International Journal of Medical Microbiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Petting zoos as sources of Shiga toxin-producing *Escherichia coli* (STEC) infections

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ARTICLE INFO

Keywords:
Shiga toxin-producing
Escherichia coli
Petting zoo
Hemolytic uremic syndrome
Transmission chain
Next generation sequencing

ABSTRACT

Despite their general low incidence, Shiga toxin-producing *Escherichia (E.) coli* (STEC) infections are considered an important public health issue due to the severity of illness that can develop, particularly in young children. We report on two Austrian petting zoos, one in Tyrol (2015) and one in Vorarlberg (2016), which were identified as highly likely infection sources of STEC infections. The petting zoo related cases involved a case of hemolytic uremic syndrome (HUS) due to STEC O157:HNM in 2015 and an outbreak of STEC O157:H7 infections affecting five young children and two adults in 2016. The HUS case accounted for 2.8% of the 36 STEC O157:HNM/H7 infections notified in Austria in 2015 (5,9% of 17 HUS cases). The seven cases described for 2016 accounted for 4.0% of the 177 human STEC infections documented for Austria in 2016, and for 19.4% of the 36 STEC O157:HNM/H7 infections notified that year. The evaluation of the STEC infections described here clearly underlines the potential of sequence-based typing methods to offer suitable resolutions for public health applications. Furthermore, we give a state-of-the-art mini-review on the risks of petting zoos concerning exposure to the zoonotic hazard STEC and on proper measures of risk-prevention.

1. Introduction

Microbiological and epidemiological investigations of transmission chains play a central role in prevention and control of infections (Krause, 2009). Even investigations that identify the source of an outbreak after its natural ending can contribute to preventing re-emergence and avoiding similar future occurrences. Whereas the incidence of sporadic notifiable illness can be seen as unavoidable, the occurrence of an outbreak almost always indicates inadequate application of hygiene standards. The core function of local public health authorities is to identify and verify such poor standards (Krause, 2009). Investigations of transmission chains are thus an instrument for evaluating and improving existing preventive measures (Reingold, 1998).

Although the majority of Shiga toxin-producing *Escherichia (E.) coli* (STEC) outbreaks occur as a result of contaminated food or water, numerous outbreaks have been traced back to direct contact with animals or indirect contact via fair, farm, and petting zoo environments (Conrad et al., 2017). Research suggests that individuals with repeated exposure to enteric pathogens, such as those living or working on farms, may become less susceptible to infection (Belongia et al., 2003; Hale et al., 2012). However, today most members of the public do not have direct interaction with farms in their daily lives and therefore are more susceptible.

Over the last decades, there has been a significant rise in the popularity of open farms, farm holidays and petting zoos (Stirling et al., 2007). Visitors have access to animals such as goats, sheep, lambs, rabbits, kittens, donkeys, guinea pigs and puppies, which makes these attractions particularly popular among children. This close association, promoted through activities such as feeding and handling the animals, has led to reports of zoonotic transmission of several mainly gastrointestinal infections, including STEC (Conrad et al., 2017; Stirling et al., 2007; McMillian et al., 2007; Weese et al., 2007; Erdozain et al., 2013; Heuvelink et al., 2002; Warshawsky et al., 2002; Goode et al., 2009).

Besides the ability of a typing method to clearly identify isolates that are involved in an outbreak, the typing method must accurately differentiate outbreak strains from non-outbreak isolates. Pulsed-field gel electrophoresis (PFGE) was initially described in 1983 (Schwartz et al., 1983) and still represents the *gold standard* in molecular typing of most bacterial species. In contrast to the situation in forensic human genetics, where—with the exception of monozygotic twins –

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https://doi.org/10.1016/j.ijmm.2018.06.008
Received 27 March 2018; Received in revised form 20 June 2018; Accepted 24 June 2018

Table 1
Animals sampled in an epidemiologically linked petting zoo in Tyrol in 2015.

	Number of individual animals sampled	STEC-positive	STEC O157:HNM-positive	Analyzed in PFGE and NGS	By PFGE, indistinguishable from HUS case isolate
Goat	20	15/20	1	0	–
Sheep	26	26/26	7	6	1 (sheep no. 4)
Llama	2	2/2	1	1	0
TOTAL	48	43/48	9/48	7/48	1/48

indistinguishable DNA fingerprinting patterns prove epidemiological relatedness, in bacteriology even epidemiologically unrelated STEC isolates can yield PFGE patterns indistinguishable from each other, without any causal relation (Schmid et al., 2014). Explanations for this might be a monomorphic population structure, which is known from *E. coli* O157:H7, or the limitation of the PFGE typing method used. On the other hand, the maximum resolution of next generation sequencing (NGS) poses a major challenge in determining meaningful similarity thresholds for grouping related isolates, to provide an appropriate level of discrimination for source attribution. The definition of such thresholds will be a major task for public health authorities, using well-defined outbreak scenarios of the pathogen species of interest. The applied method has to allow for some genetic diversity between isolates from human and animal/environmental sources, but only to the degree that it can still be assumed that they originate from the same source (Schmid et al., 2014).

Here we report on two events of STEC transmission related to Austrian petting zoos. A German case of HUS due to STEC O157:HNM in 2015 and an outbreak of STEC O157:H7 infection affecting five young children and two adults in 2016 were investigated using PFGE-typing. Using these strains from successfully investigated STEC infections, we compared newly generated NGS-results with the retrospective historic PFGE analyses results. The evaluation of the STEC infections described here clearly underlines the potential of sequence-based typing methods to offer suitable resolutions for public health applications. Furthermore, we give a state-of-the-art mini-review on the risk of petting zoos concerning exposure to the zoonotic hazard STEC and on proper measures of risk-prevention.

2. Two episodes of illness related to Austrian petting zoo visits

2.1. Patient populations

A German one year old girl suffered from HUS due to STEC O157:HNM (isolate titled: case 2015). Onset of illness was 19th of May 2015. A petting zoo in Tyrol was identified epidemiologically (and microbiologically; see below) as the highly likely source of infection.

An outbreak of STEC O157:H7 infection affecting five young children and two adults occurred in Austria in 2016 (for age and gender see Table 2, isolates titled: cases 2016). A petting zoo in Vorarlberg was identified epidemiologically as the highly likely source of infection. All dates of STEC O157:HNM/H7-isolation are mentioned in Table 4.

2.2. Material and methods

Organisms were cultured in EHEC-Direkt-Medium (Axon Lab, Baden, Switzerland) for 18–24 h and PCR was used to test for *stx1* plus *stx2* (Reischl et al., 2002) and for a O157-serotype specific gene according to EU-Reference Laboratory for *E. coli*, VTEC_Method_02 (EU Reference Laboratory for E. coli, 2013). In case of positivity isolation of STEC was conducted on SMAC, CT-SMAC (Oxoid, Basingstoke, UK) and CHROMagar O157 (CHROMagar, Paris, France). All gained STEC O157 isolates were O-and H-typed according to the protocol published by (Ørskov et al. (1977)). Pulsed-field gel electrophoresis (PFGE) profiles were generated according to the standardized laboratory protocol developed by the Centers for Disease Control and Prevention, USA (Standard Operating Procedure for PulseNet PFGE of Escherichia coli O157:H7, 2018). Genomic DNA isolation, whole genome sequencing using an Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA), assembly and contig filtering were performed as described previously (Lepuschitz et al., 2017; Hartl et al., 2017). Assembled genomes were compared using the Enterobase core genome (cg)MLST scheme (Enterobase.warwick.ac.uk. Available at: http://enterobase.warwick.ac.uk) using SeqSphere + with a cluster type threshold of ten allelic differences. Minimum spanning tree was visualized in SeqSphere+.

2.3. Initial analysis using pulsed-field gel electrophoresis

Concerning the German HUS case in 2015, we received the patient's STEC O157:HNM-isolate in June 2015 from the Bavarian health authorities. Fecal samples from 48 animals of an epidemiologically linked Tyrolean petting zoo (20 goats, 26 sheep, 2 llamas) were gained four days later. Fifteen of the 20 goats, all of the 26 sheep and both llamas were found STEC positive (Table 1). Results of PFGE analysis of STEC isolates from the HUS case and from 7 of 9 STEC O157:HNM positive animals are depicted in Fig. 1. Sheep no. 4 yielded an STEC O157:HNM isolate indistinguishable from the patient's strain by PFGE using *Xba*I as restriction enzyme. The remaining six animal isolates clustered with the patient's isolate with two bands difference. For the 2015 German HUS case we were able to provide epidemiological and PFGE microbiological proof of a causative connection to an Austrian petting zoo.

For the outbreak investigation of the 2016 cases we analyzed stool samples from the 7 persons involved, 5 children between 14 months and 5 years of age and 2 adults (Table 2). Dates for onset of illness ranged from 10. July till 23. September. From an epidemiologically linked petting zoo in Vorarlberg individual fecal samples from 5 goats, 1 pooled fecal sample from goats, individual fecal samples from 2 donkeys and pooled fecal samples from the donkeys and from alpine ibexes were collected on 01.09.2016 (Table 3). Results of PFGE analysis of isolates from 5 cases and from 2 goats are depicted in Fig. 2. In this outbreak of gastroenteritis a petting zoo in Vorarlberg was epidemiologically identified as common source: all six goat samples and an ibex sample were STEC positive. The donkey samples remained STEC negative. Fourteen individual STEC O157:H7 strains were cultured from the two goats. Using *Xba*I as restriction enzyme, they were clearly distinguishable from the patients' isolates. Epidemiological investigation showed that all seven cases had direct contact to goats. In this 2016 outbreak, a causative connection could be proven only epidemiologically. The lack of microbiological prove does not surprise, as it is well known that ruminants shed the pathogen intermittently.

2.4. Retrospective analysis using next generation sequencing

From the 2015 German HUS case investigation, the patient's STEC O157:HNM isolate and seven animal isolates (the llama isolate and 6 sheep isolates) (entitled Complex 1 in Fig. 3) and from the 2016 outbreak, five patients' strains (entitled Complex 2 in Fig. 3) and two goat isolates (entitled Complex 3 in Fig. 3) were available for NGS analysis.