EXAMINATION ON THE OCCURRENCE OF SELECTED ZOONOTIC PATHOGENS REGARDING THE CURRENT SITUATION OF THE FINNISH REINDEER HUSBANDRY

Nicole Kemper[1], Ansgar Aschfalk[2], Christiane Höller[3] & Jörg Hartung[1]

[1]Institute for Animal Hygiene, Animal Welfare and Behaviour of Farm Animals, Hannover, Germany [2]Section of Arctic Veterinary Medicine, Department of Food Safety and Infection Biology, Tromsø, Norway [3]Bavarian Agency for Health and Food Safety, Oberschleißheim, Germany

Introduction

Reindeer husbandry represents an important economic factor and a valuable cultural heritage. About 185 000 semidomesticated reindeer (*Rangifer tarandus tarandus*) live in the northern regions of Finland. Transmission of infectious agents to man may occur through direct contacts to free-ranging animals including cervids, by contamination of the environment through faecal shedding or by consumption of venison. 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* (*E. 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 and were isolated before from healthy and dieseased domestic ruminants (De Rycke *et al*., 1986; Munoz *et al*., 1996; Busato *et al*., 1998 and 1999). The objectives of this study, that was performed as part of the EU-project RENMAN, were to figure out the occurrence and prevalence of important zoonotic pathogens in reindeer.

Material and Methods

In this study, 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 until further processed.

The examination for *Campylobacter* spp was done by incubating faecal material in Preston broth for 24 h in a microaerophilic atmosphere at 37 °C. A loopful of the enriched suspension was plated on Preston agar and incubated for 48 h under the above described conditions. *Campylobacter*-like colonies were analysed by Gram-staining, catalase and oxidase tests, and further biochemical reactions.

To detect *Enterococcus* spp, faecal material was diluted in glucose-azide broth and incubated for 48 h at 37 °C. A loopful broth was then spread both on kanamycin-aesculin-azide agar and Slanetz and Bartley agar. After 48 h at 37 °C suspicious colonies were Gram-stained and their biochemical reactions were analysed further by catalase and oxidase tests.

Escherichia coli was isolated by adding faeces to Gram-negative broth. After 24 h of incubation at 37 °C a loopful of broth was plated onto Endo-c agar and incubated under the above mentioned conditions for 24 h. Typical metallic shiny colonies were subcultured on blood agar, incubated for 24 h at 37 °C and tested for their biochemical reactions. PCR was used to detect the occurrence of shigatoxin1 and 2 genes (*stx1*, *stx2*), the intimin gene (*eae*) and EHEC-hemolysin gene (hly_{EHEC}). *EHEC EDL 933* (*stx1,2* positive) was used as a positive control and *E. coli ATCC 11 229* (*stx1,2* negative) was included as negative control. The amplified products were analysed by electrophoresis and were visualised following ethidium bromide staining (100 µl/100 ml gel) at UV-light.

For the selective enrichment of *Salmonella* spp faeces was inoculated into tetrathionate broth and incubated for 24 h at 37 °C. Two enrichment steps were repeated the following two days. On the fourth day one loopful of the cultured medium was plated both on *Salmonella-Shigella* agar and Leifson agar. After 24 h of incubation at 37 °C presumptive *Salmonella* spp colonies were Gram-stained and tested biochemically.

Cultural examination of *Yersinia* spp was performed by adding faeces to Gram-negative broth and incubating for 48 h at 21 °C. One loopful of broth was then plated on *Yersinia*-selective agar and incubated for another 48 h at 21 °C. Colonies with the typical bull's-eye-appearance were subcultured on blood agar and Gram-stained and biochemical tests were subsequently carried out. To detect the *Yersinia*-genes encoding *16SrRNA*, *yadA* and *v-antigen* PCR was performed.

For the detection of *Cryptosporidium* oocysts, immunomagnetic separation was applied using Dynabeads anti-*Cryptosporidium*. Twenty µl of the immunoconcentrate were used for a direct immunofluorescence test (*Cryptosporidium*-Antigen-IFT). *Cryptosporidium parvum* oocysts from a calf served as the positive control. Using a fluorescence microscope at x400–x1000 magnification *Cryptosporidium* oocysts appear as 6-10 µm in size, round or oval in shape with bright green fluorescence.

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. Only few of the isolated *E. coli*-strains possess genes encoding *stx1* (0.14%), *stx2* (0%), *eae* (0.61%) and hly_{EHEC} (1.08%), There was no evidence of the occurrence of *Salmonella* spp nor *Cryptosporidium* spp. These results are shown in **Table 1**. 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 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.

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. 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). 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). *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. *Campylobacter hyointestinalis* was isolated from one sample only. As the cultivation of *Campylobacter* spp. is exceedingly difficult, the real prevalence might be higher. Hitherto *Campylobacter 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 *Campylobacter hyointestinalis*. This is approved by another study detecting *Campylobacter hyointestinalis* in a prevalence of 6% in Finnish reindeer faeces (Hänninen *et al*., 2002).

Conclusion

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.

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Figure 1. Prevalences of analysed pathogens in faeces of reindeer (n= 2 243)