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Short Communication

Prevalence of Diarrheagenic *Escherichia coli* Bacteria in Fecal Samples from Dogs Showing Signs of Gastrointestinal Disorders and a Clinically Healthy Control Group

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Abstract

Infections with enteropathogenic bacteria are a common cause for gastrointestinal symptoms in dogs. However, screening for *Escherichia coli* (*E. coli*) strains such as EHEC, EPEC and ETEC is seldom part of the veterinary approach to establishing differential diagnoses. Consequently, little is known, especially in Germany, about the prevalence of diarrheagenic *E.coli* strains and their role in gastrointestinal diseases in dogs. This paper will contrast the frequency of virulence markers from *E.Coli* pathotypes demonstrated in dogs with gastrointestinal symptoms as well as a control group without clinical findings. Fecal samples from 410 dogs in Germany were cultured, of which 368 were obtained from symptomatic dogs and 42 samples served as control. *E.coli* isolates were subsequently analyzed for the presence of virulence factor encoding genes specific to diarrheagenic *E. coli* pathotypes by PCR (*stx1*, *stx2*, *sta* and *eae*). *Eae* genes were detected in 17.1% of samples from symptomatic dogs and 26.2% of control group samples, respectively. *Sta* genes were detected in 2.4% of samples from symptomatic dogs and 9.2% of samples in the control group, respectively. The Shiga toxinen coding genes *stx1* and *stx2* could only be detected in *E. coli* cultures from dogs with gastrointestinal symptoms. These results suggest that while infections with diarrheagenic strains of *E.coli* can be associated with gastro intestinal illness, these strains may be present in absence of a clinical manifestation. Considering the possibility of a zoonotic potential of Diarrheagenic *E. coli* strains, a differentiation between non-pathogenic and virulent strains may yield valuable additional information for diagnostic and therapeutical purposes.

Keywords: Enterotoxins; Shigatoxin; *Shigella spp;* Zoonotic; *Yersinia spp*

Introduction

Escherichia coli (*E. coli*) are aerobic, gram-negative rod-shaped bacteria that belong to the family of Enterobacteriaceae. Unlike obligate enterpathogenic bacteria such as *Salmonella spp.* or *Yersinia spp.*, they are part of the normal colonic microbiota of many mammals and have established a commensal or mutualistic status. In this symbiotic relationship, the microbial collective supports nutrient degradation and metabolism, forms and maintains a mucosal barrier against pathogen colonization and is important for proper immune modulation of the gut associated lymphoid tissue (GALT) [1-3]. The host provides ecological niches and metabolites for microbial growth and survival [4].

However, several strains of *E. coli* are considered true pathogens and can cause clinical presentations such as hemorrhagic colitis and hemolytic-uremic syndrome [5]. These strains express virulence factors such as enterotoxigenic and enteropathogenic metabolites [6]. The most commonly detected types of enterotoxins are Shigatoxin (ST) or Verocytotoxin (VT), as well heat stable toxins (e.g. heat-stable toxin 1, STa). Shigatoxins Stx1 and Stx2 are secreted by EHEC and STEC (Shigatoxin producing *E. coli*) and bind to the host cell receptor globo triaosylceramide (Gb3) found on intestinal endothelium. They inhibit protein biosynthesis, which leads to premature cell death causing extensive GI mucosal damage [7]. *E. coli* strains that express the virulence factor intimin in addition to Shigatoxin encoding genes *stx1* and/or *stx2* are classified as EHEC (enter hemorrhagic *E. coli*), while strains with only *stx1* and/or *stx2* are classified as STEC.

The heat-stable enterotoxin STa is synthesized by several enterotoxigenic *Escherichia coli* strains (ETEC). It is not present in EPEC (enteropathogenic *E. coli*) and EHEC bacteria. It can bind to specific SoTa-receptors that consist of the extracellular domain of a trans-membrane guanylate cyclase type C (GC-C) protein, which is located on the surface of mammalian enterocytes [8]. Immediately after receptor binding the toxin over activates guanylate cyclase, which causes an increase in intracellular cyclic guanosine monophosphate (cGMP) [9]. This inhibits the Na^+/Cl^--coupling mechanism in enterocytes of the small intestine, which leads to decreased resorption of electrolytes and H_2O causing a disruption of intestinal fluid homeostasis and the clinical manifestation of secretory diarrhea [10,11].

Some diarrheagenic *E. coli* strains express additional virulence factors. For example, EPEC contain a 'locus of enterocyte

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effacement' (LEE) pathogenicity island that carries genes such as *eae* and is responsible for the attachment and effacement (A/E) phenotype on host enterocytes [12,13]. The gene product of *eae* is Intimin. It allows bacteria to intimately attach to intestinal epithelium to cause lesions in the intestinal wall. These EPEC strains can also harbor the EPEC adherence plasmid (EAF), which contains several genes (e.g. *bfpA*) encoding the bundle-forming pilus (BFP) [14]. Strains with *bfpA* and *eae* are defined as typical EPEC and strains that only contain *eae* without *bfpA* as atypical EPEC. Due to the non-invasive nature of EPEC-bacteria, the main cause for diarrhea and intestinal inflammation accompanying an infection are ultra structural changes in intestinal cells [15].

Production animals like cattle or pigs are major reservoirs for diarrheagenic strains of *E. Coli* [16,17]. Neonatal diarrhea is one of the most common diseases in calves and pigs worldwide and responsible for significant losses in large-scale farms [18]. Several epidemiologic studies conducted in multiple regions of the world have demonstrated that the domestic swine can carry and shed STEC. It is widely accepted that specific serotypes and pathotypes of ETEC and STEC strains are responsible for most neonatal diarrhea in pigs and cattle [19-21]. The role that production animals play in STEC transmission to humans and the contribution to human disease frequency has been analyzed extensively. However, little is known about pathogenic *E. coli* strains in companion animals and the role these animals might play as vectors for zoonoses. Some German reports from 1993 indicate that STEC strains were prevalent in 4.8% of sampled dogs, but no data was collected regarding their health status at the time [22]. A different study analyzed fecal samples from both, dogs with diarrhea and clinically healthy household dogs by hybridization, using DNA probes to detect EPEC and other diarrheagenic *E. coli.* The rate of EPEC excretion was found to be significantly higher in symptomatic dogs (35.1%) than in the asymptomatic control group (6%) [23].

The clinical presentation of dogs infected with diarrheagenic *E. coli* is highly variable in severity. In most cases, patients suffer from diarrhea, nausea and abdominal pain. Immunosuppressed patients like puppies or senior dogs can become critically ill and may present with hemolytic anemia, thrombocytopenia, and ultimately kidney failure [24]. An important reason for the low rate of enteropathogenic *E. coli*-specific diagnostics in companion animals is that infections are often clinically silent. Consequently, studies analyzing the transmission of enteropathogenic *E. coli* from domestic companion animals to humans are rare. There is only one report from Germany that documents a possible transmission of an EHEC from a cat to a child. Both excreted EHEC for 3 months and although the girl had clear symptoms of gastrointestinal disease, the cat was asymptomatic [25]. This case report demonstrates a possible zoonotic potential of diarrheagenic *E. coli* strains in household animals and leads to the conclusion that further evaluation of the role companion animals play in EHEC, EPEC, ETEC and STEC transmission to humans is necessary.

Objective

Most epidemiological studies have focused on the role of production animals as reservoirs for enteropathogenic *E. coli*. The prevalence rates of diarrheagenic *E. coli* in dogs are not well documented; even less so regarding associations between infections and gastrointestinal health status. This study aims to determine the frequency of the virulence marker genes *eae*, *sta*, *stx1*- and *stx2* in *E. coli* cultivates from fecal samples of dogs with gastrointestinal symptoms as well as an asymptomatic control group.

Material and Methods

Fecal samples and bacterial cultivation

A total of 410 fecal samples from routine diagnostics at Laboklin GmbH (Bad Kissingen, Germany) were analyzed. Of the samples, 368 were from dogs presenting with gastrointestinal symptoms and 42 from clinically healthy dogs with no gastrointestinal symptoms and normal fecal consistency. Fecal specimens were examined by standard methods for the presence of *Shigella spp*., *Salmonella spp*., endoparasites and viral diseases. Dogs tested positive for one or more of these were excluded from this study. Samples were cultured on selective growth media (BD Columbia Agar with 5% Sheep Blood and BD MacConkey II Agar) for 24h. Isolates were streaked onto MacConkey II agar and the identity of overnight cultures was confirmed as *E. coli* using MALDI-TOF mass spectrometry (MALDI BioTyper [Bruker Daltonik]).

Detection of genes

After culture, 5 phenotypically identical bacterial colonies were transferred into 200 µl of Tris-EDTA buffer and genomic DNA-extraction was performed with a MagNA Pure 96® System (Roche). To detect the presence of virulence markers from ETEC, STEC, atypical EPEC and EHEC bacteria, *sta, stx1, stx2* and *eae*-genes were detected via TaqMan real time PCR [26,27]. Detection of the *eae*-gene was performed via conventional PCR-methods [28]. Verocytotoxin-producing *E. coli* (VTEC), entero invasive *E. coli* (EIEC), entero aggregative E coli (EAggEC) and other diarrheagenic strains of minor importance were excluded from this analysis.

Statistical analysis

Associations between gastrointestinal health statuses with virulence marker gene presence were investigated using Pearson's chi-squared test. A p value equal to or less than 0.05 was considered statistically significant.

Results

The prevalence of virulence factor encoding genes in *E. coli* cultures in the symptomatic cohort was 17.1% *eae* (63/368), 2.4% *sta* (9/368), 1.6% *stx1* (6/368),and 0.5% (2/368) of *stx2*-genes respectively (Figure 1). In six samples of this cohort, two or more virulence factors could be detected. In samples from asymptomatic controls, frequencies of 26.2% (11/42) for the *eae*-gene and 9.5% (4/42) for STa-toxin encoding genes could be detected. The control group contained no samples with *stx1*- or *stx2*-genes, or with two or more genes encoding different virulence factors (Figure 2).

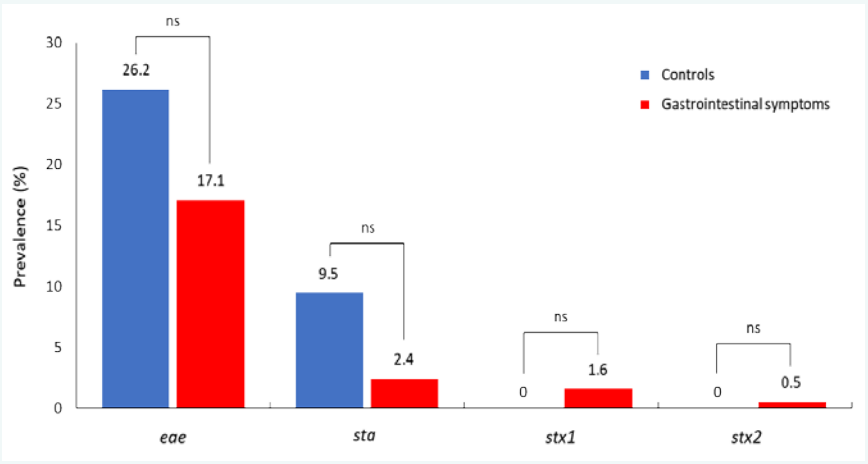

Figure 1 Comparison of the prevalence rate of virulence marker encoding genes from diarrheagenic *E. coli* strains in fecal samples from dogs with gastrointestinal symptoms (n=368) and clinically healthy dogs (n=42); p<0.05, Pearson's chi-squared test.

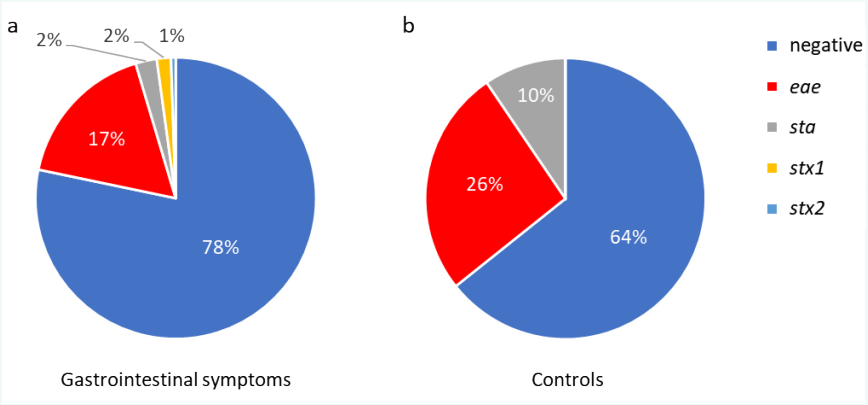

Figure 2 Distribution patterns of virulence marker encoding genes from diarrheagenic *E. coli* strains in fecal samples from dogs with gastrointestinal symptoms (a; n=368) and clinically healthy dogs (b; n=42).

Discussion

This study aimed to detect diarrheagenic *E. coli* strains that were described to have high prevalence rates in dogs, as they consequently may be more relevant for transmission events to and from humans [22-24]. Studies have shown that prevalence rates of atypical EPEC strains are increasing worldwide in humans with diarrhea and may be associated to childhood diarrhea [29,30]. Therefore, we decided to focus on atypical EPEC and excluded a detection of the *bfpA*-gene (a defining characteristic for typical EPEC strains).

More than 400 fecal samples from dogs with gastrointestinal symptoms (n=368) as well as an asymptomatic control group (n=42) were tested for the presence of four genes encoding specific virulence factors present in diarrheagenic *E. coli* (*sta, stx1, stx2* and *eae*). To our knowledge, this is the first survey on the prevalence of diarrheagenic *E. coli* strains in clinically healthy and diseased dogs in Germany. The results show that *sta*- and *eae*-genes are present in *E. coli* cultures in both groups. This finding is consistent with previous studies investigating *eae* genes in *E. coli* found in dogs [31]. However, a significant overrepresentation of EPEC strains in dogs with gastrointestinal symptoms as reported by Sancak *et al.*, in 2004 could not be confirmed [23]. The two Shigatoxin enoding genes *stx1* and *stx2* could only be detected in clinically affected animals (n=6 and n=2, respectively). This may suggest a correlation between the expression of Shigatoxins and a clinical presentation of gastrointestinal disease in dogs. However, due to the limited number of asymptomatic controls, one cannot confidently exclude the possible presence of Shiga toxin encoding genes *stx1* and/or *stx2* in clinically healthy dogs, and further research in this area may therefore be warranted. Furthermore, as additional clinical data was not obtained, there may be variability in terms of clinical presentation in the symptomatic group that could not be considered. Nevertheless, the diagnostic value of pathotype differentiation in dogs that are infected with diarrheagenic *E. coli* remains undisputed. The findings of this study strongly contradict the consensus that *E. coli* pathotype differentiation is virtually negligible in companion animals due to its overwhelmingly benign nature even in the presence of gastrointestinal symptoms.

Conclusion

Dogs may serve as reservoirs for EHEC. Therefore, screening for diarrheagenic *E. coli* virulence factor encoding genes is a potentially valuable tool in the veterinary field. It can initially serve to exclude infections with enteropathogenic and enterotoxigenic *E. coli* strains, which may be the cause for gastrointestinal presentations. Furthermore, it facilitates subsequent follow-up measures like bacterial viability and susceptibility testing for therapeutical purposes. Incidences of diarrheagenic *E. coli* strains like EHEC, EPEC, ETEC and STEC in dogs should be discussed as a potential reservoir/risk factor for human pathology, possibly even by way of vectors for bovine strains [32-34].

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