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Carrier prevalence, secondary household transmission and long-term shedding in two districts during the Escherichia coli O104:H4 outbreak in Germany, 2011

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Abstract

Background

From May-July 2011, Germany experienced a large Shiga toxin-producing E. coli (STEC) O104:H4 outbreak. Our objective was to identify the prevalence of STEC O104:H4 carriers in households in highly affected areas, the rate of secondary household transmissions, and the duration of long-term shedding.

Methods

In a cross-sectional study, we recruited case and control households to determine STEC household prevalence; we then conducted a prospective cohort study (≥2-persons households with ≥1 case) for rates of household transmission and shedding duration.

Results

For part 1, we recruited 57 case households (62 cases and 93 household contacts) and 36 control households (89 household members). We only detected cases in previously known case households and identified 1 possible adult-to-adult household transmission. For part 2, we followed 14 households and 20 carriers. No secondary household transmission was detected in the prospective follow-up. The longest prolonged shedding lasted >7 months, however, median estimated shedding time was 10-14 days (95% CI: 0-33 days). Three carriers showed intermittent shedding.
Conclusions

Prevalence of STEC O104:H4 carriers even in highly affected areas appears to be low. Despite prolonged shedding in some patients, secondary adult-to-adult household transmissions seem to be rare events in the post-diarrheal disease phase.
INTRODUCTION

From May-July 2011, Germany experienced a large outbreak of Shiga toxin-producing Escherichia coli (STEC) O104:H4 associated with fenugreek sprouts [1], causing a total of 2,987 gastroenteritis, 855 hemolytic-uremic syndrome (HUS) cases and 53 deaths [2]. The outbreak’s epidemic profile that significantly differed from previous Escherichia (E.) coli outbreaks and notification data can be explained to a great extent by the causing vehicle: cases were predominantly adults with a median age of 46 years for gastroenteritis and 42 years for HUS; furthermore, cases mostly occurred among women (58% and 68%, respectively) [2]. The estimated median incubation period was 8 days (IQR: 6-10) [2]. The highest incidence was reported for gastroenteritis cases in the district of Herzogtum Lauenburg (60.55/100,000 inhabitants) and for HUS cases in the district of Schleswig-Flensburg (13.1/100,000 inhabitants), both located in the most Northern federal state Schleswig-Holstein [2, 3].

At initiation of our study, almost no information was available on household-level prevalence of STEC O104:H4 carriers and their role in secondary transmissions. For E. coli O157, secondary transmissions have been reported in association with sporadic cases and may account for up to 20% of cases in outbreaks [4-8]. Young age of primary and secondary cases was identified as risk factor for secondary transmission [5, 8]. A study by Ludwig et al. has shown that asymptomatic E. coli O157 infections in household contacts are common and undiagnosed asymptomatic infections may contribute to secondary transmission [9].
Knowledge of E. coli carrier prevalence and rates of secondary household transmissions is essential for the decision on further short and long-term containment measures. Starting 3 weeks after the first outbreak notification, we conducted an investigation to identify the prevalence of STEC O104:H4 carriers in households, the risk for secondary household transmission, and the duration of long-term shedding.

**METHODS**

In a cross-sectional study we first screened non-single person households with and without reported STEC gastroenteritis or HUS cases for STEC O104:H4 carriers. Screening of households was performed 3-10 weeks after first notification of the outbreak. In the second part, we followed households with a carrier in a prospective cohort study to determine rates of secondary household transmission and duration of shedding among STEC O104:H4 carriers.

**Definitions**

A case was defined as a notified STEC gastroenteritis or HUS case with serogroup O104 or unknown serogroup and onset of disease after April 30, 2011, residing in the district of Herzogtum Lauenburg or Schleswig-Flensburg, or a case with symptomatic or asymptomatic STEC O104:H4 infection diagnosed during our investigation by detection of STEC O104:H4 in a stool sample. Disease onset was defined as onset of bloody or non-bloody diarrhoea, and diarrhoea as ≥2 loose stools within 24 hours. A person with detection of STEC O104:H4 after symptomatic or asymptomatic infection was
considered a carrier. A secondary household transmission was defined as STEC O104:H4 infection with disease onset or STEC O104:H4 detection $>10$ days after disease onset in the primary case, referring to the estimated incubation period of STEC O104:H4 [2]. A case household was defined as a non-single household with $\geq 1$ reported STEC gastroenteritis or HUS case; a control household as a non-single household without a notified STEC gastroenteritis or HUS case.

**Study design**

The screening of case households and the prospective cohort study were conducted in the districts of Herzogtum Lauenburg and Schleswig-Flensburg, whereas screening of control households was only performed in Herzogtum Lauenburg. We recruited 52 and 40 case households based on mandatory notification to the public health authorities of Herzogtum Lauenburg and Schleswig-Flensburg, respectively. In Herzogtum Lauenburg, 52 control households were randomly selected by recruiting a household in direct neighbourhood of the case household. In Herzogtum Lauenburg, households were contacted face-to-face, whereas in Schleswig-Flensburg study materials were mailed after phone contact.

For screening, all participants were asked to provide a stool sample and answer a questionnaire collecting information on demographic data, symptoms, symptom onset, and food items consumed. Household members of cases were asked about symptoms in the period between 14 days prior to disease onset of index case and time of screening, participants of control
households about symptoms in the 14 days prior to screening. Instructions on stool sampling and information on basic infection control/hygiene precautions to prevent secondary transmission were provided orally by public health authorities once and in printed format enclosed for each stool sampling.

Households with $\geq 1$ carrier and $\geq 1$ household member without reported STEC gastroenteritis or HUS prior to and at time of screening were enrolled in the prospective cohort study. All participants were asked to provide stool samples and information on gastrointestinal symptoms since last stool sampling on a 14 days interval. At first follow-up, the questionnaire also contained questions on household and participants characteristics and hand hygiene practice. The sampling was continued until all household carriers tested negative. A negative test had to be confirmed by 2 additional negative samples on 2 consecutive days.

**Laboratory investigation**

Stool samples and questionnaires were sent by regular mail to the Robert Koch Institute. Upon arrival, samples were transported at 4-8° Celsius to the National Reference Centre for Salmonella and other Bacterial Enteric Pathogens. Isolation and identification of the outbreak strain were performed by culturing a stool sample aliquote for 6-18h in modified Tryptic Soy-enrichment broth with 10mg/l novobiocin and 6mg/l cefsulodin and subsequent plating on extended-spectrum beta-lactamase (ESBL) selective agar medium (tryptone bile X-glucuronide-agar containing 1mg/l
cefotaxime). Presence of stx2, rfb O104 and fliC H4 genes was determined by means of multiplex PCR of single bacterial colonies and is described elsewhere [3, 10].

**Statistical analysis**

We used Wilcoxon rank sum test for differences between distributions and calculated odds ratios for exposure variables. A 5%-significance level was used for testing. Duration of shedding was quantified by the survival function, which was estimated using nonparametric maximum likelihood for interval-censored data as follow-up was not from time of diagnosis and performed on a 14 days interval for cases tested positive at time of screening [11]. Resulting intervals for quantiles of the shedding distribution could then be calculated from this curve. Uncertainty in the quantile estimation was quantified by 95%-confidence intervals (CI) based on percentile bootstrap on 999 re-samples of the data. Further detailed descriptions and examples on the use of interval-censored data analysis can be found elsewhere [12].

Stata® version 11.0, StataCorp, Texas, USA and R version 2.12.0, Vienna, Austria applying the R package “interval” was used for statistical analysis [13].

**Ethical approval**

In accordance with Article 25 paragraph 1 of the German Infection Protection Act of 2001 and in agreement with the responsible ethical review board regarding ongoing outbreaks, a formal ethical review process and approval was not required. We informally discussed the study design with the
ethical review board of the Charité University Medicine, Berlin, Germany, charged to oversee compliance with the Declaration of Helsinki, and no objections were expressed.

Results

Part 1

Response of case and control households was 88% (46/52) and 69% (36/52), respectively, in Herzogtum Lauenburg, and 45% (18/40) in case households in Schleswig-Flensburg. After excluding 7 case households that provided ≤1 stool sample per household, 57 (90%) case households with a total of 62 cases and 93 household contacts, and 36 (100%) control households with 89 participants met the inclusion criteria. Twenty of 62 (32%) cases tested positive for STEC O104:H4 at time of screening. Median time between disease onset and stool sampling was 26 days (range 10-53) for cases tested positive for STEC O104:H4 in our screening, and 33 days (range 10-58) for cases tested negative (p=0.21). Median time for stool samples from collection to arrival at RKI and the laboratory was 2 (range 1-5) and 3 days (range 1-6), respectively, for all participants and did not significantly differ for cases with and without STEC O104:H4 detection in our screening (p=0.6 and p=0.59, respectively).

Disease onset of cases occurred between May 9 and June 20. The majority of cases were females (34/62; 55%) and ≥18 years of age (61/62; 98%). Four (6%) of 62 cases were reported as HUS cases. Of the 57 cases with available information, 40 (70%) were hospitalized.
Symptoms of diarrhoea and bloody diarrhoea were reported for 100% (62/62) and 74% (46/62) of cases, respectively. Nine of 93 (10%) case household contacts reported diarrhoea ranging from 6 days prior to 4 days after disease onset of the index case. Diarrhoea ≤14 days prior to screening was stated by 16% (14/89) of participants from control households. None of them reported bloody diarrhoea or sprout consumption. Cases were more likely to report consumption of sprouts, cucumber, raw tomatoes and lettuce than non-cases with the highest odds ratio for sprout consumption (see table).

**STEC O104:H4 household prevalence**

All 89 stool samples from control households were tested negative for STEC O104:H4. Among case households, 42/62 (68%) cases had negative testing results. All but one of the 20 positive stool samples could be attributed to previously known cases.

**Households with multiple cases**

Including the previously undetected case, 5 households had 2 cases per household. In 3 households, disease onset for the second case was 1, 5, and 6 days, respectively, after disease onset of the primary case. In the fourth household, disease onset for the symptomatic but previously undiagnosed second case was 34 days after disease onset of the primary case. The questionnaire and interview exploration of this second case revealed no
evidence for late sprout exposure. There was no information available on disease onset of the second household case for the fifth household.

Part 2

We identified a total of 17 households with ≥1 STEC O104:H4 case still positive at time of screening: 14 households with 1 case and 3 households with 2 cases. One 2-person household of the 14 households with 1 case had to be excluded as both members had been previously reported as cases; in addition, 2 of the 3 households with 2 cases had to be excluded for household transmission follow-up, as the households solely consisted of the 2 cases. However, we included them for determination of shedding duration. The included 14 households comprised of 4 (29%) 4-persons households, 2 (14%) 3-persons households, and 8 (57%) 2-persons households.

The median age of the 15 cases was 48 years (range 20-77) and 47 years (range 1-76) for the 21 contacts. Only 1 household member was <6 years. Ten (67%) of the cases and 6 (29%) of the contacts were female. Three cases and 3 contacts reported a chronic disease.

The 14 households participated with a total of 132 samples during the prospective study. Three households were lost to follow-up after 1 prospective sampling. For the remaining 11 households, the median time of participation per household was 26 days (range 0-237). The median time interval between each household sampling was 22 days (range 14-37).

Thirty (83%) of the 36 participants answered all questions on hand hygiene practices. Twenty-six (87%) of these 30 participants reported to
“always” or “most of the times” wash their hands when coming home (15 cases, 11 contacts), 24 (78%) before preparing meals (13 cases, 11 contacts; 6 do not prepare meals), and all after using the toilet. Seventeen (57%) participants reported to wash their hands more frequently after the STEC diagnosis of themselves (n=10) or their family member (n=7), the remaining 13 did not change behaviour (5 cases, 8 contacts).

**Rate of household transmissions**

No household transmissions were detected during the prospective study period.

**Duration of shedding**

We followed-up 20 carriers that tested positive in our screening. The median time interval between each sampling was 19 days (range 14-37). A total of 12 carriers sent in 2 final consecutive stool samples with a median of 21 days (range 16-44) after having tested negative. Two consecutive negative stool samples were reported by 4 carriers at their primary care physician. One carrier was lost to follow-up; another 3 with negative test result withdrew from the study after the first prospective sampling. Three carriers showed a period of intermittent shedding. The longest duration of shedding was 237 days between disease onset and last positive O104:H4 test result; no underlying chronic disease was reported for this case. Details of the follow-up are shown in figure 1.
Fifty-seven of the 62 (92%) cases that participated in the screening part had a reported date of disease onset and could therefore be included in the calculation of time intervals in which cases became negative. Figure 2 shows the estimated survival curve for interval-censored data. Grey areas of the curve show indistinguishable values of the survival function, i.e. the exact value is not known, except that the function cannot increase within the interval. Estimated median shedding time was 10 to 14 days (95% CI: 0-33), with the 75% and 90% quantile being 44 to 45 days (95% CI: 23-70) and 67 to 70 days (95% CI: 44-123), respectively. For comparison, figure 2 also contains Kaplan-Meier estimated survival curves when using either the beginning, midpoint or endpoint as imputed event time.

**Discussion**

The German STEC O104:H4 outbreak was caused by a previously rarely-reported strain and resulted in considerable morbidity and mortality [3, 14, 15]. Uncertainty was high about the extent of asymptomatic infections, rate of person-to-person transmission, long-term carriers in the community and their potential public health impact.

In our investigation, no symptomatic or asymptomatic cases in control households were identified, even though we chose the districts with the highest incidence of STEC gastroenteritis and HUS cases, respectively. About 10% of the household contacts reported diarrhoea 0-6 days around disease onset in the index case and 16% of the participants of control households reported diarrhoea 14 days prior to sampling, but were tested negative in the
screening. However, cases could have been missed due to test sensitivity (as screening was based on only 1 sampling), short duration of shedding, or intermittent shedding, as it has been previously described for E. coli O157 in children [16]. To limit the effect of potential recall bias controls were not interviewed about symptoms regarding the same time period as cases; exposure and illness assessments have therefore limited comparability.

Descriptions of STEC O104:H4 secondary transmissions have meanwhile been published. Hauri et al. described at least 6 possible secondary transmissions within 4 families among a total of 179 STEC gastroenteritis or HUS cases in the state of Hesse [17]. All primary cases were adults, 1 of the secondary cases occurred in a child [17]. Kuijper et al. published 1 case of possible secondary transmission in a child from the Netherlands whose mother had acquired the infection during a visit to Germany [18]. Finally, 2 more cases (involving 1 child) of potential secondary transmission within 1 household were reported from a STEC O104:H4 outbreak in France [19]. All secondary household transmissions occurred early after disease onset of the primary case and mainly affected persons taking care of the primary case [16] or in children of primary cases [17-19].

We identified only 1 potential secondary household transmission in the screening part of our study and no further secondary transmissions in the follow-up. In contrast to other publications, our potential secondary transmission occurred late after disease onset of the primary case. Furthermore, both cases were adults. For E. coli O157, 2 studies showed that the majority of secondary household cases were seen early after disease
onset of the primary case, and no secondary cases in household contacts occurred when the primary case was an adult [5, 8]. A population-based survey in Scotland attributed 9% of all secondary cases to transmission among adults [7]. The STEC O104:H4 outbreak predominantly affected the adult population which is mirrored by our all-adult cohort apart from one child. Hygiene measures are more easily followed by adults, and regular reminders on basic infection/hygiene precautions during the prospective follow-up could have lowered the risk of household transmissions. Seto et al. showed in a model based on an E. coli O157 outbreak in the United States that even a modestly effective strategy to interrupt secondary transmission can result in a significant reduction of symptomatic cases [4].

As we could not follow carrier households from disease onset of the primary case, and first screening starting with a median of 31 days after disease onset, we might have missed secondary cases occurring early during disease of the primary case. Patients with STEC O104:H4 from households with early primary cases have been sporadically reported through mandatory notification after the official end of the outbreak, suggesting late secondary transmissions could have occurred.

We were able to document long-term shedding after STEC O104:H4 infection for >7 months in one adult carrier without underlying chronic disease and could observe a negative test followed by a positive test result in carriers what may be attributed to test sensitivity or suggest intermittent shedding. The follow-up of carriers starting from time of screening instead of disease onset resulted in large CI for estimates of shedding duration and thus the estimated
survival function should be interpreted accordingly. However, the estimated median duration of shedding in our study is similar to results by Karch et al. in children with E. coli O157 [16].

We did not assess risk factors associated with long-term shedding, as for example antibiotic treatment. Yet this information could be relevant for containment measures. A study published by Nitschke et al. showed an inverse association between macrolide therapy and duration of shedding of STEC O104:H4 [20].

STEC O104:H4 carriers employed in risk areas like food handling and medical or institutional settings are excluded from work and have usually to provide 3 consecutive negative stool samples before re-admittance to work [21]. Yet extended work absences in long-term carriers have not only financial implications both for employer and employee. Our findings could contribute to the discussion about earlier work re-admittance for some adult long-term carriers. However, as STEC O104:H4 can cause severe morbidity and mortality, any earlier re-admittance needs to be carefully weighted and further be based on a comprehensive individual risk assessment, incorporating other individual aspects such as work environment, performed work procedures, and adherence to hygiene measures. It has to be noted that undiagnosed asymptomatic carriers may pose a higher transmission risk than known long-term carriers.

The small sample size of our study and therefore limited power makes conclusive statements on STEC O104:H4 carrier prevalence and household transmissions difficult. Mainly due to logistic reasons, the aimed for time
interval for consecutive sampling was not met. Furthermore, the study population was biased towards having knowledge about carrier(s) in the household, and it consisted mainly of cases with longer shedding time who may have distinguishing characteristics from other cases in the outbreak. Response in our study differed between districts that could be due to different recruitment methods. In Schleswig-Flensburg, disease severity of cases may have resulted in a lower response as this district had the highest reported HUS incidence; compared to 25% of cases that developed HUS during the outbreak [3], our study population included only 6% HUS cases. Stool sampling procedures and transport conditions until arrival at the laboratory may have resulted in lower sensitivity. Before screening, information on the serotype was not always available. Therefore, cases could have been caused by non-outbreak serotypes that would not have been detected by our laboratory approach. We assume not to have missed a significant number of cases by employing the ESBL phenotype to detect the outbreak strain, although Aldabe et al. reported the loss of the characteristic ESBL pattern in an O104:H4 isolate within a family cluster [19]. The ESBL phenotype appeared very stable during the acute outbreak, as none of the >300 STEC isolates detected by routine diagnostic methods, i.e. without ESBL phenotype selection during the acute outbreak was of serotype O104:H4 (unpublished data, National Reference Centre for Salmonella and other Bacterial Enteric Pathogens).

Several questions of public health and clinical relevance that arise in the context of long-term shedding need to be addressed in further studies such as
the potential of STEC O104:H4 to become endemic, identification of risk factors for long-term shedding, association between long-term shedding, comorbidities and concomitant medical interventions, potential risks and benefits of antibiotic eradication in carriers, stability of the ESBL phenotype of the outbreak strain, and possible ESBL plasmid transfer and its significance on individual and population level. In addition, seroprevalence studies could possibly give important insights to estimate the proportion of asymptomatic infections. Finally, further studies are needed to assess the risk of secondary transmission in E. coli long-term carriers, potentially leading to revised recommendations regulating work re-admittance of carriers of STEC O104:H4 and other pathogenic E. coli.

In conclusion, our results suggest that the prevalence of STEC O104:H4 long-term carriers is low even in highly affected areas. Although shedding of STEC O104:H4 may persist >7 months in some patients, secondary transmissions among adult household contacts in the post-diarrheal phase of infection appear to be rare events, particularly when long-term carriers are regularly followed-up. Therefore, clinicians and public health authorities should be aware that extended duration of shedding does occur and that reminders about consequent hygiene precautions can help to prevent secondary transmissions within patients’ households and among contacts.
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Footnotes Page

Conflict of Interest:
M. H. is co-author of a recently submitted paper on a multi-centre study on the duration of shedding time during the STEC O104:H4 outbreak. All other authors state that they do not have a financial or other association that might pose a conflict of interest.

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**Figure 1:** Follow-up of screening-positive carriers. Time (days) of symptom onset before screening, time (days) of stool sampling after screening, and laboratory result; Shiga toxin-producing E. coli O104:H4 outbreak, Germany, 2011.
Figure: Estimated survival function from 57 cases when taking interval-censoring into account (Turnbull’s method). For comparison, Kaplan-Meier estimated survival curves are given when imputing the event time as beginning, mean or end of the intervals, respectively. Note that the figure contains no indication of estimation uncertainty. Only cases with available information are included. Cross-sectional and prospective cohort study part; Shiga-toxin producing E. coli O104:H4 outbreak, Germany, 2011.
**Table:** Consumption of food items among cases and non-cases and association with disease outcome; only participants with available information are included. Cross-sectional study part; Shiga-toxin producing *E. coli* O104:H4 outbreak, Germany, 2011.

<table>
<thead>
<tr>
<th>Food item</th>
<th>Cases, no. (%)</th>
<th>Non-cases, no. (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprouts</td>
<td>31 (60.8)</td>
<td>10 (6.3)</td>
<td>23.1</td>
<td>9.9-54.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cucumber</td>
<td>39 (72.2)</td>
<td>64 (39.5)</td>
<td>4.0</td>
<td>2.0-7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Raw tomatoes</td>
<td>46 (78.0)</td>
<td>80 (49.1)</td>
<td>3.7</td>
<td>1.8-7.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lettuce</td>
<td>41 (71.9)</td>
<td>73 (46.2)</td>
<td>3.0</td>
<td>1.5-5.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Ground meat (beef)</td>
<td>18 (38.3)</td>
<td>75 (47.5)</td>
<td>0.7</td>
<td>0.4-1.3</td>
<td>0.269</td>
</tr>
<tr>
<td>Unpasteurized milk products</td>
<td>11 (20.4)</td>
<td>32 (20)</td>
<td>1</td>
<td>0.5-2.2</td>
<td>0.953</td>
</tr>
</tbody>
</table>