1759\,mg\,kg^{-1}$ for organic and 652–$1367\,mg\,kg^{-1}$ for conventional products (Table 2). In the case of organic head lettuce produced outside (19 samples) and the greenhouse (ten samples), one sample of each exceeded the limit. In the case of conventional head lettuce, this frequency was much higher. Levels above the limit were detected in 18 out of 19 samples grown outside and four out of 14 samples from the greenhouse. In comparison with conventional head lettuce, nitrate levels were much lower for organic products. This confirms results from previous studies (Woese et al. 1997; Soil Association

Table 2. Nitrate and cadmium levels in organic (O) and conventional (C) iceberg and head lettuce produced outside and in the greenhouse.

			Nitrate[b] (mg NO_3 kg^{-1})				
Type	Production		Median	Range	*n*	Cadmium (mg kg^{-1}), range	*n*
Iceberg	Field	O	939	370–1759	13	<0.02–0.022	5
Iceberg	Field	C	966	652–1367	13	<0.02–0.038	4
Head lettuce	Field	O	1275[a]	139–3212	19	<0.02–0.042	6
Head lettuce	Field	C	3280[a]	1818–4357	19	<0.02–0.052	8
Head lettuce	Greenhouse	O	3223	946–4129	10	<0.02–0.043	9
Head lettuce	Greenhouse	C	3515	2439–5197	14	<0.02–0.066	8

Notes: [a]Differences between nitrate in conventional and organic head lettuce from the field were statistically significant (Student's *t*-test, $p < 0.001$).
[b]European Union limits for head lettuce from the field are 2500 mg kg^{-1}, harvested between 1 April and 30 September, and 4000 mg kg^{-1} when harvested between 1 October and 31 March; for head lettuce from the greenhouse 3500 mg kg^{-1}, when harvested between 1 April and 30 September, and 4500 mg kg^{-1} when harvested between 1 October and 31 March; for iceberg lettuce from the field 2000 mg kg^{-1} (EC 2001, 2002b).

2001; Consumentenbond 2002). In the case of iceberg lettuce no differences were observed.

Levels of arsenic and the heavy metals lead, cadmium and mercury were below the limits. Cadmium levels showed a large variation, as shown in Table 2. However, no differences were observed between organic and conventional products.

Carrots (pesticides, nitrate, heavy metals)

Organic carrots were sampled at farms with varying experience, ranging from two to 18 years. Most of them switched to organic less than five years ago. More than eleven different varieties were used but Nerac was most popular. Yields varied between 35 and 75 tons $hectare^{-1}$. Four pooled samples of organic carrots were investigated for non-polar pesticides but none of the more than 300 different compounds could be detected.

Nitrate levels in organic carrots showed a large variation, with a range in 2004 of 11–864 mg kg^{-1} and a median of 244 mg kg^{-1} (Table 3). This was three times higher than in conventional carrots harvested in the same year, showing a median of 58 mg kg^{-1} and a range from 16 to 180 mg kg^{-1}. Previous studies showed lower levels in organic carrots (Consumentenbond 2002) but the present data confirm those from other studies showing an increase in nitrate levels in organic carrots (Bokhorst, personal communication). Malmauret et al. (2002) also observed higher levels in organic carrots, i.e. 394 versus 113 mg kg^{-1} product in conventional carrots. Studies at the farms showed that a relatively high use of dung and plant waste materials, as compared with previous years, is probably the cause of the rise in nitrate levels. This is partly related to farms that switched only recently to organic practices.

Table 3. Nitrate levels in organic (O) and conventional (C) carrots.

		Nitrate (mg NO_3 kg^{-1})		
Production	Year	Median	Range	*n*
Organic	2003	192	34–449	20
Organic	2004	244	11–864	15
Conventional	2004	58	16–180	15

Note: Differences between nitrate in conventional and organic carrots from 2004 were statistically significant (Student's *t*-test, $p < 0.001$).

Heavy metals and arsenic were only analysed in the organic carrots samples in 2003. None of the levels exceeded the limits, although some cadmium levels were close to the limit of 0.1 mg kg^{-1}.

Potatoes (pesticides, nitrate, heavy metals)

Organic potatoes were sampled at the farms; conventional potatoes were not investigated. Most of the farms were also sampled for carrots, again most of them with less than five years of experience. Agria and Sante were the most popular races with yields between 23 and 47 tons $hectare^{-1}$. Four pooled samples of organic potatoes were investigated for non-polar pesticides but none of the more than 300 different compounds could be detected.

The nitrate level in organic potatoes was low with an average of 87 mg kg^{-1} but the variation was quite large (range = 8–390 mg kg^{-1}). There were no Dutch data on conventional potatoes for comparing the data. However, levels were lower than those reported by Hajšlová et al. (2005) for organic potatoes. In that study levels in conventional potatoes were higher than in organic ones.

Table 4. Description of the farms with organic pigs, including the results from the studies on *Salmonella* and *Campylobacter*.

Farm number	Organic since	Animals	*Salmonella*	*Campylobacter*[a]	Rough fibre-rich feed
1	1997	340	Negative	5/5	+
2	1990	75	Negative	5/5	+++
3	1998	500	Negative	4/5	+
4	1998	122	Negative	3/5	++
5	1998	200	Negative	2/5	+
6	1995	140	Negative	4/5	+
7	1999	120	Negative	5/5	+
8	1999	525	Negative	5/5	+
9	1997	850	Negative	5/5	+
10	1990	450	Negative	0/5	+
11	2003	500	Positive	3/5	+
12	2004	700	Negative	4/5	±
13	1996	460	Positive	3/5	−
14	2003	400	Positive	2/5	+
15	2002	325	Negative	5/5	+
16	2002	257	Positive	3/5	+
17	2002	950	Negative	5/5	−
18	2004	400	Positive	3/5	−
19	2003	106	Negative	5/5	±
20	2002	620	Negative	1/5	+
21	2001	400	Negative	2/5	−
22	2002	120	Positive[b]	2/5	+
23	1998	400	Negative	5/5	−
24	2003	1100	Positive[b]	0/5	−
25	2003	450	Positive	0/5	+
26	2002	80	Negative	4/5	+
27	2002	440	Negative	1/5	+
28	1998	700	Negative	0/5	−
29	1998	300	Negative	0/5	+
30	2002	450	Negative	1/5	+
31	1999	20	n.i.[c]	0/5	−

Notes: [a]Number of faeces samples from which *Campylobacter* could be isolated for antibiotic resistance.
[b]Negative with the MSRV method.
[c]Not investigated due to loss of sample.

None of the samples levels exceeded the limits for heavy metals, although some cadmium levels were close to the limit of $0.1\,mg\,kg^{-1}$.

Pigs (veterinary drugs, heavy metals, microorganisms, antibiotic resistance)

In total 31 organic farms with pigs were visited, representing 53% of the organic pig farms in the Netherlands. Fourteen farms were more experienced (6–14 years), the other 17 switched to organic only recently (1–4 years). The number of pigs varied between 20 and 1100 (Table 4).

No residues of antibiotics were detected with a bacterial test in samples of kidneys and meat of 20 organic pigs, sampled at the slaughterhouse. Only one kidney sample showed a slight inhibition of bacterial growth on the macrolides plate but below the action level. Levels of arsenic in meat were below the detection limit of $0.1\,mg\,kg^{-1}$. Similar was true for levels of lead and mercury in the kidneys of these animals, being below, respectively, 0.05 and $0.005\,mg\,kg^{-1}$ with one exception, being a mercury level of $0.008\,mg\,kg^{-1}$. Cadmium levels varied between less than 0.005 and $0.38\,mg\,kg^{-1}$ (median $= 0.11\,mg\,kg^{-1}$), all of them being below the European Union limit of $1\,mg\,kg^{-1}$.

Initially ten farms were visited in the winter of 2003 and all the pooled faeces samples of the twelve animals per farm scored negative for *Salmonella*. Another set of 20 farms was sampled in the spring of 2005 and this time eight farms were positive for *Salmonella*. Interestingly, this included seven farms that only recently switched to organic (three years or less). The other farm turned to organic in 1996 and on this farm three barns were sampled. One of these samples, derived from a stable with young pigs that were purchased elsewhere, was positive. Overall the incidence of *Salmonella* was 27% and comparable with data known from conventional farms in the Netherlands. However, the fact that *Salmonella* could not be isolated from dung collected at the more experienced organic pig farms, is of potential interest. Possible causes could be the use of more rough fibre rich and more diverse feeds at organic farms

Table 5. Resistance of bacteria from pig faeces against different classes of antibiotics. Data are expressed as the percentage of bacteria resistant against zero, one, two, three, four or more than four classes of antibiotics. Conventional data from the MARAN study (MARAN 2004).

	E. coli		*E. faecium*		*Campylobacter*	
Resistant to classes	Organic	Conventional	Organic	Conventional	Organic	Conventional
0	43	27	3	5	22	5
1	25	19	22	21	33	15
2	15	14	43	28	22	26
3	7	16	22	17	14	25
4	6	17	9	17	4	16
>4	4	8	2	12	5	13

compared with conventional pig farms, which may influence the colonization of *Salmonella* in the gastrointestinal tract (Knudsen et al. 2001).

Campylobacter was one of the bacteria generally used for the assessment of antibiotic resistance and could be isolated from 56% of the faeces samples, being similar to conventional farms. When focusing on the farms, *Campylobacter* could be isolated from one or more of the five sampled pigs at 81% of the farms. *E. coli* O157 was not detected in any of the samples, but this was only analysed in samples from the first ten farms. This was decided because the data were in line with conventional farms where the prevalence of *E. coli* O157 is 0–2%.

The use of antibiotics at organic farms is restricted although 19 of the 31 farms mentioned the occasional use of antibiotics. The resistance level to a number of antibiotics was investigated in *E. coli*, *E. faecium* and *Campylobacter* spp., isolated from faeces of the five animals per farm (one isolate per sample). *E. coli* could be isolated from all 155 animals and resistance was only observed for older drugs like amoxicillin, neomycin, tetracycline, sulfamethoxazole, trimethoprim and chloramphenicol. No resistance was observed in *E. coli* from organic farms against newer drugs like cefotaxim, gentamicin, the quinolones ciprofloxacin and nalidixic acid, and florfenicol. Figure 1(a) shows that in general the resistance in organic pigs is lower than observed in conventional pigs, based on data from the Dutch regular monitoring programme (MARAN 2004). In this programme one animal per slaughter batch is sampled, according to guidelines of the European Food Safety Authority. Regarding the limited number of farms, it was decided to use five animals per farm in the present study, which could have some influence on the results. *E. Faecium* was isolated from 80% of the samples or on 97% of the farms. Significant resistance was only observed against doxycycline, quinupristin/dalfopristin (Synercid®) and bacitracin, and to flavomycin but this *Entorococcus* species is intrinsically resistant to this drug. Figure 1(b) shows that also for this species in general the resistance is lower than in conventional pigs, in particular true for erythromycin and salinomycin. Bacitracin has been used as a growth promoter until 1999 and resistance is rather widespread in conventional pigs, and apparently also in organic pigs. Synercid is related to virginiamycin, also used as a growth promoter until 1999.

As mentioned above, *Campylobacter* (*jejuni* and *coli*) could be isolated from 80% of the samples, or at 77% of the farms. In comparison with *E. coli* and *E. faecium*, *Campylobacter* showed resistance to many of the tested drugs (nine out of twelve). Remarkable was the resistance against the quinolones ciprofloxacin and nalidixic acid, since this type of drugs is only used to treat individual pigs. In general, resistance in organic pigs was lower than in conventional pigs (Figure 1c). When looking at the fraction of bacteria resistant to one, two, three, four or more than four antibiotics, 32% of the *E. coli* isolates from organic pigs were multiple drug resistant (resistant to two or more independent classes of antibiotics), as compared with 55% in conventional pigs (Table 5). For *E. faecium* figures were similar for organic and conventional, being, respectively, 75% and 74%, but for *Campylobacter* spp. lower for organic, i.e. 45% versus 80%. Although the lack of difference for *E. faecium* seems remarkable, differences became more clear when focusing on multiple resistance against three or more antibiotic classes, showing 33% resistance for organic versus 46% for conventional. Furthermore, low-level resistance (<10%) was observed in *Enterococci* isolated from organic farms for erythromycin and salinomycin compared with the resistance levels found in conventional animals.

Lactating cows (veterinary drugs, microorganisms)

Ten kidney samples of cows, reared in organic farms, were collected at the slaughterhouse and tested for bacterial growth inhibition. Three samples showed a slight inhibition at the aminoglycosides plate but below the action limit.

Ten farms with lactating cows were visited in September–November 2003 and samples of dung were collected. Farm size varied between 35 and 74 cows and one bigger farm with 250 animals. *E. coli* O157 was detected in all three samples of one farm and in one of four samples of another farm, the latter collected at a pasture with non-lactating cows and young animals. The first farm was revisited in September 2004 and found negative than, as was the case for the second farm revisited in June 2004.

Laying hens (veterinary drugs, microorganisms, heavy metals)

Ten farms out of a total of 100 were visited and both eggs and dung collected. Number of hens per farm varied between 170 and 10,900, and farms switched to organic between 1994 and 2003. Dung was tested for the presence of *Salmonella* using different methods, including the standard MSRV method. Most samples scored negative, except for one farm where one of the two barns tested positive. Except for one, hens were vaccinated against *Salmonella*, but antibiotics were reported not to be applied. All eggs tested negative for toltrazuril, and for the presence of aminoglycosides, sulfonamides, beta-lactam, tetracyclins, quinolones and colistin. Levels of cadmium, lead, arsenic and mercury were below the detection limits of, respectively, 0.005, 0.05, 0.1 and 0.005 mg kg^{-1}. This is in contrast to the rather high levels of heavy metals observed by Van Overmeire et al. (2006) in eggs from private owners in Belgium, indicating that heavy metals may be transferred to eggs, as previously shown for dioxins.

Broilers (veterinary drugs, microorganisms, heavy metals, antibiotic resistance)

The number of organic farms with broilers is rather limited in the Netherlands and these were all visited. Most farms turned organic since 1998 and the number of animals varied between 380 and 8000. Animals belonged to slow-growing varieties, as reflected by the age at slaughter of 70–81 days (for conventional broilers this is around 40 days). Antibiotics were reported not to be used. Other veterinary drugs and anthelmintics were used on two and one farms, respectively. Livers were checked for nitrofuran drugs and all tested negative. Lead, arsenic and mercury in livers were below the detection limits of, respectively, 0.05, 0.1 and 0.01 mg kg^{-1}. Cadmium levels varied between <0.005 and 0.029 mg kg^{-1}, well below the European Union limit of 0.5 mg kg^{-1}.

On none of the nine organic farms, animals were carriers of *Salmonella* as determined with the MSRV method. Of course, the number of farms is very small, but the data are in line with those observed by the VWA in 2003 and 2004 in meat, showing incidences of 2 and 3%, respectively, as compared with 11 and 7% in meat from conventional broilers (VWA 2004b, 2005). *Campylobacter* was detected in faeces samples at all farms, and overall could be isolated from 32 of the 45 samples. In conventional broilers the incidence is much lower, in 2000, 2001 and 2002, respectively, at 24, 16 and 27% (VWA 2004a). The VWA detected *Campylobacter* in 36 and 44% of the meat products of organic broilers as compared with 26 and 29% of the conventional products (VWA 2004b, 2005). Heuer et al. (2001) examined organic and conventional farms in Denmark and detected *Campylobacter* in, respectively, 49 and 100% of the faeces samples collected during slaughter. Remarkable was the high incidence of 91% for *Campylobacter jejuni* and only 9% *C. coli*, whereas Rodenburg et al. (2004), who also observed a high incidence of *Campylobacter* in organic broilers, reported incidences of 30% for *C. jejuni* and 70% for *C. coli*. This is relevant since in particular *C. jejuni* is responsible for infections in humans. Our data are, however, in line with those of Heuer et al. (2001), also reporting primarily *C. jejuni*, independent of the type of production.

In *Campylobacter* highest resistance against antibiotics was found against amoxicillin, doxycycline, metronidazole, and the quinolones nalidixic acid and ciprofloxacin (Figure 2c). There were no clear differences in resistance proportions with data from 57 samples from conventional broilers. This was also the case for multidrug resistance (resistant against two or more independent classes of antibiotics) showing figures of 72 and 63% in organic versus conventional broilers (Table 6).

E. coli could be isolated from all 45 faecal samples, *E. faecium* from 89% of the samples. Considerable resistance was observed in *E. coli* against amoxicillin, tetracycline, sulfamethoxazole, trimethoprim and to a lesser extent nalidixic acid, but not against the newer antibiotics. Figure 2(a) shows a comparison with data from conventional broilers, demonstrating that in conventional broilers resistance occurred much more frequently than in organic animals. *E. faecium* showed considerable resistance against doxycycline, bacitracin, synercid, and to a lesser extent to erythromycin, salinomycin and streptomycin, but again much lower than in conventional broilers. This organism is known to be resistant against flavomycin as shown in Figure 2(b). Overall, 27% of the *E. coli* were multiple resistant (two or more independent classes of antibiotics) as compared with 81% of the conventional broilers. For *E. faecium* figures are, respectively, 69 and 94% (Table 6). The observed reduced resistance against antibiotics confirms previous studies by the Dutch Food and Consumer Products Safety Authority in the Netherlands (VWA 2005).

Table 6. Resistance of bacteria from broiler faeces against different classes of antibiotics. Data are expressed as the percentage of bacteria resistant against zero, one, two, three, four or more than four classes of antibiotics. Conventional data are from the MARAN study (MARAN 2004).

	E. coli		*E. faecium*		*Campylobacter*	
Resistant to classes	Organic	Conventional	Organic	Conventional	Organic	Conventional
0	53	10	3	1	28	19
1	20	9	30	6	0	18
2	16	8	38	10	31	23
3	2	13	20	16	22	26
4	9	22	8	22	13	7
>4	0	38	3	46	6	7

The fact that in organic broilers in the commensal gut flora (*E. coli* and *E. faecium*) less antimicrobial resistance occurs than in conventional broilers can mainly be explained by the absence of selection pressure in organic animals. The fact that in *Campylobacter* spp. this difference was not observed is likely to be caused by the fact that in broilers *Campylobacter* colonization occurs after environmental contamination with this organism. In a densely populated country like the Netherlands it can be speculated that the *Campylobacter* spp. isolated from the organic broilers originate from conventionally reared poultry and were already resistant at the moment of colonization.

Conclusions

Although some of the results suggest a difference between organic and conventional, it should be stressed that the present study was only a survey focusing on one set of samples and one or two production years. However, in a number of cases the results are similar to those from other studies, like the lower nitrate levels in head lettuce, the lack of increased mycotoxin levels in organically produced grains, less *Salmonella* but more *Campylobacter* in broilers, and less antibiotic-resistant bacteria in pigs and broilers. In the case of nitrate in carrots, there are indications for increasing levels in organic cultivation and further studies are required to confirm this. Also, the lower incidence of antibiotic-resistant bacteria in pigs and the lower incidence of *Salmonella* in more experienced pig farms deserve more attention.

Acknowledgements

This project was sponsored by the Dutch Ministry of Agriculture, Nature and Food Quality, and by the Food and Consumer Products Safety Authority. The authors thank Kees Veldman, Marga Japing, Jeannette Wup (Central Veterinary Institute), Riekje Bruinenberg (LBI), Grada Hazeleger-Druten, Ciska Jansen-Schalk, Wilma Jacobs-Reitsma, Wendy Van Overbeek, Mariel Pikkemaat, Harry Van Egmond, Cissy Warmerdam, Henk Bannink, Theo de Rijk and Paul Zomer (RIKILT), and Hilko Van der Voet (Biometris) for their valuable contribution.

References

Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' for the determination of pesticide residues in produce. J AOAC Int. 86:412–431.

Bernhoft A, Torp M, Clasen P-E, Uhlig S, Heier BT. 2003. Less Fusarium infection and mycotoxins in organically than in conventionally grown grain. In Steinnes E, editor. Proceedings of a symposium held at the Norwegian Academy of Science and Letters, Oslo, Norway, 16–17 October 2003.

Consumentenbond. 2002. De schaduwkant Van groente. Consumentengids February:34–37.

Cooper KM, Mulder PPJ, Van Rhijn JA, Kovacsics L, McCracken RJ, Young PB, Kennedy DG. 2005. Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV. Food Addit Contam. 22:406–414.

Czerwiecki L, Czajkowska D, Witkowska-Gwiazdowska A. 2002. On ochratoxin A and fungal flora in Polish cereals from conventional and ecological farms. Part 2: occurrence of ochratoxin A and fungi in cereals in 1998. Food Addit Contam. 19:1051–1057.

Díez C, Traag WA, Zomer P, Marinero P, Atienza J. 2006. Comparison of an acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' method with classical multi-residue methods for the extraction of herbicide residues in barley samples. J Chromatograph A. 1131:11–23.

Döll S, Valenta H, Dänicke S, Flachowsky G. 2002. Fusarium mycotoxins in conventionally and organically grown grain from Thuringia/Germany. Landbauforschung Völkenrode. 2:91–96.

Dutka-Malen S, Evers S, Courvalin P. 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 33:24–27.

EC. 2001. Commission Regulation (EC) No. 466/2001, setting maximum levels for certain contaminants in foodstuffs. Off J Eur Comm. L77:1–13.

EC. 2002a. Commission Decision of 12 August 2002, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off J Eur Comm. L221:8–36.

EC. 2002b. Commission Regulation (EC) No. 563/2002, amending Regulation No. 466/2001 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Comm. L86:5–6.

Hajšlová J, Schulzová V, Slanina P, Janné K, Hellenäs KE, Andersson Ch. 2005. Quality of organically and conventionally grown potatoes: four-year study of micronutrients, metals, secondary metabolites, enzymic browning and organoleptic properties. Food Addit Contam. 22:514–534.

Heuer OE, Pedersen K, Andersen JS, Madsen M. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. Lett Appl Microbiol. 33:269–274.

Hoogenboom LAP, Van Kammen M, Berghmans MCJ, Koeman JH, Kuiper HA. 1991. The use of pig hepatocytes to study the nature of protein-bound metabolites of furazolidone; a new analytical method for their detection. Food Chem Toxicol. 29:321–328.

Kijlstra A, Traag WA, Hoogenboom LAP. 2007. Effect of flock size on dioxin levels in eggs from chickens kept outside. Poultry Sci. 86:2042–2048.

Knudsen KEB. 2001. Influence of feed and feed structure on disease and welfare of pigs. In Hovi M, Baars T, editors. Proceedings of the 4th NAHWOA Workshop, Network for Animal Health and Welfare in Organic Agriculture; Wageningen, the Netherlands; 24–27 March 2001. Available from: http://orgprints.org/876/

Magkos F, Arvaniti F, Zampelas A. 2006. Organic food: buying more safety or just peace of mind? A critical review of the literature. Crit Rev Food Sci Nutr. 46:23–56.

Malmauret L, Parent-Massent D, Hardy JL, Verger P. 2002. Contaminants in organic and conventional foodstuffs in France. Food Addit Contam. 19:524–532.

MARAN. 2004. Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands, in 2004. CIDC Report. Available from: http://www.cidc-lelystad.nl/

Mulder PPJ, Balzer-Rutgers P, Brinke EMT, Bolck YJC, Berendsen BJA, Gercek H, Schat B, Van Rhijn JA. 2005. Deposition and depletion of the coccidiostats toltrazuril and halofuginone in eggs. Analytica Chimica Acta. 529:331–337.

Nouws J, Van Egmond H, Smulders I, Loeffen G, Schouten J, Stegeman H. 1999. A microbiological assay system for assessment of raw milk exceeding EU maximum residue levels. Int Dairy J. 9:85–90.

Pikkemaat MG, Mulder PPJ, Elferink JWA, De Cocq A, Nielen MWF, Van Egmond HJ. 2007. Improved microbial screening assay for the detection of quinolone residues in poultry and eggs. Food Addit Contam. 8:842–850.

Pikkemaat MG, Oostra-van Dijk S, Schouten J, Rapallini M, Van Egmond HJ. 2007. A new microbial screening method for the detection of antimicrobial residues in slaughter animals: the Nouws antibiotic test (NAT-screening). Food Contr. 19:781–789.

Pussemier L, Piérard J-Y, Anselme M, Tangni EK, Motte J-C, Larondelle Y. 2006. Development and application of analytical methods for the determination of mycotoxins in organic and conventional wheat. Food Addit Contam. 23:1208–1218.

Rodenburg TB, Van der Hulst-Van Arkel MC, Kwakkel RP. 2004. *Campylobacter* and *Salmonella* infections on organic broiler farms. Neth J Agri Sci. 52:101–108.

Rossi F, Bertuzzi T, Comizzoli S, Turconi G, Roggi C, Pagani M, Cravedi P, Pietri A. 2006. Preliminary survey on composition and quality of conventional and organic wheat. Ital J Food Sci. 18:355–367.

Schneweis I, Meyer K, Ritzmann M, Hoffmann P, Dempfle L, Bauer J. 2005. Influence of organically or conventionally produced wheat on health, performance and mycotoxin residues in tissues and bile of growing pigs. Arch Anim Nutr. 59:155–163.

Schoeters G, Hoogenboom LAP. 2006. Dioxin contamination in laying hens and eggs. Mol Nutr Food Res. 50:908–914.

Soil Association. 2001. Organic farming, food quality and human health, a review of the evidence. Soil Association (available at www.soilassociation.org).

Traag WA, Portier L, Bovee TFH, Van der Weg G, Onstenk C, Elghouch N, Coors R, Van de Kraats C, Hoogenboom LAP. 2002. Residues of dioxins and coplanar PCBs in eggs of free range chickens. Organohalogen Compnd. 57:245–248.

Van Overmeire I, Pussemier L, Hanot V, De Temmerman L, Hoenig M, Goeyens L. 2006. Chemical contamination in eggs of free-range hens in Belgium. Food Addit Contam. 23:1109–1122.

VWA. 2004a. Report of trends and sources of zoonotic agents, the Netherlands 2003. Report. VWA (available at www.vwa.nl).

VWA. 2004b. Survey pathogenen in kipproducten uit biologische teelt 2003. Report. VWA.

VWA. 2005. Survey pathogenen en bacteriële resistentie in kipproducten uit biologische teelt 2004. Report. VWA.

Woese K, Lange D, Boess C, Bögl KW. 1997. A comparison of organically and conventionally grown foods – results of a review of the relevant literature. J Sci Food Agric. 74:281–293.