

Examination on Shedding of Shigatoxin producing *Escherichia coli* in Dairy Cattle in Northern Germany

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SUMMARY

The relevance of Shigatoxin producing *Escherichia coli* (STEC) is justified by the caused serious diseases in combination with a low infectious dose. This study aimed for the identification and evaluation of risk factors in fecal shedding of STEC in dairy cows. The longitudinal design of the study permitted to follow the STEC-shedding of selected cows over the period of twelve months. Overall, 24.7% (407) of 1,646 fecal samples were positive for STEC by PCR. The inner herd prevalence varied from 11.1% to 32.3%. The month of sampling, the number of lactations and days in milk, the nutritional condition, the somatic cell count, the content of urea and protein in milk and the presence of a continuously positive cow in the herd were identified to have a significant influence on shedding of STEC. As already confirmed for the serogroup O157, heifers, cows in early lactation and dry cows also formed a group of higher risk for the other STEC-serogroups. The late summer months (August, September, and October) were analyzed as season with higher prevalences. Isolated bovine STEC mainly possessed the genes for *stx*₂ and *EHEC-hly*_A (434 isolates, 39.3%), and *stx*₁, *stx*₂ and *EHEC-hly*_A (311 isolates, 28.1%), which represent potentially pathogenic virulence profiles for humans.

INTRODUCTION

Shigatoxin producing *Escherichia coli* (STEC) are an emerging issue for veterinary public health. The highly cytotoxic Shigatoxin combined with other virulence factors causes gastroenteritides and severe disease patterns in children with a low, but increasing incidence. Most research to date has concentrated on the STEC-serovar O157; most methods have been developed to detect only this most important STEC. However, only 71.0% of the German cases of hemolytic-uremic syndrome and 17.0% of the reported STEC-infection in humans in 2007 were caused by this serovar [9]. To avoid this diagnostic problem, it is essential to establish methods in routine usage that are capable to detect also non-O157-Serovars. The only difference of these

serovars to a pathogenic *E. coli* is the possession of *stx*-genes. Ruminants, and especially cattle are the natural reservoir of STEC. Extended risks originate in domesticated cattle kept in close contact to humans. Furthermore, cattle derived foods are often contaminated and represent an important source of infection as well [10]. Bovine carriers of STEC show no sign of clinical disease whilst shedding this pathogen.

Risk factors for faecal shedding by dairy cattle are various, including different factors of management like hygiene, the kind of diet, husbandry and changes in housing or structure of the herd [1,4,5]. Moreover, also individual characteristics of each cow seem to have an influence [2].

ANIMALS, MATERIAL AND METHODS

This study was conducted on six dairy farms in Schleswig-Holstein/Germany in the period of 02/2007 until 01/2008. The design of the study was longitudinal with the intent to follow the fecal excretion patterns of STEC. Therefore, cows in different numbers of lactation were randomly selected. Moreover, the analysis of several risk factors for the individual animal and the herd was performed with additional information about the herd management, the health status, body condition scores and data on the amount and contents of milk, the number of passed lactations and the days

in milk. A number of 140 cows were sampled monthly, resulting in a total of 1,646 examined fecal samples. Following cultural isolation, STEC were identified by colony-hybridization and characterized by PCR. The data were statistically analyzed using the program 201A00C400F2Excel201A00C400F4 and the procedure 201A00C400F2logistic201A00C400F4 in 201A00C400F2SAS201A00C400F4. Furthermore, selected STEC-isolates were tested for their biochemical reaction and their serotypes.

RESULTS

In 24.7% (407) of all samples, STEC were detected. Within the herds, the mean prevalence varied between 11.1% and 32.3%. As significant factors for shedding of STEC several influences were shown: the month of sampling, the number of lactations and days in milk, the nutritional condition, the somatic cell count, the content of urea and protein in milk and the presence of a continuously positive cow in the herd. These cows were classified as continuously positive when showing equal or more than four consecutive positive samplings. The prevalence was highest in the late summer months (August, September, and October) with 27.9%, 28.8% and 35.0% in contrast to the spring months with lower prevalences (February: 20.5%, March: 17.6%, April: 16.3%). Heifers, dry cows, and cows with 50 to 150 days in milk showed a significant higher risk for shedding STEC. However, cows with a body condition score lower than 3.50, a protein and urea content lower than 3.0%, respectively 150mg/L, or with a somatic cell count higher than 100.000 cells/mL have a reduced risk to be identified as STEC-shedder.

In twelve month of sampling, only 13.6% (19) of the cows were detected as constantly negative. Most of the animals excreted STEC at

least once (45.5%, 55 cows), and 17.1% (24) were detected as positive in more than 50% of their samples. Of these cows, 14 (10.0%) were identified as continuously shedding. Isolated STEC from these cows were selected (n=70) for further analysis. These STEC belonged to 24 Sorbitol-fermenting non-O157-STECSerovars. Twelve of these serovars have already been associated with disease in human [7]. Only two isolates did not ferment Sorbitol. The five most predominant serovars, in descending order, were serovar O133:H-, O22:H8, Ont:H25, O130:H11 and O8:H19. Overall, 1,105 STEC were isolated. Dominating combinations of virulence genes were *stx*₂ and *EHEC-hly*_A (434 isolates, 39.3%), and *stx*₁, *stx*₂ and *EHEC-hly*_A (311 isolates, 28.1%) (Table 1).

Tab. 1: Incidence of virulence factors from isolated STEC

virulence factor	number of pos. isolates	proportion of pos. isolates
<i>stx</i> ₁	93	8,4%
<i>stx</i> ₂	626	56,7%
<i>stx</i> ₁ + <i>stx</i> ₂	386	34,9%
<i>eae</i>	91	8,2%
<i>EHEC-hly</i> _A	862	78,0%

n=1,105 samples

DISCUSSION

Congruent to other literature [11], a prevalence of 24.7% was detected in healthy dairy cows in Northern Germany in this study. Seasonality for the serogroup O157 showed highest prevalence in summer [8], in contrast to those for STEC in general with a prevalence peak in autumn [3].

The higher risk for heifers and for cows with 50 to 150 days in milk could be explained by metabolic and emotional stress of these animals and was already shown for serovar O157 [6]. Only few studies have dealt with the influence

of milk contents on the shedding of serovar O157:H7 [5]. This study clearly showed the impact of milk contents, probably related to diet, health and stress on the shedding of Non-O157-STEC as well. Hitherto, the presence of a continuously shedding cow in the herd as a persistent source of infection was shown to be a risk factor for the shedding of serogroup O157 [2]. In this study, the important role of these animals for the infection cycle within the herd for STEC in general was confirmed.

CONCLUSION

Pathogen virulence profiles, common in human isolates, are frequently found in bovine STEC. Consequently, cattle represent an important reservoir and might act as potential human health risk. Even though risk factors for the excretion of STEC have been examined before, the main influencing factors still remain unknown. This study confirms the importance of continuously shedding cows as a major source of STEC. These cows maintain the infectious cycle of STEC in the herd; therefore, interventions on individual animal level might serve as the base for an effective pathogen control.

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