

# ***Campylobacter* spp., *Salmonella* spp., Verocytotoxic *Escherichia coli*, and Antibiotic Resistance in Indicator Organisms in Wild Cervids**

By A. Lillehaug<sup>1</sup>, B. Bergsjø<sup>2</sup>, J. Schau<sup>2</sup>, T. Bruheim<sup>3</sup>, T. Vikøren<sup>1</sup> and K. Handeland<sup>1</sup>

<sup>1</sup>Section for Wildlife Diseases, <sup>2</sup>Section for Bacteriology, National Veterinary Institute, Oslo Norway, <sup>3</sup>Regional Laboratory Trondheim, National Veterinary Institute, Trondheim Norway.

**Lillehaug A, Bergsjø B, Schau J, Bruheim T, Vikøren T, Handeland K: *Campylobacter* spp., *Salmonella* spp., verocytotoxic *Escherichia coli*, and antibiotic resistance in indicator organisms in wild cervids. Acta vet. scand. 2005, 46, 23-32.**

– Faecal samples were collected, as part of the National Health Surveillance Program for Cervids (HOP) in Norway, from wild red deer, roe deer, moose and reindeer during ordinary hunting seasons from 2001 to 2003. Samples from a total of 618 animals were examined for verocytotoxic *E. coli* (VTEC); 611 animals for *Salmonella* and 324 animals for *Campylobacter*. A total of 50 samples were cultivated from each cervid species in order to isolate the indicator bacterial species *E. coli* and *Enterococcus faecalis* / *E. faecium* for antibiotic resistance pattern studies. *Salmonella* and the potentially human pathogenic verocytotoxic *E. coli* were not isolated, while *Campylobacter jejuni jejuni* was found in one roe deer sample only. Antibiotic resistance was found in 13 (7.3%) of the 179 *E. coli* isolates tested, eight of these being resistant against one type of antibiotic only. The proportion of resistant *E. coli* isolates was higher in wild reindeer (24%) than in the other cervids (2.2%). *E. faecalis* or *E. faecium* were isolated from 19 of the samples, none of these being reindeer. All the strains isolated were resistant against one (84%) or more (16%) antibiotics. A total of 14 *E. faecalis*-strains were resistant to virginiamycin only. The results indicate that the cervid species studied do not constitute an important infectious reservoir for either the human pathogens or the antibiotic resistant microorganisms included in the study.

***Campylobacter*; *Salmonella*; verocytotoxic *Escherichia coli*; VTEC; antibiotic resistance; cervids; moose; red deer; roe deer; reindeer; faeces.**

## **Introduction**

In recent years, the annual culling of moose (*Alces alces*) by hunting in Norway has been in the order of 38,000, while 24,500 red deer (*Cervus elaphus*) were culled in 2002. The latter figure represents an approximately doubling of the number of red deer shot ten-years earlier. Both roe deer (*Capreolus capreolus*) and reindeer (*Rangifer tarandus tarandus*) hunting levels have, however, been decreasing over the last few years with current estimated figures for the

roe deer hunt being 30,500, while roughly 6,600 reindeer are felled annually. The meat gain of these game animals amounted to approximately 13,000 metric tons in 2002, or in the magnitude of 3.3 kg *pro capita* in Norway (Anonymous 2003).

The venison is to a great extent consumed by the hunters, their families and acquaintances. Only minimal amounts are sold on the regular market. Therefore, only a small proportion of

this meat is subject to stringent meat inspection. The game animals are killed and slaughtered in outlying fields, with the skinning and butchering of carcasses usually being performed outdoors or in private barns, garages, basements or kitchens. Such practices may greatly increase the risk of faecal contamination from the animal's own intestines as well as reducing product quality. Potentially, faeces from wild cervids could also contaminate surface water, which may then go on to be used as drinking water for humans and/or domestic animals.

The enteropathogenic bacteria *Salmonella*, *Campylobacter* and certain serovars of verocytotoxic *E. coli* (VTEC) are pathogenic to humans, and all are notifiable diseases, subject to extensive national surveillance programmes. There has been a gradual increase in the number of reports of salmonellosis during the last years, and in 2002, 1495 human cases were reported in Norway. Of these, 75% were presumed to be of foreign origin. The incidence of campylobacteriosis has also increased significantly during the last decade, and from 1998 on, reports have exceeded those for salmonellosis. In 2002, a total of 2192 cases of enteritis caused by *Campylobacter* were reported, 52% of these infections were acquired abroad. During the period 1992-2002, 79 cases of VTEC infections were reported in Norway, 16 of these during 2002 (of which seven were infected abroad), and 15 in 2001 (Hofshagen et al. 2002). Screening studies of meat products have revealed relatively high frequencies of antibiotic resistant indicator organisms, particularly in pork and poultry products (Kruse 1999). The aims of the present study were to investigate the occurrence of *Salmonella*, *Campylobacter*, VTEC and the antibiotic resistance patterns in indicator organisms in faecal samples collected from moose, red deer, roe deer and reindeer killed during the hunting season in Norway.

## Material and methods

### Faecal samples

Material is collected from cervids during the hunting season each year, as part of the National Health Surveillance Program for Cervids (HOP). During 2001, fresh faecal samples from 135 red deer were provided from five municipalities in western and mid-Norway and frozen for later use. These samples were used in the examinations for *Salmonella*, VTEC and antibiotic resistance patterns in indicator organisms. The same type of material was collected from 53 red deer in five different municipalities in western Norway in 2003. These samples were examined fresh for *Campylobacter*.

In 2002, a total of 127 moose samples were submitted from three different municipalities in southern and eastern Norway, and 206 samples from roe deer were provided from 12 municipalities in eastern, southern and mid-Norway. Faecal samples from 153 wild reindeer were collected from six municipalities in one mountain district in mid-Norway during 2003.

### Examinations for *Campylobacter*

Examination was carried out on the fresh samples (which had not been frozen) from 53 red deer, 82 moose, 38 roe deer and 150 wild reindeer. These were cultivated directly on *Campylobacter* blood free selective agar (Oxoid CM 739) supplemented with cefoperazone, amphotericin B and teicoplanin (Oxoid SR 174), and incubated in a microaerophile atmosphere at 37°C for 2-3 days. Presumptive *Campylobacter* colonies were confirmed by phase-contrast microscopy. The different species were identified by phenotypic assays, including growth pattern at 42°C, catalase production and hippurate hydrolysis.

### Examinations for *Salmonella*

Faecal samples from a total of 135 red deer, 127 moose, 196 roe deer and 153 reindeer were ex-

Table 1. Antimicrobial resistance in *Escherichia coli* isolated from faecal samples from reindeer (n=42) or "other" cervids (n=137); moose (n=48), red deer (n=45) and roe deer (n=44). The figures give the percentage distribution of isolates to the actual minimum inhibitory concentration (MIC) values. Bold vertical lines denote breakpoints for resistance. White fields denote the range of dilutions tested for. MIC-values higher than the highest concentration tested for are given as the lowest MIC-value above the range. MIC-values equal to or lower than the lowest concentration are given as the lowest tested concentration.

Antimicrobial substance	Cervid species	Resistant strains, %	Distribution of <i>Escherichia coli</i> isolates (%) to different MIC-values (mg/L)															
			0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Amoxicillin/ Clavulanic acid	"Other"	0							5.8	65	27	2.2						
	Reindeer	0							64.3	35.7								
Ampicillin	"Other"	0						8	72.3	17.5	1.5	0.7						
	Reindeer	0						14.3	76.2	9.5								
Ceftiofur	"Other"	0			2.4	21.9	74.5	3.6										
	Reindeer	0				16.7	81											
Chloramphenicol	"Other"	0							10.2	49.6	40.1							
	Reindeer	0							16.7	52.4	31							
Enrofloxacin	"Other"	0	34.3	64.2	1.5													
	Reindeer	0	35.7	61.9	2.4													
Florfenicol	"Other"	0							6.6	34.3	57.7	1.5						
	Reindeer	0							54.8	42.9	2.4							
Gentamicin	"Other"	0			0.7	29.9	63.5	5.1	0.7									
	Reindeer	0				50	45.2	4.8										
Nalidixic acid	"Other"	0						3.6	53.3	43.1								
	Reindeer	0							50	50								
Neomycin	"Other"	0						47.4	48.2	4.4								
	Reindeer	0							97.6	2.4								
Oxytetracycline	"Other"	1.5				1.5	20.4	40.1	35.8	0.7			1.5					
	Reindeer	7.1					52.4	40.5						7.1				
Streptomycin	"Other"	0.7							0.7	38	56.2	4.4			0.7			
	Reindeer	21.4								33.3	40.5	2.4	2.4			9.5	9.5	2.4
Sulphamethoxazole	"Other"	1.5													98.5			
	Reindeer	9.5													88.1			2.4
Trimethoprim	"Other"	0.7		5.8	20.4	64.2	7.3		1.5									9.5
	Reindeer	0			28.6	66.7	4.8								0.7			

aminated. The samples were typically 5 g faeces per roe deer, 10 g per red deer and reindeer, and 15 g per moose.

The method used for examination was ME02\_046, National Veterinary Institute, complying with the Nordic Committee on Food Analysis requirements for the detection of *Salmonella* in such material, and accredited according to ISO 17025. The principle for the method is non-selective pre-enrichment in phosphate buffered peptone water, selective enrichment in Rappaport-Vassiliadis soya peptone broth, and plating out on red violet bile agar plates and bromthymol blue lactose, sucrose agar. Colonies are then isolated and tested, both biochemically and serologically, for confirmation. Samples were pooled in groups of three from each animal species for examination.

*Examinations for verocytotoxic E. coli (VTEC)*  
Samples from 135 red deer, 127 moose, 206 roe

deer and 150 wild reindeer were tested. One faecal "pearl", or a corresponding amount of faeces was examined, from every animal. Pools of three samples were tested by methods based on the protocols of "Dynal". After non-selective enrichment, specific O-serovars of *E. coli* (O26, O103, O145, O111 and O157) were concentrated by immunomagnetic separation, followed by cultivation on selective agar plates. *E. coli* being potentially positive for the actual serovars, were tested for agglutination with the respective antisera.

The isolates of these O-serovars were tested by PCR at The Norwegian School of Veterinary Science, for the presence of the gene-sequences *stx* (shigatoxin) and *eae* (intimin), crucial for the pathogenicity of VTEC.

#### *Isolation of indicator organisms for antibiotic resistance testing*

Cultivation from the 50 faecal samples from each of the four cervid species, was made di-

Table 2. Antimicrobial resistance in *Enterococcus* spp. isolated from faecal samples from wild cervids (n=19): *E. faecium* (n=4); moose (n=1) and red deer (n=3), and *E. faecalis* (n=15); moose (n=2), red deer (n=5) and roe deer (n=8). The figures give the percentage distribution of isolates to the actual minimum inhibitory concentration (MIC) values. Bold vertical lines denote breakpoints for resistance. White fields denote the range of dilutions tested for. MIC-values higher than the highest concentration tested for are given as the lowest MIC-value above the range. MIC-values equal to or lower than the lowest concentration are given as the lowest tested concentration.

Antimicrobial Substance	Bacterial species	Resistant strains, %	Distribution of <i>Enterococcus</i> isolates (%) to different MIC-values (mg/L)														
			0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048
Ampicillin	<i>E. faecium</i>	0		25	25		50										
	<i>E. faecalis</i>	0		6,7	80	13,3											
Avilamycin	<i>E. faecium</i>	0					50	50									
	<i>E. faecalis</i>	0			26,7	40	33,3										
Bacitracin	<i>E. faecium</i>	25									46,7	75	25				
	<i>E. faecalis</i>	0									53,3						
Chloramphenicol	<i>E. faecium</i>	0					25	75									
	<i>E. faecalis</i>	0					13,3	80	6,7								
Erythromycin	<i>E. faecium</i>	0			50	25		25									
	<i>E. faecalis</i>	6,7			6,7	6,7	13,3	66,7				6,7 <sup>1</sup>					
Flavomycin	<i>E. faecium</i>	100															
	<i>E. faecalis</i>	0				20	40	20	13,3	6,7			100				
Gentamicin	<i>E. faecium</i>	0						75	25			NT <sup>2</sup>	NT	NT			
	<i>E. faecalis</i>	0								53,3	40	NT	NT	NT	6,7		
Neomycin	<i>E. faecium</i>	0						25	50	25					NT	NT	
	<i>E. faecalis</i>	0									6,7	66,7	20	NT	NT	6,7	
Narasin	<i>E. faecium</i>	0		25	75												
	<i>E. faecalis</i>	0	6,7	20	73,3												
Oxytetracycline	<i>E. faecium</i>	25				75						25					
	<i>E. faecalis</i>	6,7				46,7	46,7						6,7				
Streptomycin	<i>E. faecium</i>	0										75	25	NT	NT		
	<i>E. faecalis</i>	6,7										6,7	66,7	NT	NT	20	6,7
Vancomycin	<i>E. faecium</i>	0			50	25		25									
	<i>E. faecalis</i>	0			6,7	80	13,3										
Virginiamycin	<i>E. faecium</i>	0		50	25	25											
	<i>E. faecalis</i>	100									53,3	46,7					

<sup>1</sup> *Enterococcus*-strains with MIC > 4 using VetMIC-plates (National Veterinary Institute, Sweden) was retested using Etest strips (Biodisk, Solna, Sweden)

<sup>2</sup> NT – Not Tested

rectly on bromthymol blue lactose, sucrose agar plates (37°C, 24 h) for the isolation of the indicator bacterial species *E. coli*, and on Slanetz & Bartley enterococcus agar plates (44°C for two days) for *Enterococcus faecalis* or *E. faecium*. Typical enterococcus colonies were confirmed by a negative catalase reaction, and *E. faecium* and *E. faecalis* were identified by a PCR method described by Dutka-Malen et al. (1995a, 1995b) (Hot-Start ddIIID-PCR). Presumptive *E. coli* colonies were sub-cultured on blood agar and confirmed using the indole test.

#### Testing for antibiotic resistance patterns in indicator organisms

VetMIC-plates from the National Veterinary Institute in Sweden were used for the testing of antibiotic resistance patterns. The method is

based on broth dilution in microtiter plates, with the wells containing the antibiotics as a dry substance. There are special plates for each bacterial species. Briefly, the method is carried out by diluting the test bacterium to 0.5 McFarland in 5 mL sterile distilled water. For *E. coli* and enterococci, respectively, 10 and 50 µL were further diluted in 10 mL cationic adjusted Mueller Hinton-broth, and 50 mL of this dilution was distributed in the wells on the microtiter plate. After incubation at 35°C for 18-20 h for *E. coli* and 20-22 h for enterococci, the plates were read visually (magnification mirror). Minimum inhibitory concentration (MIC) was read as the lowest concentration of antibacterial showing inhibition of bacterial growth, i.e. the bacterial pellet was not present. The antibacterial substances and concentrations tested

for are given in Tables 1 and 2. For *Enterococcus*-strains with MIC > 4 for erythromycin, the MIC-values were obtained using Etest (Bio-disk, Solna, Sweden).

## Results

### Examinations for *Campylobacter*

Out of a total of 324 samples tested, one positive sample was found in a roe deer. This isolate was determined to be *Campylobacter jejuni jejuni*. The positive animal was an adult buck from Hedmark county.

### Examinations for *Salmonella*

Among the 611 individuals tested for *Salmonella*, no positive samples were found.

### Examinations for *VTEC*

A total of 104 isolates of potentially pathogenic serovars of *E. coli* were found in the 207 pooled samples examined. *E. coli* O103 was found in 41% of the pooled samples, while O26 and O145 were found less frequently. O111 and O157 were not detected (Table 3).

All 104 isolates were examined for the shiga-toxin gene by PCR-analyses, of which 102 isolates were definitely negative. One O103 isolate from red deer was slightly positive for *stx*<sub>1</sub> and another O103 isolate from red deer was positive for both *stx*<sub>1</sub> and *stx*<sub>2</sub>.

A total of 79 isolates were tested for the pres-

ence of the gene sequence (*eae*) that codes for the production of intimin. The two isolates of O103 being positive with regards to *stx* in PCR were included in this analysis, and tested negative. Two isolates from reindeer were found to be positive in the *eae* test: one an O103 and the other an O145. However, both these isolates were found to be negative for the *stx* gene sequence.

Hence, no isolates were found to be potentially pathogenic to humans in the sense that gene sequences coding for shigatoxin and intimin were not identified in the same strain.

### Antibiotic resistance in *E. coli*

*E. coli* was isolated from 45 red deer, 48 moose, 44 roe deer and 42 wild reindeer out of a total of 50 faecal samples from each animal species tested. As shown in Table 1, ten of the reindeer isolates were resistant to one or more of the antibiotics. Nine strains were resistant to streptomycin, and three of these were resistant to both oxytetracycline and sulfamethoxazole, as well. A further six strains were resistant to streptomycin only. Finally, one strain was only resistant to sulfamethoxazole. All the isolates from moose were demonstrated to be sensitive to each of the 14 types of antibiotics tested for. One isolate from red deer (2.2%) was resistant to two types of antimicrobials; namely streptomycin and sulfamethoxazole. Two strains from

Table 3. Numbers of different *E. coli* serovar isolates, with verocytotoxic potential, found in faecal samples from different cervid species.

Cervid species	No of individual samples	No of pooled samples	<i>E. coli</i> O26	<i>E. coli</i> O103	<i>E. coli</i> O111	<i>E. coli</i> O145	<i>E. coli</i> O157
Red deer	135	45	1	23	0	5	0
Moose	127	43	0	27	0	1	0
Roe deer	206	69	2	23	0	7	0
Reindeer	150	50	2	12	0	1	0
Total	618	207	5	85	0	14	0

roe deer (4.4%) were shown to be resistant, one to oxytetracycline only, and the other to oxytetracycline, sulfamethoxazole and trimethoprim. Differences in proportions of resistant isolates between animal species were statistically significant, calculated using the chi-squared test ( $p < 0.001$ , three degrees of freedom).

*Antibiotic resistance in E. faecium / E. faecalis*  
In the 50 reindeer samples, neither *E. faecalis* nor *E. faecium* isolates were found. In moose, *E. faecium* was found in one sample and *E. faecalis* in two samples. In red deer, *E. faecium* was found in three samples and *E. faecalis* in five samples. In roe deer *E. faecalis* was found in eight samples, giving a total of 19 faecal enterococci isolates for antibiotic sensitivity testing. The results, using 13 different types of antimicrobials, are given in Table 2.

Resistance to one (84%) or more (16%) antibacterials was found in all of the enterococci strains. All *E. faecalis* strains were resistant to virginiamycin. One *E. faecalis* strain from a roe deer was resistant to erythromycin, oxytetracycline and streptomycin, as well.

All four *E. faecium* strains were resistant to flavomycin. Two of these strains, from a red deer and a moose, were also resistant to one other antimicrobial each, oxytetracycline and bacitracin, respectively.

## Discussion

The examination of faecal samples from Norwegian free ranging cervids; moose; red deer; roe deer and wild reindeer; for the presence of important pathogens to humans, revealed only one positive sample for *Campylobacter* and none for either *Salmonella* or verocytotoxic *E. coli*. The antibiotic resistance patterns found also indicate low levels of resistant strains among the indicator organisms of *E. coli* and faecal enterococci, except for the *E. coli* isolates from reindeer.

Verocytotoxic *E. coli* potentially pathogenic to humans was not found in cervid faeces, which is in agreement with results of previous studies in Norway (Wasteson et al. 1999) and Sweden (Wahlström et al. 2003). VTEC O157 is very rarely found even in domestic ruminants in Norway; five positive cattle carcasses were identified in 2002, while during the period 1996 - 2001, a total of three carcasses were found, after the testing of approximately 2300 cattle annually (Hofshagen et al. 2002). However, the proportion of cattle and sheep harbouring shiga-toxin producing *E. coli* in the faeces seem to be high (Urdahl et al. 2003), in contrast to what we found in wild cervids. Aschfalk et al. (2003b) found one shiga-toxin producing strain out of 31 *E. coli* isolates from semi-domesticated reindeer in Norway.

In America, deer have been related to human VTEC outbreaks (Keene et al. 1997, Cody et al. 1999). Screenings of white tailed deer faeces found *E. coli* O157:H7 prevalences of approximately 1-2% (Sargeant et al. 1999, Rice et al. 2003), whereas samples from elk (*Cervus elaphus nelsoni*, i.e. red deer) were negative (Rice et al. 2003). VTEC has also been identified in roe deer in Germany (Thoms 1999) and in a moose in Canada (Todd et al. 1999). In the present study, PCR-products were found in varying amounts and of different lengths (number of base pairs) in some of the isolates, but they were not identical to the positive control strains. This indicates these isolates contain gene sequences that are related to the recognised variants of *stx* and *eae*. Whether potential gene products of such sequences have any importance for the pathogenicity of the host strains, cannot be deduced. This would need to be investigated further.

The negative results for *Salmonella* in this screening, together with similar results in a study of 332 cervid carcasses in 1997 (Hofshagen et al. 2001), suggest that cervids do not

constitute a significant reservoir for human salmonellosis in Norway, although a low prevalence of seropositive moose has been reported (Aschfalk *et al.* 2003a). This is also the case with regard to domestic ruminants in Norway, with the exception of sheep; *S. diarizonae* is found relatively frequently in this species (Hofshagen *et al.* 2002). The situation in Norway seems to be similar to that in Sweden; Wahlström *et al.* (2003) did not find *Salmonella* in any of the faecal samples from 295 cervids. In a similar investigation of roe deer meat from Germany, all 73 samples were negative (Weber & Weidt 1986). However, there are occasional reports of *Salmonella* in deer, which present a public health threat from the contaminated venison (Jaksic *et al.* 2003), as well as causing clinical disease in the deer (Sato *et al.* 2000). In Norway, other wild animals and birds, with no importance as sources of game meat, have been shown to be carriers of *Salmonella* contributing to human contamination, for example hedgehogs (Handeland *et al.* 2002), various stationary passerine birds and sea gulls (Kapperud *et al.* 1998, Refsum *et al.* 2002). Our results indicate, however, that wild cervids do not contract *Salmonella*-infection to any significant extent from other wildlife and sheep, although they obviously have to be exposed through faecal contamination of pastures.

*Campylobacter jejuni jejuni* was isolated from one roe deer sample only (2.6%). This level is in accordance with a Swedish study where 4% of 172 roe deer samples and one out of 86 moose samples were positive for *Campylobacter* (Wahlström *et al.* 2003). In a German study of roe deer at the meat inspection, *Campylobacter* sp. was found in 3% of the carcasses (Paulsen *et al.* 2003). In an earlier report, all rectal swabs from Norwegian moose (n = 372); reindeer (n = 94) and roe deer (n = 8) were negative for *Campylobacter* (Rosef *et al.* 1983). There are also other studies reporting negative

*Campylobacter* results; in mule deer and pronghorns in Saskatchewan (Van Donkersgoed *et al.* 1990), and in roe deer in Germany (Weber & Weidt 1986). Altogether, this strongly implies that cervids are of limited importance with respect to sources of *Campylobacter* infection. Examinations of Norwegian domestic animals have demonstrated that cattle (Hofshagen *et al.* 2002) and sheep (Rosef *et al.* 1983) relatively frequently act as carriers for thermophilic *Campylobacter*. Moreover, greater frequencies of *Campylobacter*-positive individuals have been found in Norwegian domestic (Kapperud *et al.* 1993) and wild bird populations (Kapperud & Rosef 1983).

Antibiotic resistance was found in only three out of 137 *E. coli* strains (2.2 %) from moose, red deer and roe deer. One strain may be characterised as multi-resistant. The proportion of resistant *E. coli* in moose, red deer and roe deer seems to be substantially lower than that for *E. coli* in Norwegian cattle (19%) and pig (26%) faecal samples, tested against the very same antibacterials (Kruse & Simonsen 2002), while the proportion of resistant reindeer isolates (24%) was equivalent to that reported for domestic animals. In reindeer, three of the isolates were multi-resistant. The most frequently encountered resistance was to streptomycin. This reflects similar findings in Norwegian cattle and pig *E. coli* isolates (Kruse & Simonsen 2002). The underlying reason for the greater frequency of antibiotic resistant *E. coli* in reindeer is not yet known. One could speculate that these animals are exposed to antibiotics or similar substances in connection with their food intake. Since streptomycin can be produced by certain soil-bacteria (*Streptomyces griseus*), one could imagine that such organisms are present in reindeer pastures or in their main winter feed organism, the reindeer lichen. However this hypothesis has not been investigated further.

The number of *Enterococcus* strains in this

study was low. Moreover, as there are only a few published results of antibiotic resistance patterns in enterococci, from faecal samples of domestic animals, to compare with, it is difficult to characterise the levels of antibiotic resistance in the *Enterococcus* isolates from cervids. Fifteen of the 19 *Enterococcus* strains were resistant to virginiamycin.

However, *E. faecalis* is taken to be naturally resistant to this antibiotic, while *E. faecium* should be sensitive, the latter taken to be naturally resistant to flavomycin (Kruse & Simonsen 2002). One isolate of *E. faecium* was resistant to bacitracin, and one was resistant to oxytetracycline, indicating levels of resistant isolates similar to that found in domestic ruminants (Kruse & Simonsen 2002).

### Conclusion

The examination of samples from Norwegian cervids indicates that their faeces do not constitute an important source of infection, with respect to *Salmonella*, *Campylobacter* and verocytotoxic *E. coli*. Moreover, the levels of antibiotic resistant indicator organisms seem to be low in these animals which have not been exposed to therapeutic use of antibacterials. However, the *E. coli* isolates from reindeer constitute an interesting exception.

### Acknowledgements

The authors wish to thank local wildlife management authorities and hunters who contributed with the organising or sending of faecal samples. We would also like to acknowledge the help of medical laboratory engineer Kristin O'Sullivan, Norwegian School of Veterinary Science, for the running of the PCR-tests for gene-sequences coding for toxin production of VTEC.

### References

*Anonymous*: Official Statistics of Norway. Hunting Statistics 2002. Statistics Norway. 2003, 50 pp. ISBN 82-537-6493-6.

Aschfalk A, Hundertmark KJ, Bendiksen HR, Arnemo JM, Denzin N: Serosurvey for antibodies against *Salmonella* species in free-ranging moose (*Alces alces*) from Norway. Berl. Münch. tierärztl. Wschr. 2003, 116, 417-420.

Aschfalk A, Kemper N, Höller C: Bacteria of pathogenic importance in faeces from cadavers of free-ranging or corralled semi-domesticated reindeer in northern Norway. Vet. Res. Commun. 2003, 27, 93-100.

Cody SH, Glynn MK, Farrar JA, Cairns KL, Griffin PM, Kobayashi J, Fyfe M, Hoffman R, King AS, Lewis JH, Swaminathan B, Bryant RG, Vugia DJ: An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Ann. int. Med. 1999, 130, 202-209.

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

Dutka-Malen S, Evers S, Courvalin P: ERRATUM: Detection of glycopeptide resistance genotype and identification to the species level of clinically relevant enterococci by PCR. J. clin. Microbiol. 1995b, 33, 1434.

Handeland K, Refsum T, Johansen BS, Holstad G, Knutsen G, Solberg I, Schulze J, Kapperud G: Prevalence of *Salmonella* Typhimurium infection in Norwegian hedgehog populations associated with two human disease outbreaks. Epidemiol. Infect. 2002, 128, 523-527.

Hofshagen M, Aavitsland P, Kruse H (eds): Trends and sources of zoonotic agents in animals, feed-stuffs, food, and man in Norway 2001. Norwegian Zoonosis Centre. 2001, 83 pp.

Hofshagen M, Aavitsland P, Kruse H (eds): Trends and sources of zoonotic agents in animals, feed-stuffs, food, and man in Norway 2002. Norwegian Zoonosis Centre. 2002, 39 pp.

Jaksic S, Uhtil S, Asaj A, Petrak T, Botka-Petrak K: Salmonellen in Wildfleisch. (Salmonells in game meat.) Allg. Forst Jagd-Ztg. 2003, 174, 57-58.

Kapperud G, Rosef O: Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway. Appl. environ. Microbiol. 1983, 45, 375-380.

Kapperud G, Skjerve E, Vik L, Hauge K, Lysaker A, Aalmen I, Ostroff SM, Potter M: Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. Epidemiol. Infect. 1993, 111, 245-255.



- Kapperud G, Stenwig H, Lassen J: Epidemiology of *Salmonella* typhimurium O:4-12 infection in Norway – Evidence of transmission from an avian wildlife reservoir. *Amer. J. Epidem.* 1998, *147*, 775-782.
- Keene WE, Sazie E, Kok J, Rice DH, Hancock DD, Balan VK, Zhao T, Doyle MP: An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *J. Amer. med. Ass.* 1997, *277*, 1229-1231.
- Kruse H: Kartlegging av forekomst av antibiotikaresistente bakterier i norsk kjøtt og kjøttprodukter. (A survey of the occurrence of antibiotic resistant bacteria in Norwegian meat and meat products.) SNT-Rapport 1-99. 1999, 33pp.
- Kruse H, Simonsen GS: NORM/NORM-VET 2001. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø / Oslo 2002. 2002, 73 pp. ISSN: 1502-2307.
- Paulsen P, Hilbert F, Winkelmayr R, Mayrhofer S, Hofbauer P, Smulders FJM: Zur tierärztlichen Fleischuntersuchung von Wild, dargestellt an der Untersuchung von Rehen in Wildfleischbearbeitungsbetrieben. (Veterinary meat inspection of wildlife, examinations of roe deer in a cutting plant.) *Arch. Lebensmitt.-Hyg.* 2003, *54*, 137-140.
- Refsum T, Handeland K, Baggesen DL, Holstad G, Kapperud G: Salmonellae in avian wildlife in Norway from 1969 to 2000. *Appl. environ. Microbiol.* 2002, *68*, 5595-5599.
- Rice DH, Hancock DD, Besser TE: Faecal culture of wild animals for *Escherichia coli* O157:H7. *Vet. Rec.* 2003, *152*, 82-83.
- Rosef O, Gondrosen B, Kapperud G, Underdal B: Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Appl. environ. Microbiol.* 1983, *46*, 855-859.
- Sargeant JM, Hafer DJ, Gillespie JR, Oberst RD, Flood SJA: Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. *J. Amer. vet. med. Ass.* 1999, *215*, 792-794.
- Sato Y, Kobayashi C, Ichikawa K, Kuwamoto R, Matsuiura S, Koyama T: An occurrence of *Salmonella* Typhimurium infection in sika deer (*Cervus nippon*). *J. vet. med. Sci.* 2000, *62*, 313-315.
- Todd ECD, Szabo RA, MacKenzie JM, Martin A, Rahn K, Gyles C, Gao A, Alves D, Yee J: Application of a DNA hybridization-hydrophobic-grid membrane filter method for detection and isolation of verotoxigenic *Escherichia coli*. *Appl. environ. Microbiol.* 1999, *65*, 4775-4780.
- Thoms B: Nachweis von verotoxinbildenden *Escherichia coli* in Rehfleisch. (Detection of verotoxin-producing *Escherichia coli* in roe deer's meat.) *Arch. Lebensmitt.-Hyg.* 1999, *50*, 52-54.
- Urdahl AM, Beutin L, Skjerve E, Zimmermann S, Wasteson Y: Animal host associated differences in Shiga toxin-producing *Escherichia coli* isolated from sheep and cattle on the same farm. *J. appl. Microbiol.* 2003, *95*, 92-101.
- Van Donkersgoed J, Janzen E, Chirino-Trejo M, Dunn C: Prevalence of *Campylobacter jejuni* in pronghorns and mule deer in southern Saskatchewan. *Canad. vet. J.* 1990, *31*, 302-303.
- Wahlström H, Tysén E, Olsson Engvall E, Brändström B, Eriksson E, Mörner T, Vågsholm I: Survey of *Campylobacter* species, VTEC O157 and *Salmonella* species in Swedish wildlife. *Vet. Rec.* 2003, *153*, 74-80.
- Wasteson Y, Arnemo JM, Klungseth Johansen B, Vold L, Mathiesen SD, Olsen MA, Wiig Ø, Derocher AE: Analysis of faecal samples from wild animals for verotoxin producing *Escherichia coli* and *E. coli* O157:H7. *Vet. Rec.* 1999, *144*, 646-647.
- Weber A, Weidt H: Untersuchungen zum Vorkommen von Salmonellen und thermophilen *Campylobacter* in Kotproben von erlegten Feldhasen und Rehwild. (Examinations for *Salmonella* and thermophilic *Campylobacter* in culled field hares and roe deer.) *Prakt. Tierarzt* 1986, *67*, 1092-1094.

## Sammendrag

Undersøkelse av fecesprøver fra hjortevilt for *Campylobacter spp.*, *Salmonella spp.*, verocytotoksiske *Escherichia coli* og antibiotikaresistens hos indikatororganismer.

Fecesprøver ble samlet inn i regi av Helseovervåkingsprogrammet for hjortevilt (HOP) fra kronhjort, rådyr, elg og villrein i løpet av jaktseasonene fra 2001 til 2003. Prøver fra i alt 618 dyr ble undersøkt for verocytotoksiske *E. coli* (VTEC), 611 dyr for *Salmonella* og 324 dyr for *Campylobacter*. For å studere antibiotikaresistens-mønstre ble indikatorbakteriene *E. coli* og *Enterococcus faecalis* / *E. faecium* forsøkt isolert fra til sammen 50 prøver fra hver dyreart. *Salmonella* og de potensielt humanpatogene verocytotoksiske *E. coli* ble ikke isolert, mens *Campylobacter jejuni jejuni* ble funnet i prøven fra ett eneste rådyr.

Antibiotikaresistens ble påvist hos 13 (7,3%) av de 179 *E. coli* isolatene som ble testet. Av disse var åtte resistente mot bare en type antibiotika. Andelen resistente *E. coli* isolater var høyere hos villrein (24%) enn hos det øvrige hjorteviltet (2,2%). *E. faecalis* eller *E. faecium* ble isolert fra 19 prøver, men ingen av disse var fra villrein. Alle stammene var resistente

mot ett (84%) eller flere (16%) antibiotika. Til sammen 14 *E. faecalis*-stammer var resistente mot bare virginiamycin. Resultatene indikerer at hjortevilt ikke utgjør et smittereservoar av betydning verken for de humanpatogene bakteriene som inngikk i studien, eller for antibiotikaresistente bakterier.

(Received June 9, 2004; accepted February 3, 2005).

Reprints may be obtained from: A. Lillehaug, Section for Wildlife Diseases, National Veterinary Institute, P.O. Box 8156 Dep., N-0033 Oslo, Norway.