
Public farms: hygiene and zoonotic agents

A. E. HEUVELINK*, S. M. VALKENBURGH, J. J. H. C. TILBURG,
C. VAN HEERWAARDEN, J. T. M. ZWARTKRUIS-NAHUIS AND E. DE BOER

Food and Consumer Product Safety Authority, Zutphen, The Netherlands

(Accepted 11 January 2007; first published online 21 February 2007)

SUMMARY

In three successive years, we visited petting farms ($n=132$), care farms ($n=91$), and farmyard campsites ($n=84$), respectively, and completed a standard questionnaire with the objective of determining the hygienic status of these farms and describing hygiene measures implemented to reduce the risk of transmission of zoonotic agents from the animals to humans. For at least 85% of the farms, the overall impression of hygiene was recorded as good. However, more attention must be paid to: informing visitors on hygiene and handwashing, provision of handwashing facilities, and a footwear cleaning facility. Examination of samples of freshly voided faeces resulted in the detection of Shiga toxin-producing *Escherichia coli* O157 and/or *Salmonella* spp. and/or *Campylobacter* spp. at almost two-thirds (64.9%) of the petting farms, and around half of the care farms (56.0%) and farmyard campsites (45.2%). These data reinforce the need for control measures for both public and private farms to reduce human exposure to livestock faeces and thus the risk of transmission of zoonotic diseases. Public awareness of the risk associated with handling animals or faecal material should be increased.

INTRODUCTION

Petting farm visits are popular leisure activities and have also become an important feature of education for young children. In The Netherlands, there are about 450 petting zoos with a total number of around 15–20 million visits annually, mainly in family groups but also in pre-arranged school parties. Such visits are highly beneficial to children in helping them to learn about aspects of animal husbandry and farm produce. Close contact with the animals is often encouraged, including the petting and feeding of animals. However, in July 2000, a 17-month-old

boy was hospitalized with haemolytic–uraemic syndrome (HUS) caused by infection with a Shiga toxin (Stx)-producing strain of *Escherichia coli* (STEC) O157 [1]. The infection was associated with a visit to a petting farm 5 days prior to onset of illness. This case together with the results of a subsequent pilot study that showed STEC O157 to be present in two out of 11 other, randomly selected petting farms [1], highlighted the need to implement control measures to reduce the risk for transmission of STEC O157 and other enteric pathogens at petting farms. Therefore, in 2001 a code of hygienic practices at petting farms in The Netherlands was issued and distributed [2]. This guidance contains information on the pathogens most commonly reported to be present in the farm environment and the steps to be taken to minimize the risk of human infection

* Author for correspondence: A. E. Heuvelink, Ph.D., Food and Consumer Product Safety Authority, PO Box 202, 7200 AE Zutphen, The Netherlands.
(Email: annet.heuvelink@vwa.nl)

by providing guidelines on both animal care and management, and farm management. Moreover, a standard information sign with directions for washing hands was created, and made available for petting farms without cost.

During the past decennia, the role of animals in health and social care has significantly increased. Animals are brought in nursing homes and hospitals and more and more farms combine agriculture and care. A care farm is a partnership between a farmer, a care team and people who will benefit from therapy. Currently, there are about 590 care farms in The Netherlands and it is expected that this number will significantly increase. Eight years ago there were only 80 care farms. The combination of agriculture and care not only benefits farmers by providing additional income but also helps the care sector to meet the health and social care needs of our society. The farms organize activities or provide supervised work or training for the mentally or physically handicapped, psychiatric patients, clients being treated for addiction, or elderly people requiring care. Similar to a petting farm, the contact between humans and animals at these care farms is closer and more frequent than at normal farms. However, most of the clients are probably unaware of the risk of zoonoses and are not unduly concerned about hygiene. In addition, some clients may have a reduced immune response and therefore a higher chance of acquiring a zoonosis.

A third type of farm that has become increasingly popular is the farmyard campsite. An increasing number of people spend their holidays in the country because of the quiet environment, to experience farm work, and to have contact with the farm animals. Within the campsite sector, staying at farms comprises about 4 million visitors *vs.* a total of 21 million at normal campsites in The Netherlands. With 45 000 campsites, farmers own about 15% of the camping market.

Despite all the benefits and pleasures of human–animal contact in the farm settings described above, the risk for disease transmission remains of concern. Infections with enteric bacterial pathogens and parasites pose the highest risk. The objectives of this study were to estimate the hygienic status, describe hygiene measures implemented to reduce the risk of human infection and to determine the prevalence of STEC O157, *Salmonella* spp., and *Campylobacter* spp. on petting farms, care farms, and farmyard campsites in The Netherlands.

MATERIALS AND METHODS

Farm visits

During the period from June to November 2002, a total of 132 petting farms throughout The Netherlands were visited, selected randomly from an address file. At each visit, a standard questionnaire regarding the hygienic status of the farms and hygiene facilities present was completed. The questions were directly related to the code of hygienic practices at petting farms in The Netherlands [2]. Samples of freshly voided faeces were collected in paddocks and pens, with a target number of 25 per petting farm. The samples were kept at 4–8 °C during transport to the laboratory and processed within 48 h after collection. The faecal samples were examined for the presence of STEC O157, *Salmonella* spp. and *Campylobacter* spp.

During the period from June to November 2003 and 2004, 91 care farms and 84 farmyard campsites located across the country were visited, respectively. The selection of care farms was based on the presence of farm animals; however, poultry farms were excluded. STEC O157, the bacterium that prompted us to perform these farm studies, has only rarely been isolated from poultry and the chance of transmission to humans via these animals was considered to be minor. The farmyard campsites were selected randomly. Similar to the petting farm study, at each visit, a standardized questionnaire regarding the hygiene facilities and hygienic status of the farms was completed and 10–25 samples of single fresh droppings were collected for bacteriological examination.

The farm manager received prior warning of a visit. In the second half of 2004, petting farms were again visited to inspect the hygienic status, this time, however, without prior notification. The petting farms visited were selected randomly. Sixty-two (50%) of the 125 farms visited in 2004 had also been visited in 2002. The inspections were carried out using the same standard questionnaire.

Bacteriological examination

STEC O157

For the isolation of STEC O157, samples (25 g) were enriched in modified tryptone soya broth (Oxoid Ltd, Basingstoke, UK) containing novobiocin (20 mg/l) (Sigma Chemical Co., St Louis, MO, USA) (mTSB) for a maximum of 20 h at 41.5 °C. Then, the enriched cultures were subjected to the Vitek Immuno

Diagnostic Assay System for immunoconcentration of *E. coli* O157 (VIDAS-ICE; bioMérieux, Lyon, France), before subculture onto sorbitol-MacConkey agar (SMAC; Oxoid) supplemented with cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l) (CT-SMAC; Oxoid) and CHROMagar™ O157 (CHROMagar, Paris, France) supplemented with cefixime (0.025 mg/l) and potassium tellurite (1.25 mg/l) (½CT-CHROM). The ICE procedure was performed according to the manufacturer's instructions. After incubation at 37 °C for 18–20 h, typical colonies from CT-SMAC were selected and screened for lactose fermentation on Levine's eosin Methylene Blue agar (Oxoid), and the absence of β-glucuronidase and sorbitol fermentation on SMAC containing 4-methylumbelliferyl-β-D-glucuronide (0.1 g/l) (Sigma Chemical Co.). Isolates thus selected were tested by latex agglutination with an *E. coli* O157 latex test kit (Murex Biotech Ltd, Dartford, Kent, UK). Typical colonies from ½CT-CHROM were directly subcultured onto tryptone soya agar (Oxoid) and tested by latex agglutination. Latex agglutinating isolates were confirmed biochemically as *E. coli* by an API 20E test (bioMérieux) and subjected to several real-time PCR assays for further confirmation and characterization (see below). Phage typing (one isolate per farm) was done at the Laboratory for Enteric Pathogens of the Central Public Health Laboratory in London (United Kingdom). The pulsed-field gel electrophoresis (PFGE) technique of contour-clamped homogeneous electric fields (CHEF) was used for genomic typing of the isolates (one isolate per positive sample) [3]. Genomic DNAs were digested in agarose plugs with *Xba*I (10 U) (Roche Diagnostics, Mannheim, Germany). The resulting fragments were resolved by CHEF-PFGE with a CHEF DR-III apparatus (Bio-Rad Laboratories, Richmond, CA, USA) at a constant voltage of 200 V for 20 h at 13 °C and a linearly ramped pulse time of 2.2–54.2 s. The fingerprints generated were processed using Bionumerics software (Applied Maths, Kortrijk, Belgium). Isolates were considered 'indistinguishable' if 100% of the fragments were identical.

Salmonella spp.

Samples (25 g) were cultured for *Salmonella* spp. by pre-enrichment at 37 °C in buffered peptone water (Oxoid) for 18 h and selective enrichment in modified semi-solid Rappaport–Vassiliadis (MSRV) agar

(Oxoid) at 41.5 °C for a maximum of 48 h. After 24 h or 48 h enrichment, a loopful of growth from the outer edge of the zone of migration on the MSRV plate was streaked onto both brilliant green phenol red agar (Oxoid) and mannitol lysine crystal violet brilliant green agar (Oxoid) and incubated at 37 °C for 24 h. Presumptive *Salmonella* spp. colonies were identified biochemically by an API 20E test. Isolates that yielded typical *Salmonella* results were subjected to a slide agglutination assay using *Salmonella* polyvalent O antiserum (Statens Serum Institute, Copenhagen, Denmark). The serovar and phage type of the isolates were determined at the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands).

Campylobacter spp.

Using a sterile swab, faecal samples were directly streaked onto charcoal cephoperazon deoxycholate agar (CCDA; Oxoid). The plates were incubated under microaerophilic atmosphere at 37 °C for 48 h. Typical colonies were confirmed to be *Campylobacter* spp. by examining microscopic cell morphology and latex agglutination (Oxoid). To identify the species of the isolates real-time PCR assays were performed (see below).

Confirmation and characterization of isolates by real-time PCR

Isolates biochemically confirmed to be *E. coli* O157 were subjected to several real-time PCR assays to determine the presence of *stx* genes (*stx*₁ and *stx*₂), the *E. coli* attaching-and-effacing gene (*eae*), the enterohaemolysin gene (*hly*EHEC), and the *rfb* (O-antigen-encoding) region of *E. coli* O157 (*rfb*O157). Following confirmation by a genus-specific real-time PCR assay, *Campylobacter* spp. isolates were subjected to real-time PCR assays specific for the species *C. jejuni*, *C. coli*, and *C. lari*.

The oligonucleotide primers and probes were designed using Primer Express software (version 2.0, Applied Biosystems, Foster City, CA, USA) (Table 1). The specificity of the sequences was tested by a blast search in GenBank (BLASTn version 2.2.10, National Centre for Biotechnology Information, Washington, DC). The oligonucleotides were purchased from Eurogentec (Seraing, Belgium). The probes were labelled at the 5'-end with the reporter dye 6-carboxyfluorescein (FAM) and at the 3'-end with the black-hole quencher (BHQ).

Table 1. Primers and probes used for the confirmation and characterization of STEC O157 and *Campylobacter* spp. isolates by real-time PCR

Target gene	Designation*	Sequence (5'→3')	Position	GenBank accession no.
<i>stx</i> ₁	ST1-F	TCTGATGCGCAGAACTATTAGCA	1608–1630	AF461172.1
	ST1-R	TACTCCACCTTTCCAGTTACACAATC	1736–1711	
	ST1-P	CAAGTGCCTGGCGACGCC	1691–1709	
<i>stx</i> ₂	ST2-F	ATATCAGTGCCCCGRTGTGACA	525–545	AF500192.1
	ST2-R	TTAACGCCAGATATGATGAAACAA	660–637	
	ST2-P	CCGGAATGCAAATCAGTCGTCCTCA	610–635	
<i>eae</i>	EAE-F	AGCTTCAGTCGCGATCTCTGA	732–752	X60439
	EAE-R	GAAGCCTGGTTACCAGCGATAC	804–783	
	EAE-P	CGGCGATTACGCGAAAGATAACCGC	754–777	
<i>hly</i> _{HEC}	Hly-F	GAGAATGGATATGACGCAAGACAT	563–586	U12572
	Hly-R	GCAAGTTCACCGATCTTCTCATC	702–680	
	Hly-P	AAGAGCAGTCGCAATAACCCAGCAACAT	649–676	
<i>rfb</i> _{O157}	RfbO157-F	TCAGCGCAATCTTCAATTACAAA	7949–7971	AE005429
	RfbO157-R	CTAAAGCTATTATGTGTGCCATTTATACG	8054–8025	
	RfbO157-P	TGGCCAGTTCTACAATTTGTTCCATATCACATG	7986–8018	
23S	Camp23S-F	GCTAATGTTGCCCGTACCGTAA	77 700–77 721	AL139076
	Camp23S-R	TCTTCCACGCGCCTTAGAAT	77 766–77 747	
	Camp23S-P	TCATCCCACCCACCTGTGTCCG	77 744–77 723	
<i>glyA</i>	Cjej-orfC-F	TTGGTATGGCTATAGGAACTCTTATAGCT	203 377–203 349	AL139078
	Cjej-orfC-R	CACACCTGAAGTATGAAGTGGTCTAAGT	203 262–203 289	
	Cjej-orfC-P	TGGCATATCCTAATTTAAATTATTAGCAGGAG	203 341–203 310	
<i>glyA</i>	Ccoli-glyA-F	TTGGAGCTTATCTTTTTGCAGACA	422–445	AF136494
	Ccoli-glyA-R	GCATGAGGAAATGGACTTGG	503–483	
	Ccoli-glyA-P	TGCTACAACAAGTCCAGCAATGTGTGCA	474–447	
<i>glyA</i>	Clari-glyA-F	TGGTGCCTATTTGTTTGTCTGAT	423–444	AF136495
	Clari-glyA-R	TGAGCATAAGGGAATGGACTAGGA	506–483	
	Clari-glyA-P	ACCTGCTACAACCAAGCCTGCAATATGTG	477–449	

* F, forward primer; R, reverse primer; P, probe.

For selectivity tests, target and non-target strains were grown onto TSA (overnight at 37 °C) and one loopful of bacterial culture was suspended in 500 µl sterile distilled water for DNA extraction. After centrifugation (5 min, 14 000 g) the pellet was re-suspended in 300 µl 6% (w/v) Chelex 100 (Bio-Rad, Veenendaal, The Netherlands) and incubated for 15–20 min at 56 °C. After heating for 8 min at 100 °C, the suspension was immediately put on ice and centrifuged again (5 min, 14 000 g) and the resulting supernatant was diluted 1:5 in TE buffer (10 mM Tris, 0.1 mM EDTA; pH 8.0) and then used as template in the PCR assay.

Amplification and detection were carried out in a LightCycler Instrument (version 2.0, Roche Diagnostics) and the 20-µl PCR mixture contained 2.5 µl of the sample DNA, 500 nM each of the primers, 250 nM of the probe, and the LightCycler

TaqMan Master mix kit (Roche Diagnostics). Samples were amplified with an initial denaturation step at 95 °C for 10 min to activate the FastStart Taq DNA polymerase and 40 cycles of denaturation at 95 °C for 10 s and annealing and extension at 60 °C for 15 s. The temperature transition rate was 20 °C pers. Samples positive for the target gene were identified by the instrument at the cycle number where the fluorescence attributable to the target sequences exceeded that measured for background.

Statistical analysis

To calculate whether the results of the petting farms regarding the hygienic status and hygiene facilities were significantly different in 2002 vs. 2004, we performed a χ^2 test ($P=0.05$).

Table 2. Summary of the results of the questionnaire concerning hygiene facilities and hygienic status

Question	Answer	No. of petting farms (%)				No. of care farms (%)		No. of farmyard campsites (%)	
		2002		2004					
Is the code of hygienic practices at petting farms present?	Yes	111*/132	(84)†	89/125	(71)†	41/91	(45)	35‡/83	(42)
Is there an information sign for visitors present?	Yes	90§/132	(68)	92/125	(74)	—	—	—	—
Is there a handwashing facility present?	Yes	121/132	(92)	108/125	(86)	86/91	(95)	79/83	(95)
Is the handwashing facility equipped with a soap dispenser?	Yes	92/121	(76)	90/108	(83)	73/86	(85)	28/79	(35)
Is the handwashing facility equipped with paper towels?	Yes	54/121	(45)	53/108	(49)	21/86	(24)	8/79	(10)
Is there (near the gate) a facility to clean footwear present?	Yes	56/132	(42)	68/125	(54)	—	—	12/83	(15)
Is there a distinct area to eat for employees present?	Yes	118/132	(89)	—	—	83/91	(91)	—	—
Is there a distinct area to eat for visitors present?	Yes	54/123	(44)†	83/125	(66)†	—	—	—	—
Is there an isolation ward present?	Yes	83/130	(64)	88/125	(70)	—	—	—	—
Is the isolation ward equipped with distinct clothing/materials?	Yes	36/83	(43)	42/88	(39)	—	—	—	—
What is the overall impression of hygiene at the farm?	Good	112/132	(85)	108/125	(86)	79/91	(87)	76/83	(92)
	Moderate	19/132	(14)	17/125	(14)	10/91	(11)	7/83	(8)
	Bad	1/132	(1)	0/125	(0)	2/92	(2)	—	—
Are the pathways/animal contact areas free of faeces?	Yes	124/132	(94)	111/125	(89)	83/91	(91)	75/83	(90)
What is the impression of the cleanliness of the animals that can be touched by visitors?	Good	125/132	(95)	121/125	(97)	86/91	(95)	79/83	(95)
	Moderate	3/132	(2)	4/125	(3)	4/91	(4)	4/83	(5)
	Bad	4/132	(3)	0/125	(0)	1/91	(1)	—	—

* For five farms it was noted that the code was handed out during the visit, raising the percentage of petting farms having the code to 88%.

† Statistically significant difference (χ^2 test, $P=0.05$) between 2002 and 2004.

‡ For 28 farms it was noted that the code was handed out during the visit, raising the percentage of farmyard campsites having the code to 76%.

§ For 10 farms it was noted that the standard handwashing sign was handed out during the visit, raising the percentage of petting farms having an information sign to 76%.

We analysed whether the bacterial contamination rates found on the farms depended on the hygienic status (the overall impression of hygiene, the cleanliness of the pathways/animal contact areas and of the animals) by a one-way ANOVA ($P=0.05$).

RESULTS

Hygiene inspection

The data collected from the questionnaires are summarized in Table 2. Some of the results recorded for the petting farms visited in 2002 could not be interpreted unequivocally. Since no conclusions could be drawn from these results, we discarded them. As

a result, on some aspects the total number of results is less than 132, i.e. the number of petting farms visited.

The results of the hygiene inspections at petting farms in 2004 were not significantly different from 2002, except for the presence of the code of hygienic practices (lower percentage of farms in 2004) and a distinct eating area for visitors (higher percentage of farms in 2004) (Table 2).

Zoonotic agents

The results of the bacteriological examination are summarized in Table 3.

Table 3. Isolation of STEC O157, *Salmonella* spp., and *Campylobacter* spp. from animal faecal samples collected on petting farms, care farms, and farmyard campsites

Farm	Zoonotic agents	No. positive/total no. (%)	
		Farms	Faecal samples
Petting farm*	STEC O157	13/127 (10.2)	58/2293 (2.5)
	<i>Salmonella</i> spp.	19/131 (14.5)	41/2341 (1.8)
	<i>Campylobacter</i> spp.	74/131 (56.5)	225/2365 (9.5)
Care farm†	STEC O157	14/91 (15.4)	61/1333 (4.6)
	<i>Salmonella</i> spp.	7/91 (7.7)	12/1319 (0.9)
	<i>Campylobacter</i> spp.	46/91 (50.5)	194/1341 (14.5)
Farmyard campsites‡	STEC O157	10/84 (11.9)	18/1117 (1.6)
	<i>Salmonella</i> spp.	2/83 (2.4)	6/1086 (0.6)
	<i>Campylobacter</i> spp.	30/84 (35.7)	115/1109 (10.4)

* At two petting farms, from one and three faecal samples, respectively, biochemically typical *E. coli* O157 strains were isolated, positive for *eae* and *hly*_{EHEC}, but negative for *stx*. When these isolates are included, the number of positive petting farms becomes 15 (11.8%) and the number of positive droppings 62 (2.7%).

† At three care farms, i.e. from four faecal samples, biochemically typical *E. coli* O157 strains were isolated, positive for *eae* and *hly*_{EHEC}, but negative for *stx*. When these isolates are included, the number of positive care farms becomes 17 (18.7%) and the number of positive droppings 65 (4.9%).

‡ At one other farmyard campsites, i.e. from one faecal sample, a biochemically typical *E. coli* O157 strain was isolated, positive for *eae* and *hly*_{EHEC}, but negative for *stx*. When this isolate is included, the number of positive farmyard campsites becomes 11 (13.1%) and the number of positive droppings 19 (1.7%).

The minimum number of samples collected at a petting farm was two, the maximum 34, the median 22, and on average 18 samples were collected. At care farms, the minimum number was two, the maximum 26, the median 14, and the mean 15 samples. At farmyard campsites these numbers were 3, 25, 12, and 13, respectively.

The percentage of STEC O157-positive samples at the petting farms, care farms, and farmyard campsites that tested positive for STEC O157 varied from 4% to 60% (mean 26% and median 20%), 4–70% (mean 23% and median 20%), and 5–44% (mean 16% and median 11%), respectively. All isolates carried both the *eae* and *hly*_{EHEC} gene, and tested positive with the PCR assay for the *rfb* (O-antigen-encoding) region of *E. coli* O157. The percentage of samples positive at *Salmonella* spp.-positive petting farms varied from 3% to 50% (mean 12% and median 7%) and at care farms from 4% to 17% (mean 9% and median 9%). The contamination percentages at the two *Salmonella* spp.-positive farmyard campsites were 14% and 71%. The percentage of positive

samples at *Campylobacter* spp.-positive petting farms, care farms, and farmyard campsites varied from 3% to 69% (mean 18% and median 13%), 4–85% (mean 27% and median 20%), and 9–100% (mean 29% and median 20%), respectively.

After combining the results of the three zoonotic agents, on 85 (64.9%) of the petting farms, 51 (56.0%) of the care farms, and 38 (45.2%) of the farmyard campsites STEC O157 and/or *Salmonella* spp. and/or *Campylobacter* spp. were found.

From four faecal samples, collected on two different petting zoos, *E. coli* O157 strains were isolated biochemically characteristic of human pathogenic STEC O157 (non-sorbitol-fermenting and negative for the enzyme β -D-glucuronidase) which also carried both the *eae* and *hly*_{EHEC} gene, but were negative for the *stx* genes (see Table 3 note). These isolates do not belong to the STEC group and therefore the respective samples and farms were not recorded as STEC O157-positive. The same applies to four faecal samples collected from three different care farms, and one isolate from a farmyard campsites (see Table 3 notes).

Furthermore, it is noteworthy that from two out of 20 samples collected on another petting farm biochemically typical *E. coli* O157 strains (non-sorbitol-fermenting and negative for the enzyme β -D-glucuronidase) were isolated that appeared to be negative for all four virulence genes (*stx*₁, *stx*₂, *eae*, *hly*_{EHEC}) (result not included in Table 3).

Among the 146 *E. coli* O157 isolates (137 STEC O157 and nine *stx*-negative *E. coli* O157 strains) originating from 43 farms, 12 different phage types were identified, specifically phage types 1, 2, 4, 8, 14, 32, 34, 43, 49, 50, 51, and 54. Isolates from three farms reacted with the typing phages but did not conform to a recognized phage type. PFGE analysis showed 48 different fingerprints. Isolates originating from different farms were of distinct strain types. However, the *Xba*I restriction pattern generated by one of the two isolates from a certain petting farm could not be distinguished from the one isolate from another petting farm. For 21 of the 25 farms with more than one positive sample it was observed that all isolates from the same farm generated identical *Xba*I restriction patterns. The remaining four farms harboured 2–4 different STEC O157 subtypes, their fingerprints differing by 3–8 bands.

Among the 59 *Salmonella* isolates originating from 28 farms, 10 different serovars were identified, specifically *S.* (di)arizone, *S.* Brandenburg, *S.* Dublin, *S.* Enteritidis, *S.* Hadar, *S.* Infantis, *S.* Livingstone, *S.* Mbandaka, *S.* Ohio, and *S.* Typhimurium. The *Campylobacter* isolates from 339 (63.5%) of the 534 positive samples were subjected to the species-specific PCR assays. *C. jejuni* was found in 71.1% of the samples, *C. coli* in 21.8%, and *C. lari* in 0.9%. In 4.1% of the samples mixed *Campylobacter* cultures were found and the isolates from the remaining 2.1% of the samples were confirmed as *Campylobacter* spp. by PCR but gave negative results in the species-specific PCR assays.

No statistically significant association was found between the bacterial contamination rates of the farms and hygienic status, with two exceptions. The *Salmonella* spp. contamination level of the petting farm that scored 'bad' regarding the overall impression of hygiene was significantly higher than the mean level of both the petting farms that scored 'good' and those that scored 'moderate'. Second, farmyard campsites with faecally contaminated pathways/animal contact areas appeared to have significantly higher rates of contamination with *E. coli* O157 than farmyard campsites with pathways/animal contact areas free of faeces.

DISCUSSION

In three successive years, we visited petting farms, care farms, and farmyard campsites, respectively, with the objective of determining the hygienic status of these farms, describing hygiene measures implemented to reduce the risk of human infection and determining the prevalence of zoonotic agents. For at least 85% of the farms visited, the overall impression of hygiene was recorded as good. Only at one petting farm and two care-related farms was the status judged as poor. However, it was suspected that these good results had been biased by the fact that the inspections were done at a pre-arranged visit. To map out the real situation, in 2004 petting farms were subjected to a similar inspection without prior notification. This time, none of the petting farms scored 'bad', neither on hygienic status nor on the cleanliness of animals that can be contacted by visitors. The importance of the creation of a safe farm environment for both visitors and employees is being underlined by the results of the bacteriological examination: at almost two-thirds (64.9%) of the petting farms, and around half of the care farms (56.0%) and farmyard campsites (45.2%), STEC O157 and/or *Salmonella* spp. and/or *Campylobacter* spp. were detected. At some of the farms positive for STEC O157 the percentage of positive samples ranged from 50% to as high as 70%. The farms were visited and sampled only during the period from June to November, when the farms are open to the public; they are closed during the winter months. It is very likely that the period of sampling has affected the prevalence rates found. Previous farm studies in The Netherlands have shown a peak for the prevalence of STEC O157 in animals during the summer and autumn [4, 5]. It is probable that the same seasonal pattern in shedding can be observed at public farms. This would mean a higher risk of zoonotic transmission during the time of the year the farms are open to the public. As could be expected, the highest isolation rates for STEC O157 were found in cattle and small ruminants. Subtyping of the STEC O157 isolates by PFGE showed that all isolates from one farm belonged to one subtype and there was no overlap between different farms, with a few exceptions. The occurrence of a single strain type at individual farms supports the idea of a common infection source and/or horizontal spread within the animal population of a farm. The potential of the eight *E. coli* O157 isolates that did not carry *stx* genes to cause human disease is unclear.

However, non-toxicogenic *E. coli* O157 strains have been associated with sporadic cases and outbreaks of human disease, including HUS in Europe [6, 7]. In addition, loss of *stx* genes have been documented, both during human infection and during *in vitro* cultivation [8, 9].

Salmonella spp. and *Campylobacter* spp. were found in faecal samples of several animals, with the highest prevalence for *Salmonella* spp. in poultry and pigs and for *Campylobacter* spp. in pigs, poultry, cattle and small ruminants. The *Salmonella* isolates belonged to serotypes commonly found in farm animals [10]. The high prevalence of *C. coli* found in *Campylobacter*-positive faecal samples from care farms is due to the high number of porcine isolates, which often belong to this species [10].

It is known that animals are carrying microorganisms (bacteria, viruses and parasites) and can excrete these microorganisms in milk, urine and faeces, without being ill themselves. This way, the environment of the animal can become contaminated [11–15]. Visitors and employees who mix with, touch, pet, and cuddle the animals, and also walk in paddocks where contaminated animals are present (or have been present), are at real risk of becoming infected with these microorganisms. Fortunately, most of these microorganisms are not pathogenic to humans. Apart from parasites, the classical bacterial enteropathogens STEC O157, *Salmonella* spp., and *Campylobacter* spp. are considered to form the major risk.

For all three pathogens examined, human cases of infection have been reported that resulted from contact of the patients with the faeces of animals that carried the bacteria [16–18]. In particular for STEC O157, there is growing evidence that exposure to livestock faeces has long been an underestimated route of transmission [19–26]. In January 1999, an enhanced laboratory-based surveillance of STEC O157 was implemented in The Netherlands [27]. From January 1999 to December 2005, 315 symptomatic cases were diagnosed with STEC O157 infection (annual incidence 0.22–0.35/100 000 inhabitants), predominantly sporadic infections. Since the summer of 2000, contact with the farm environment was investigated for 31 cases. For nine (29%) patients the source could be confirmed by indistinguishable PFGE profiles for isolates from patients and animal faecal samples. However, for two cases, a young girl and her mother, the transmission of the pathogen from the animal to the patient was probably not caused by

animal contact but by the consumption of raw cow's milk. For another four cases, the farm tested positive for STEC O157, but the animal isolates did not match the isolates from the patients ($n=3$) or no patient isolate was available for comparison ($n=1$). In the surveillance questionnaire, of the known risk factors exposure to (faeces from) farm animals was reported most frequent (annually 20–27% of the cases, except in 2004: 12%), although reports of consumption of raw or undercooked beef has increased since 2003 (1999–2002: 16%, 2003–2005: 34% or 25%, including or excluding outbreak cases). These results also highlight the fact that in The Netherlands exposure to livestock faeces is an important transmission route of STEC O157. The risk of human infection of visitors and employees of farms can be reduced significantly, and relatively easily by the implementation of a well-thought-out, well-structured hygiene policy [28].

Although the overall impression of hygiene at the petting farms visited in 2002 was good, a number of points for improvement became apparent, e.g. informing visitors on hygiene and handwashing, provision of handwashing facilities, a footwear cleaning facility, a specifically designated area where visitors can eat that is strictly separated from the animals, and an isolation ward with distinct clothing and boots. The inspections carried out in 2004, 3 years after the code of hygienic practices had been issued, showed a slight improvement with respect to most of these points of interest. However, the scores for 2002 and 2004 were not significantly different ($P=0.05$), except for the presence of a separate eating area for visitors (Table 2). Therefore, there is still a need for improvement. A recent Swiss study at petting farms, also concluded the need for better visitors' information on hygiene and a strict separation between picnic areas and animals [29].

The results obtained for care farms and farmyard campsites were equivalent to those of the petting farms, with provision of handwashing facilities and a footwear cleaning facility coming up as the main points for attention. The code of hygienic practices at petting farms was also present on almost half of the care farms and farmyard campsites, which pleasantly surprised us because it had only been distributed among petting farms. The code must have been downloaded from our website (www.vwa.nl) on the farm managers' own initiative.

Finally, there is a responsibility for visitors and employees themselves. It is important that they are

aware of the risk associated with animal contact and know how to reduce the risk. When the farm manager makes every effort to minimize the spread of pathogens possibly present by offering good hygienic farm management and hygiene facilities that further reduce the risk of transfer from animals to humans, it is up to the visitors and employees to follow proper hygiene practices.

In conclusion, the data of this study reinforce the need for control measures for public and private farms to reduce human exposure to livestock faeces and thus the risk of transmission of zoonotic diseases. Although the overall impression of hygiene at the farms enrolled in this study was good, there is still a need for improvement. Public awareness of the risk associated with handling animals or faecal material should be increased. Furthermore, the data plead for a commonly supported quality system based on the code of hygienic practices at petting farms and if possible, secured by a certification system. Although the code is ~80% applicable to care farms, farmyard campsites, and normal animal farms, it is recommended specific codes are introduced for each type of farm. Lastly, we are still continuing work on the risk of zoonoses in human–livestock contact settings and an integrated risk factor analysis potentially could make an important contribution in this area.

ACKNOWLEDGEMENTS

We thank all farms that voluntarily participated in this study for their cooperation and the specific farm sectors for their support. We also thank Thomas Cheasty of the Central Public Health Laboratory, London, UK, for phage typing the STEC O157 strains and the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands) for determining the serovars and phage types of the *Salmonella* isolates.

DECLARATION OF INTEREST

None.

REFERENCES

1. Heuvelink AE, *et al.* *Escherichia coli* O157 infection associated with a petting zoo. *Epidemiology and Infection* 2002; **129**: 295–302.
2. Valkenburgh SM. *Code of hygienic practices at petting farms in the Netherlands* [in Dutch]. Food and Consumer Product Safety Authority, Zutphen, The Netherlands, 2001. pp. 16 (<http://www.vwa.nl/>).
3. Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. One-day (24–28 h) standardized laboratory protocol for molecular subtyping of *Escherichia coli* O157:H7 by pulsed-field gel electrophoresis (PFGE). Centers for Disease Control and Prevention, Atlanta, GA, USA, 1999.
4. Heuvelink AE, *et al.* Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. *Journal of Clinical Microbiology* 1998; **36**: 3480–3487.
5. Schouten JM, *et al.* Prevalence estimation and risk factors for *Escherichia coli* O157 on Dutch dairy farms. *Preventive Veterinary Medicine* 2004; **64**: 49–61.
6. Schmidt H, *et al.* *Escherichia coli* O157:H7 and O157:H(-) strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. *Journal of Clinical Microbiology* 1999; **37**: 3491–3496.
7. Allerberger F, *et al.* Nontoxigenic sorbitol-fermenting *Escherichia coli* O157:H⁻ associated with a family outbreak of diarrhoea. *Wiener Klinische Wochenschrift* 2000; **112**: 846–850.
8. Karch H, *et al.* Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. *Journal of Clinical Microbiology* 1995; **33**: 1602–1605.
9. Murase T, Yamai S, Watanabe H. Changes in pulsed-field gel electrophoresis patterns in clinical isolates of enterohemorrhagic *Escherichia coli* O157:H7 associated with loss of Shiga toxin genes. *Current Microbiology* 1999; **38**: 48–50.
10. European Food Safety Authority (EFSA). *Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004*. EFSA, Parma, Italy, 2006, pp. 275.
11. Duffy G. Verocytotoxigenic *Escherichia coli* in animal faeces, manures and slurries. *Journal of Applied Microbiology* 2003; **94**: 94S–103S.
12. Guan TY, Holley RA. Pathogen survival in swine manure environments and transmission of human enteric illness – a review. *Journal of Environmental Quality* 2003; **32**: 383–392.
13. Johnson JY, *et al.* Prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources. *Canadian Journal of Microbiology* 2003; **49**: 326–335.
14. Hutchison ML, *et al.* Analyses of livestock production, waste storage, and pathogen levels and prevalences in farm manures. *Applied and Environmental Microbiology* 2005; **71**: 1231–1236.
15. Keen JE, *et al.* Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerging Infectious Diseases* 2006; **12**: 780–786.

16. **Merritt TD, Herlihy C.** *Salmonella* outbreak associated with chicks and ducklings at childcare centres. *Medical Journal of Australia* 2003; **179**: 63–64.
17. **Hendriksen SW, et al.** Animal-to-human transmission of *Salmonella Typhimurium* DT104A variant. *Emerging Infectious Diseases* 2004; **10**: 2225–2227.
18. **Smith KE, et al.** Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. *Pediatric Infectious Disease Journal* 2004; **23**: 1098–1104.
19. **Payne CJI, et al.** Vero cytotoxin-producing *Escherichia coli* O157 gastroenteritis in farm visitors, North Wales. *Emerging Infectious Diseases* 2003; **9**: 526–530.
20. **Varma JK, et al.** An outbreak of *Escherichia coli* O157 infection following exposure to a contaminated building. *Journal of the American Medical Association* 2003; **290**: 2709–2712.
21. **David ST, et al.** Petting zoo-associated *Escherichia coli* O157:H7 – secondary transmission, asymptomatic infection, and prolonged shedding in the classroom. *Canada Communicable Disease Report* 2004; **30**: 173–180.
22. **Kassenborg HD, et al.** Farm visits and under-cooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data from a case-control study in 5 FoodNet sites. *Clinical Infectious Diseases* 2004; **38** (Suppl. 3): S271–278.
23. **Centers for Disease Control and Prevention (CDC).** Outbreaks of *Escherichia coli* O157:H7 associated with petting zoos – North Carolina, Florida, and Arizona, 2004 and 2005. *Morbidity and Mortality Weekly Report* 2005; **54**: 1277–1280.
24. **Durso LM, et al.** Shiga-toxigenic *Escherichia coli* O157:H7 infections among livestock exhibitors and visitors at a Texas County Fair. *Vector Borne and Zoonotic Diseases* 2005; **5**: 193–201.
25. **Ejidokun OO, et al.** Human Vero cytotoxigenic *Escherichia coli* (VTEC) O157 infection linked to birds. *Epidemiology and Infection* 2006; **134**: 421–423.
26. **Locking M, et al.** VTEC infections and livestock-related exposures in Scotland, 2004. *Eurosurveillance Weekly* 2006; **11**: 060223.
27. **Van Duynhoven YT, et al.** Enhanced laboratory-based surveillance of Shiga toxin-producing *Escherichia coli* O157 in the Netherlands. *European Journal of Clinical Microbiology & Infectious Diseases* 2002; **21**: 513–522.
28. **Centers Disease Control and Prevention (CDC).** Compendium of measures to prevent disease associated with animals in public settings. *Morbidity and Mortality Weekly Report* 2005; **54**: 1–12.
29. **Butikofer B, et al.** Incidence of zoonoses in petting zoos and evaluation of hygiene measures to prevent the transmission to humans [in German]. *Schweizer Archiv für Tierheilkunde* 2005; **147**: 532–540.