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Semi-domesticated Reindeer in Fennoscandia – a Risk to Human Health? (Workpackage 9.2)

Introduction

As one main point of the RENMAN project was the investigation of the environment, the objective of this workpackage was to examine and evaluate the relations between reindeer, the environment and human health regarding microorganisms that might cause serious diseases in both, animal and man. Free-ranging animals may serve as sentinels or reservoirs for diseases in livestock and man. Transmission of infectious agents to man may occur through direct contacts to free-ranging animals including cervids (Fanning & Edwards, 1991), by contamination of the environment through faecal shedding (Aavitsland & Hofshagen, 1999) or by consumption of venison (Keene *et al.*, 1997). In contrast to domestic animals, however, the epidemiological situation in free-ranging animals and in their habitat is difficult to assess.

There is a lack of information regarding the human health risk due to faecal shedding of pathogens by reindeer. Bacteria, such as *Campylobacter* spp., *Enterococcus* spp., *Escherichia coli*, *Salmonella* spp., *Yersinia* (*Y.*) spp. and the parasites *Cryptosporidium* spp. are among the most important agents in causing zoonosis like enteric and other diseases. They were isolated before from healthy and diseased domestic ruminants (De Rycke *et al.*, 1986; Munoz *et al.*, 1996; Busato *et al.*, 1998 and 1999, Tham *et al.*, 1999). In *E. coli*, the ability to cause severe illness in humans and animals is associated to the occurrence of several virulence factors like the production of shigatoxins. Therefore, the presence of shigatoxin genes can be an allusion for the virulence of certain strains, also known as shigatoxin producing *E. coli* (STEC).

Besides STEC, *Campylobacter* spp., *Salmonella* spp. and *Yersinia* spp. are a problem for meat production with a high infection risk for humans consuming contaminated products. In addition, these bacteria are known to cause illness especially in young animals. Thus, a certain importance of these pathogens as well in reindeer production may not be excluded. *Salmonella* spp. (Kuronen *et al.*, 1998) and *Yersinia* spp. (Rehbinder & Nikander, 1999) have been found associated to mortality in reindeer in Finland and Sweden. *Campylobacter* spp., that have not been associated to mortality in reindeer yet, may also occur (Kobayashi *et al.*, 1999; Lahti *et al.*, 2001).

The impact of *Cryptosporidium* spp. on human health has become more important during the recent years as waterborne cryptosporidiosis outbreaks cannot be excluded. As free-ranging reindeer can contaminate surface waters by faecal shedding, the occurrence of these pathogens should also be determined. Regarding *Cryptosporidium* spp. in northern European reindeer, no data is available. Ruminants, such as reindeer, may be a reservoir for all these pathogens and more knowledge is required to better protect man, reindeer, wild and farm animals against outbreaks of diseases caused by these infectious organisms. This is of special importance as corralling of reindeer for winter feeding is increasing, eventually raising the prevalence of infectious microorganisms and the incidence of infectious diseases in reindeer.

Materials and Methods

During the RENMAN project, 2 243 faeces samples from healthy reindeer, adults and calves, of both genders were examined for the occurrence of *Campylobacter* spp., *Enterococcus* spp., *E. coli*, *Salmonella* spp., *Yersinia* spp., and in addition, for the occurrence of the parasites *Cryptosporidium* spp.. The samples were taken in the course of one year (June 2001 - April 2002) from Finnish and Norwegian free-ranging and corralled reindeer herds, considering parameters such as degree of intensification of husbandry, location and season. Samples were taken off the ground or per rectum from slaughter animals, sent to Kiel, Germany, directly after collection and conserved frozen at - 4 °C until further processed.

The examination of the occurrence of the different bacteria species was performed according to standard procedures. PCR was used to detect the occurrence of shigatoxin1 and 2 genes (*stx1*, *stx2*), the intimin gene (*eae*) and EHEC-hemolysin gene (hly_{EHEC}) in *E. coli*. To detect various *Yersinia*-genes, PCR was performed using primers for the virulence gens 16S rRNA, yadA, V-antigen and 16S rRNA. For the detection of *Cryptosporidium* oocysts, immunomagnetic separation and immunofluorescence microscopy were applied. Furthermore, 392 soil samples and 50 water samples were examined for these pathogens. For statistical analyses, the data was evaluated with the Statistica 5.0 software (StatSoft GmbH, Hamburg, Germany), following the instructions of Trampisch & Windeler (1997). For all analyses, differences were considered significant at $p \leq 0.05$.

Results

In 2 224 (99.2%) out of the total number of 2 243 faecal samples one or more of the examined bacteria species were isolated. *Campylobacter* sp, identified as *C. hyointestinalis*, was detected in one sample only (0.04%). *Enterococcus* spp. were isolated in 2 084 (92.9%) samples. *Escherichia coli* were isolated in 2 123 (94.7%) samples. There was no evidence of the occurrence of *Salmonella* spp. nor *Cryptosporidium* spp. Table 1 shows the results for the detection of the *E. coli* toxin genes by PCR. One hundred and eight (4,8%) strains of *Yersinia* spp. were

isolated, consisting of *Y. enterocolitica* Biogroup 1A (n=29), *Y. intermedia* (n=2), *Y. kristensenii* (n=72), *Y. mollaretii* (n=3) and *Y. rhodei* (n=2).

Regarding the degree of intensification of reindeer husbandry, the season or the

	stx1	stx2	***eae***	hly_{EHEC}	$eae+hly_{EHEC}$
$n_{E.\ coli}$=2 123	3 (0.14%)	0	11 (0.52%)	21 (0.99%)	2 (0.09%)

Table 1 *Occurrence of E. coli toxin genes in 2 123 isolated strains (prevalences in parentheses)*

geographic origin, no significant differences were found for *Enterococcus* spp. and *E. coli*, whereas the prevalences of *Yersinia* spp. differed significantly: prevalences for *Yersinia* spp. in free-ranging reindeer in summer and autumn were significantly higher than in fenced reindeer during winter. Figure 1 illustrates the prevalences of *Yersinia* spp. and the other isolated bacteria in free-ranging and corralled reindeer. Prevalences of *Yersinia* spp. in Norwegian reindeer (10.24%) were significantly higher than in Finnish reindeer (3.60%). *E. coli* was isolated from 14 (3.57%) of the soil samples. *Enterococcus* spp. was detected in 6 samples (1.53.%) and *Yersinia* spp. in 3 samples (0.77%). Nine (18%) of the water samples were positive for *E. coli* and 2 (4%) for *Enterococcus* spp..

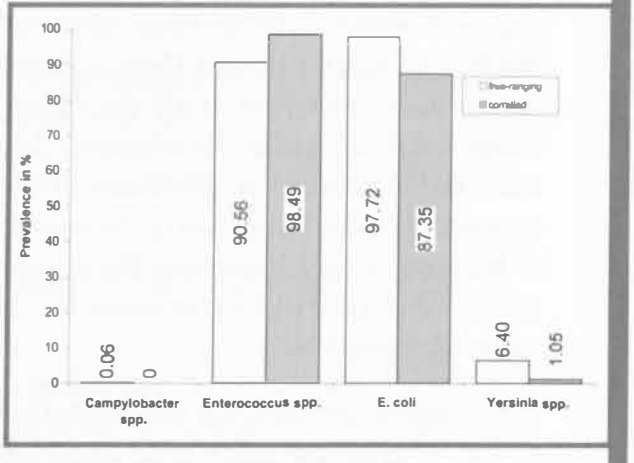

Figure 1 *Prevalences of analysed pathogens in faeces of free-ranging (n=1 579) and corralled reindeer (n=664)*

Discussion

Faecal samples of reindeer were examined for the occurrence of important enteric pathogens in order to get information about the human and animal health risk. Human infections may be accomplished through direct animal contacts or through contamination of the environment due to faecal shedding in reindeer husbandry areas, or by consumption of contaminated reindeer meat. All bacteria investigated in this study may be found in Northern Europe in the environment in aquatic, terrestrial and animal reservoirs (Kapperud, 1981) and were isolated from the intestinal tract of healthy or diseased ruminants worldwide (Adesiyun

et al. 1998; Busato *et al.*, 1998). Even though most of the isolated bacteria strains do not have the potential to cause severe human or animal health problems, certain strains might be a risk, especially for immunosupressed, old or very young persons and animals. Therefore, one has to regard the epidemiological impact of transmission of these infectious agents from the environment to reindeer and man and vice versa.

In reindeer, *Enterococcus* spp. and *E. coli* occurred in very high prevalences, showing the affiliation of these two species to the normal intestinal flora of healthy reindeer. Concerning *E. coli*, there are only few reports on diseases caused by shigatoxin-producing bacteria in ruminants (Sherwood, 1985; Mainil, 1999), however these bacteria are of extreme importance in causing severe diseases in humans (Griffin & Tauxe, 1991). As the genes encoding *stx1*, *eae* and hly_{EHEC} were detected only in very low numbers of the isolated *E. coli*-strains, the human health risk due to *E. coli* excreted by reindeer can be considered very low at the moment. These results comply with another study detecting no *E. coli* O157: H7 in 1 387 faecal and 421 meat samples from reindeer (Lahti *et al.*, 2001). But this situation might change very quickly, as it is known that STEC virulence factors are mobile within bacterial populations (Yamamoto *et al.*, 1984; Pupo *et al.*,1997).

Yersinia spp. was isolated in 108 samples. The identified species *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii* and *Y. rhodei* have been isolated before from various environmental samples (fresh water, soil, *etc.*), food, healthy animals and healthy and diseased humans (Baier & Puppel, 1981; Sulakvelidze, 2000). Even though these species are widely distributed in nature, their actual impact on human health is a matter of controversy. But as they are isolated from persons associated to gastrointestinal disorders, the role of these species should not be disregarded (Sulakvelidze, 2000). The isolated *Y. enterocolitica* strains belonged to Biogroup 1A, which embraces the nonpathogenic European *Y. enterocolitica* strains, often isolated from environmental samples, foods, animal and human faeces (Bottone, 1997).

Campylobacter hyointestinalis was isolated from one sample only. As the cultivation of *Campylobacter* spp. is exceedingly difficult, the real prevalence might be higher. Hitherto *C. hyointestinalis* has been associated only sporadically with human gastrointestinal disorders (Edmonds *et al.*, 1987, Gorciewicz *et al.*, 2002). Even though the prevalence for *Campylobacter* spp. in this study was very low, it shows that reindeer can be carriers of *C. hyointestinalis*. This is approved by another study detecting *C. hyointestinalis* in a prevalence of 6% in Finnish reindeer faeces (Hänninen *et al.*, 2002). Regarding the soil and water samples, there was no hint that the detected bacteria were reindeer-originated. All positive water samples were taken next to human settlements and human origin is the most probable reason for these positive results.

Summarizing it can be stated that the examined enteropathogens were either not detected at all (*Salmonella* spp. and *Cryptosporidium* spp.), in very small numbers (*Campylobacter* spp.) or if detected, their virulence and pathogenicity was very low (*E. coli* and *Yersinia* spp.). In the present situation in northern Europe the potential human and animal health risk by reindeer, excreting various important enteropathogenic bacteria and *Cryptosporidium* spp., has to be estimated as very low. These results are very important, especially regarding the status of reindeer meat as a natural product for the consumer, as for the production no antibiotic treatment is required so far, in contrast to the situation of ordinary farm animals. It may be expected that free-ranging ruminants develop some of the same production limiting diseases that affect domestic ruminants, if brought into an intensive farming environment (Mackintosh, 1998), that may have an impact on human health as well.

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