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ORIGINAL PAPER

Hepatitis A Outbreak in the General Population due to a MSM-Associated HAV Genotype Linked to a Food Handler, November 2017–February 2018, Germany

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Abstract
Hepatitis A (HAV) is a viral infection causing a range of symptoms, sudden onset of fever, malaise, diarrhea, and jaundice. It is mostly transmitted fecal-oral through contaminated food, with immediate household and sexual contacts having a higher risk of infection. Since 2016 an increased number of HAV infections, mostly affecting men who have sex with men (MSM) have been noticed worldwide, with three main genotypes circulating. We report here on the first spillover outbreak of the MSM-associated HAV genotype RIVM-HAV16-090 in the German general population in November 2017–February 2018. In total, twelve cases could be attributed to the outbreak with the index case and a coworker in a butchers shop being the most probable source of the outbreak. The identical HAV genotype was detected in two environmental samples in the premises of the butchers shop and in nine cases. Outbreak control measures included detailed contact tracing and stool examinations, several environmental investigations, thorough cleaning, and disinfection of the premises of the butchers shop. Post-exposure vaccination was recommended to all unprotected contacts during the investigation. Furthermore, although hand-washing facilities were in accordance with the required law, additional installment of soap and disinfectant dispensers and contactless faucets has been recommended.

Keywords Hepatitis A virus · Foodborne outbreak · MSM-cluster genotype

Introduction

Hepatitis A virus (HAV) infection causes a range of mild-to-severe symptoms with sudden onset of fever, malaise, nausea, loss of appetite, abdominal discomfort, diarrhea, dark-colored urine, and jaundice. Children are often asymptomatic, > 70% of children younger than 6 years of age and 20% of children older than 6 years have asymptomatic HAV infections (Jeong and Lee 2010). The severity of disease is higher in older age groups. Main infection route is fecal-oral, either direct human-to-human, through contaminated fomites or contaminated food. The incubation period ranges from 15 to 50 days (median 28 days) (Sattar et al. 2000; Heymann 2004). The highest infectivity due to fecal virus shedding occurs during the 2-week period preceding the onset of jaundice and declines during the week after onset (Schmid et al. 2009). Household members and sexual partners of infected persons, intravenous drug users, and international travelers to hepatitis A endemic areas have an increased risk of infection (Heymann 2004).

Since 2016 a large outbreak of hepatitis A has been occurring in the EU/EEA mostly affecting men who have sex with men (MSM). From June 2016 till September 2018, a total of 4475 confirmed cases have been notified from 22 EU/EEA countries with a nucleotide sequence homology of ≥ 99.3% to one of the three HAV genotype 1A outbreak strains: VRD_521_2016; RIVM-HAV16-090; and V16-25801 based on genomic fragments at the VP1-2a junction region (ECDC 2018). The outbreak strain, initially detected

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within the MSM risk group and a peak male-to-female ratio of 11.8 in May 2017 (ECDC 2018), has by the end of the year 2017 also been observed among the general population outside this risk group (Friesema et al. 2018).

Foodborne outbreaks with HAV have already been described, among others due to frozen (Nordic Outbreak Investigation Team 2013; Hutin et al. 1999; Niu et al. 1992; Severi et al. 2015) or fresh berries (Sane et al. 2015), dried tomatoes (Donnan et al. 2012), oysters (Bialek et al. 2007; Desenclos et al. 1991; Shieh et al. 2007), and other shellfish (Pinto et al. 2009; Sanchez et al. 2002). Another source of HAV infections in foodborne outbreaks can be the contamination through food handlers (Schmid et al. 2009; Harries et al. 2014; Schenkel et al. 2006; Wenzel and Allerberger 2014), as HAV is environmentally stable and can remain infectious for long periods in the environment and on inanimate surfaces (Heymann 2004; Sattar et al. 2000).

We here report on the first spillover outbreak in the German general population, caused by one of the three MSM-associated HAV strains and linked to a food handler as the most probable source of the outbreak. Through contact tracing, two immediate contacts have been diagnosed *via* stool examination, and over the next few weeks seven customers of the shop were identified as confirmed cases.

Methods

Epidemiological Investigations

In order to classify cases to the outbreak, the following case definition was used: a person diagnosed with hepatitis A in district X from November 2017–February 2018 with a ≥ 99.3% homology to the genotype IA outbreak strain RIVM-HAV16-090 and/or an epidemiological link to the butchers shop.

Case finding was performed prospectively from notification records of the district, starting from November 2017. An attempt was made to genotype and interview all notified cases regarding their food habits and shopping preferences. Family members and close contacts of each confirmed case including coworkers in the butchers shop and neighboring shops were interviewed regarding symptoms, and stool samples, when available, were examined for the presence of HAV-RNA by RT-qPCR. Vaccination was recommended to all contacts that were not immunized against HAV in the past.

Environmental Investigations

Three authorities were involved in the different environmental investigations: Local Health Authority (LHA), Food Inspection Authority (FIA), and Veterinary Health Authority. The first inspection was conducted on 28.11.2017 by the LHA and FIA separately (inspection round 1). The LHA performed informative interviews, immunization check-ups, and hygiene instructions. The FIA observed working procedures and working environment and ordered cleaning and disinfection procedures; however, no sample collection was performed. After the second confirmed case in the butchers shop, all three authorities conducted joint environmental inspections of the butchers shop on 15.12.2017, 19.12.17, and 01.02.2018 (inspection round 2, 3, and 4) (Fig. 1) and a professional cleaning company was appointed to perform cleaning and disinfection. All working procedures were observed, with emphasis on hygiene precautions taken, and inspection of toilets and washing facilities was performed. Environmental samples were taken from high-contact surfaces (e.g., door handles, knife, cash register) in the inspection round 2, 3, and 4. Food samples of ready-to-eat products (salami and other cold cuts to be consumed without heat treatment) were taken on the 4th inspection round and examined for the presence of HAV by RT-qPCR (Costafreda et al. 2006).

Virological Examinations and Genotyping

Molecular analyses from samples of human origin were performed at the national consultant laboratory for HAV (University Medical Centre Regensburg, Germany). Nucleic acid isolation was performed using the RNeasy Mini Kit (Qiagen, Hilden, Germany) with 100 µL elution. A 10-µL aliquot of the eluate was used for reverse transcription in a reaction volume of 20 µL using Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Applied Biosystems, Foster City, CA, USA) and random hexamers (42 °C, 30 min). Two replicates were analyzed in 30 µL PCRs each containing 10 µL of the RT product (corresponding to 5 µL eluate), ROX buffer, 5.0 mmol/L $MgCl_2$, 1.25 U AmpliTaq Gold DNA polymerase (all Applied Biosystems), dNTPs, specific primers (300 nmol/L each), and TaqMan hydrolysis probe (200 nmol/L). Primers and probe for the HAV reverse transcription quantitative real-time PCR (RT-qPCR) were in the viral polymerase gene region (SH-Poly-A, SH-Poly-1, and SH-Poly-Q) (Houde et al. 2007). Thermal cycling was performed on a StepOnePlus instrument (applied biosystems) and comprised a 10-min initial enzyme activation step at 95 °C, and 45 cycles of 95 °C for 15 s and 60 °C for 1 min. RT-qPCR-positive samples were further characterized by amplicon sequencing. The amplification was performed according to the unified HAV Net protocol (http://www.rivm.nl/en/Topics/H/HAVNET) by using specific primers for the HAV VP1/P2A genomic region (HAV 6.1, 5′-TAT GCY ITI TCW GGI GCI YTR GAY GG-3′; HAV 10, 5′-TCY TTC ATY

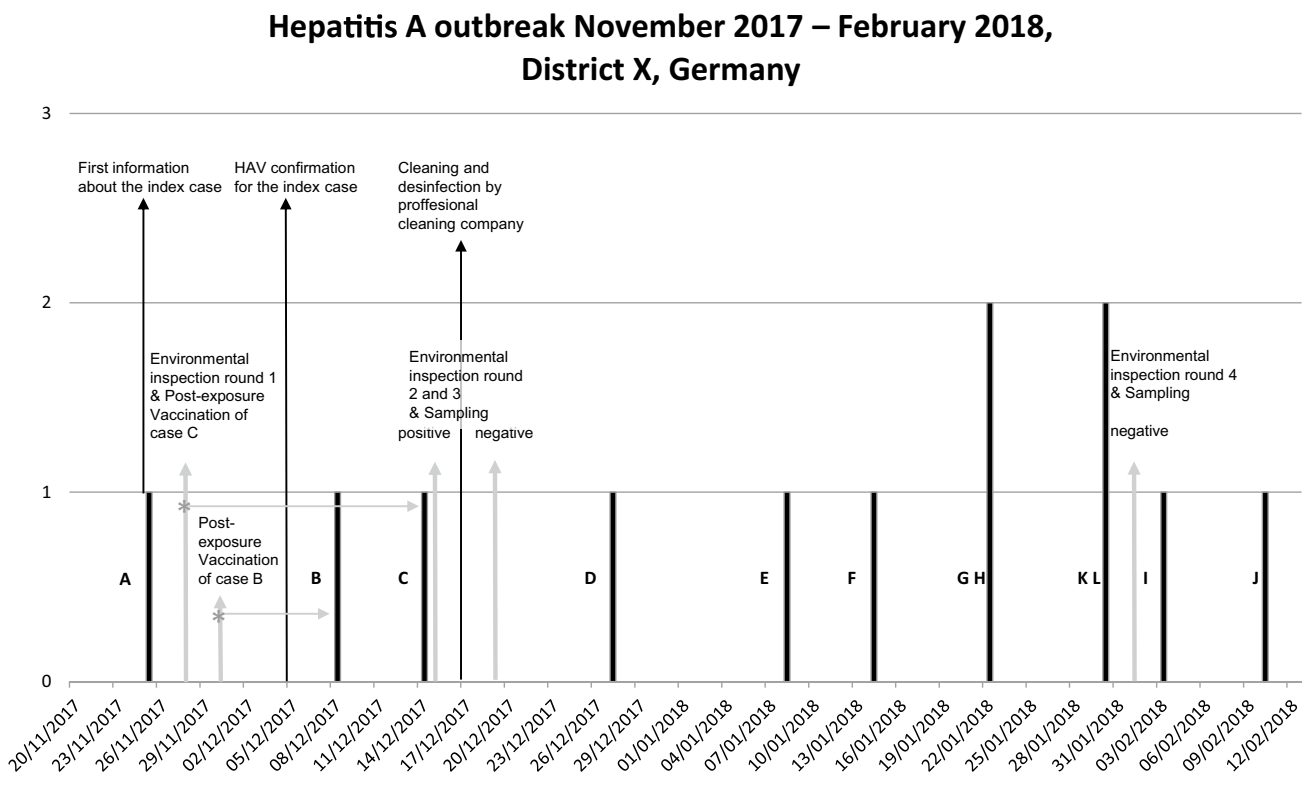

Fig. 1 Timeline of the hepatitis A outbreak November 2017–February 2018 in district X, Germany. Black–dates of notification of the HAV cases; Gray asterisk–dates of post-exposure vaccination of two cases; Gray arrows–dates of the environmental investigations

TCW GTC CAY TTY TCA TCA TT-3′, 614 nt). A 2.5-µL aliquot from the first round of PCR was then used as a template in the second round of PCR with primers HAV 8.2, 5′-GGA TTG GTT TCC ATT CAR ATT GCN AAY TA-3′ and HAV 11, 5′-CTG CCA GTC AGA ACT CCR GCW TCC ATY TC-3′ (518 nt). Nucleotide sequences were determined by using the BigDye terminator cycle sequencing kit (applied biosystems) and separated on a model 3730xl genetic analyser (applied biosystems). Sequences were analyzed using CodonCode Aligner 5.1.5 software (CodonCode Corporation, Centerville, MA, USA). GenBank and local sequence databases were searched for sequences with high similarity using the FASTA and BLAST algorithms.

All environmental samples were tested by RT-qPCR at the Bavarian Health and Food Safety Authority. The real-time assay was based on the amplification of a fragment of the highly conserved 5` non-coding region of the HAV genome (Costafreda et al. 2006).

Representative HAV genotype variants identified in this outbreak were deposited in GenBank with the following accession numbers: LS991407 (case B) and LS991408 (case K).

Results

Epidemiological Investigations

A total of 12 cases (three notified in 2017 and nine notified in 2018) were attributed to this outbreak. Gender representation was equal with six being female and six male. Four children (4–11 years old) and eight adults (46–58 years old) were affected. Nine cases were hospitalized (all adults and one child). The LHA received first information of a suspected HAV case on 25.11.2017, as the index case (A) was hospitalized with the clinical diagnosis of infectious mononucleosis, with serological markers for HAV and Epstein-Barr virus infection. The HAV diagnosis was confirmed by stool examination using RT-qPCR on 5.12.2017. The first explorative routine investigation by the LHA revealed that the patient had no prior travel history to HAV endemic regions or admitted MSM contacts and was working in a butchers shop as a sales assistant. Contact tracing identified five immediate household contacts and five work colleagues. Among the household contacts, two were vaccinated and three received post-exposure prophylaxis

(PEP) vaccination on 29.11.2017. Among the colleagues, three out of five received PEP on 28.11.2017. One colleague could not be vaccinated because of an underlying chronic illness and one refused vaccination. One out of the six persons who received post-exposure vaccination was subsequently identified as a case through stool examination, however without typical clinical symptoms for HAV (case B, the 4-year-old daughter of the index case). One coworker who had received post-exposure prophylaxis developed mild, unspecific symptoms (case C) in the first week of December, but worked until 11.12.2017. Jaundice was evident on 14.12.2018, when the patient was hospitalized. Seven cases (D–J) were notified until 10.02.2018 (Fig. 1). All could be linked epidemiologically to the butchers shop and *via* HAV genotyping. All seven subsequent cases denied any MSM contacts during the incubation period.

In addition to that, two cases (children from one family, case K and L) were notified on 30.01.2018, with one single-nucleotide polymorphism (variant E2VOa) difference to E2VO variant of the RIVM-HAV16-090 strain. No direct epidemiological link could be established to the butchers shop, and no additional cases, despite intensive investigations, were identified in the family, school, or kindergarten that the children attended.

Active contact tracing involved a total of 68 immediate household contacts or work colleagues, of which 17 were vaccinated in the past, 16 received post-exposure vaccination, and 53 provided stool for HAV detection (Table 1). One person who had received the post-exposure vaccination developed symptoms typical for an HAV infection (case C). Three cases were identified as a result of the active HAV contact tracing (case B, case J, and case L).

Environmental Investigations

During the first inspection of the butchers shop performed by the LHA and FIA, no environmental samples were taken, as the index case was not confirmed. Proper hand hygiene measures were reinforced, and cleaning and disinfection of all surfaces was ordered. After the identification of a second case (case C) in the butchers shop, a second environmental investigation and inspection at the butchers shop was performed on 15.12.2017. Although all the prerequisites were according to law, several hygienic improvements could be made: aside from the common customer and staff toilets, no contactless hand-washing facilities were available. Soap and hand disinfectants were present and in conformity with the law, however, they were in bottles, without a dispenser. Eleven environmental samples were collected from high-contact surfaces (*e.g.*, door handles, knife, cash register). Two samples, one from a door handle in the break room and another from the hand-disinfectant bottle in the washroom, were tested positive for HAV-RNA by RT-qPCR. A professional cleaning company was hired to perform detailed cleaning and disinfection, and in further environmental samplings during the 3rd and 4th inspection round, no HAV could be detected. All food samples taken during the 4th inspection round (n = 3) were negative.

Virological Examinations and Genotyping

Due to low HAV-RNA content, no reliable genotyping was performed for the index case. However, nine cases, all having epidemiological links to the butchers shop, were genotyped as the HAV genotype 1A strain, belonging to the MSM-cluster RIVM-HAV16-090. Two environmental samples from a door handle and the hand-disinfectant dispenser were positive for HAV with the same genotype

Table 1 Contact tracing performed by the LHA for each confirmed case of the hepatitis A outbreak November 2017–February 2018, Germany

Case	Interviewed contacts	Stool samples tested	Positive stool samples	Post-exposure vaccination	Vaccinated in the past
A	10	5	2 (case B and C)	6	2
B	0	0	0	0	0
C	22	25	0	0	0
D	4	2	0	0	0
E	4	4	0	2	0
F	3	3	0	2	1
G	8	6	0	3	4
H	0	0	0	0	0
I	12	3	1 (case J)	2	8
J	0	0	0	0	0
K	5	5	1 (case L)	1	2
L	0	0	0	0	0
Total	68	53	4	16	17

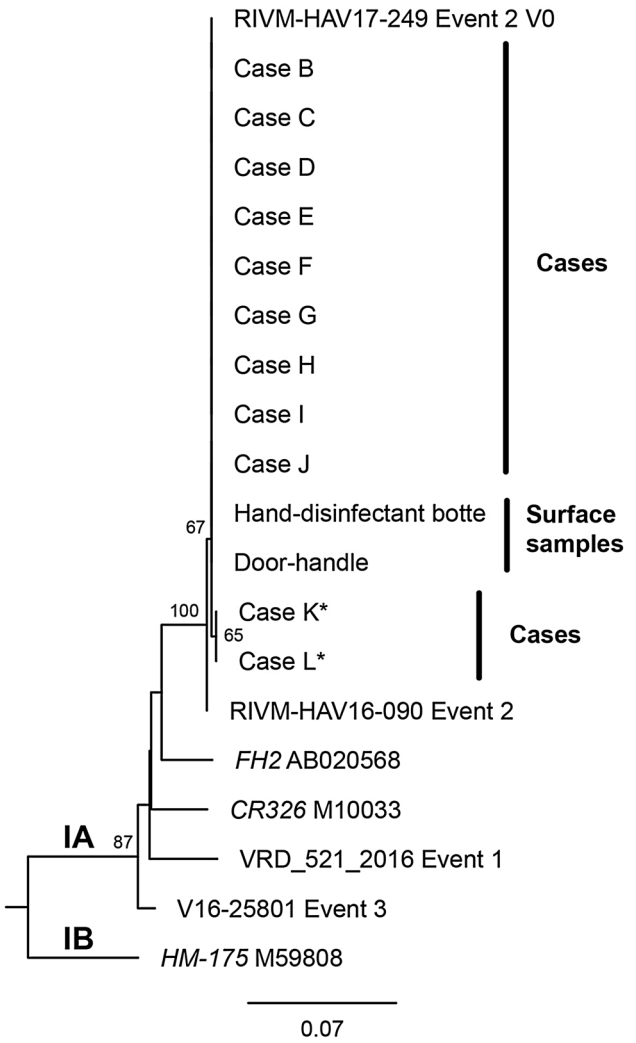

Fig. 2 Rooted maximum likelihood phylogenetic consensus tree for VP1/P2A nucleotide sequences of hepatitis A virus (HAV) isolates, hepatitis A outbreak November 2017–February 2018. All sequences of the outbreak-related strains cluster in HAV subgenotype IA. Sequences from nine cases (B–J) and two surface samples are 100% identical to isolate RIVM-HAV17-249 Event 2 Variant 0 (E2V0). Two cases with the novel HAV variant E2V0a are denoted by asterisks (99.8% similarity to E2V0, one nucleotide difference). Typical reference members of genotype IA and IB are denoted by isolate name (italic) and GenBank ID. Numbers at the nodes indicate bootstrap values of > 50%. The scale bar represents 0.07 substitutions per site. Sequence data from this paper have been deposited with the European Nucleotide Archive under Accession Nos. LS991407 (case B) and LS991408 (case K)

RIVM-HAV16-090 sequence. Additional two cases belonging to one family differed in one SNP over the 460 bp long VP1/P2a region used for genotyping HAV (Fig. 2).

Outbreak Control Measures

Outbreak control measures included detailed contact tracing and stool examinations, thorough cleaning and disinfection of the premises of the butchers shop, oral and written orders for the installment of soap and disinfectant dispensers, and recommendation of contactless taps and hand-washing facilities. PEP was also recommended to all contacts during the investigation. However, during that time, there was a considerate scarcity of the monovalent hepatitis A vaccine in Germany. This might have contributed to the small number of PEP uptake (16/51; 32%) of the unprotected contacts. Furthermore, a considerate obstacle to the implementation of post-exposure vaccination was the unclear cost takeover. Other outbreak control measures included several environmental investigations by the LHA and FIA on 28.11.2017 and all three agencies during three subsequent investigation rounds. All working procedures were observed, with emphasis on hygiene precautions and instructions. Inspection of toilets and washing facilities was performed, and environmental samples were taken. The second HAV case in the butchers shop and the detection of HAV in environmental samples on 15.12.2017 ensued broader investigations of neighboring fruit shop and bakery, as they shared the same washroom and break room with the butchers shop. No further infections among coworkers in the implicated butchers shop, or the additional two shops were detected and notified. The outbreak was considered over 60 days (two mean incubation periods for HAV) after the diagnosis and exclusion from work of the case C in the butchers shop. After the successful disinfection, no HAV was detected in environmental and food samples, therefore, the source was considered removed. No secondary cases with the identical outbreak strain or epidemiological connection to the butchers shop were notified to the LHA (December 2018).

Discussion

We report here on a foodborne hepatitis A outbreak of the RIVM-HAV16-090 genotype variant E2VO with the source of infection being the contamination through a food handler. To the best of our knowledge, this is the first-described foodborne outbreak in the literature to date of the MSM-cluster genotype RIVM-HAV16-090 among the general population. The detection of this genotype among the general population is not surprising, as infectious patients may spread the virus not only through sexual contacts but also through contamination of surfaces or other vehicles such as food. A spillover from the primary-described risk group of MSM was recently reported in the Netherlands in the year 2017 (Friesema et al. 2018). In total, 243 (72%) cases were reported with an MSM-genotype in the Netherlands. Out of those, 158 (65%) had reported MSM contacts, while 35% had no MSM contacts (Friesema et al. 2018). The spillover is also obvious in other European countries. The male-to-female ratio among all laboratory-confirmed HAV cases

decreased from 4.8 in March–May 2017 to 1.4 in August 2018 in Europe (ECDC 2018).

Similar outbreaks involving food handlers as the source of infection have already been described in literature not only with ready-to-eat products as the vehicle in question (Harries et al. 2014; Schmid et al. 2009; Schenkel et al. 2006; Wenzel and Allerberger 2014), but also in a meat production facility (Robert Koch Institut 1998). The index case of this outbreak was working in a butchers shop as a sales assistant, not being involved in the meat production facility. The butchers shop was offering, aside from meat that is heat-treated before consumption, also ready-to-eat meat products, such as ham, sausages, salami, sandwiches, and cold cuts. These ready-to-eat products were handled, prepared, and sold by sales assistants.

A total of 16 close contacts received PEP during this outbreak, two were subsequently found to be HAV positive. This failure rate of 12.5% is higher than described in literature (Victor et al. 2007; Whelan et al. 2013) among PEP-vaccinated close contacts. Several factors might contribute to this. Firstly, due to vaccine shortage, some contacts received the bivalent vaccine for PEP (case B), instead of the monovalent vaccine. While monovalent vaccine has a 95% protective effect already after one application, the bivalent vaccine expected to have this protective effect only after two applications (Robert Koch Institut 2017). Furthermore, as the median incubation period for HAV is 25–30 days, and both contacts were tested positive prior to that, it could be speculated that the PEP vaccination was most probably delivered too late for both cases, and that the infection occurred prior to first control measures in November. It is worth emphasizing that both PEP-vaccinated contact cases that subsequently were found positive for HAV were contacts of the index case, and that delivery of the PEP might have been more timely delivered to contacts of cases identified later during the outbreak. Case B (daughter) was mainly asymptomatic at the time of diagnosis, which is unsurprising, as > 70% children under six years of age develop no symptoms after an HAV infection (Jeong and Lee 2010). Case C (coworker) that received PEP during the first inspection round, and therefore was not required to submit stool for HAV detection, developed symptoms within 2 weeks. It would be interesting to investigate whether and how the PEP has influenced the molecular evolution and selection of quasispecies of these two samples. A recent study compared the HAV capsid region of vaccinated and unvaccinated patients with deep sequencing and found higher diversity in the epitope-coding regions of the vaccinated group, suggesting a positive selection (Sabria et al. 2019). Due to understaffing, the colleague worked until 11.12.2017 in the butchers' shop, although having mild unspecific symptoms. After developing jaundice, the colleague had been hospitalized on 14.12.2017. The highest viral shedding period is 2 weeks prior to the development of typical symptoms and 1 week after (Schmid et al. 2009). The virus is environmentally very stable, retained on human hands up until 7 h and on non-porous surfaces, depending on humidity and room temperature in the range of several days under experimental conditions (Sattar et al. 2000). Environmental investigations revealed the same HAV outbreak strain on a door handle and hand-disinfectant bottle in the washroom in the butchers shop during the inspection on 15.12.2017. This is an important finding, as the detection of HAV on surfaces in the food-handlers' premises implicated in a foodborne outbreak has so far been described only once in literature (Harries et al. 2014). Therefore, it can be assumed that the source of the seven infections among customers most probably was the contamination of food and/or surfaces by one of the HAV symptomatic employees in the butchers shop. No environmental samples were available from the first inspection on 28.11.2017; furthermore, first cleaning and disinfection instructions were ordered to the employees of the butchers shop, and not performed by a professional cleaning company, therefore the timepoint of the contamination cannot be clearly defined.

All previous reports of outbreaks involving food handlers were associated with higher case numbers and epidemiological case–control studies involved in order to clearly resolve the outbreak source (Robert Koch Institut 1998; Harries et al. 2014; Schenkel et al. 2006; Schmid et al. 2009). As this outbreak contained relatively low case numbers (only 12 cases), no case–control study was attempted. The reason for such low case numbers could be attributed to the fact that the suspected HAV diagnosis of the index case was immediately notified. Therefore, the LHA was attentive and ensured detailed prospective epidemiological and microbiological investigations, and performed through contact tracing. An underreporting of asymptomatic cases is possible, although thorough investigations and interviews involved repeated information dissemination among schools and kindergartens of the affected four children, information of household contacts and stool examinations of 78% of close contacts (53/68). The latter led to the identification of three of the here reported 12 cases (25%). An asymptomatic carrier among children might explain the two cases notified on 30.01.2018 infected with the HAV variant E2VOa, bearing one SNP difference in the VP1 region used for genotyping and that did not have any epidemiological link to the implicated butchers shop. As already discussed, children develop generally less severe hepatitis A symptoms in comparison to adults; therefore, an unnoticed asymptomatic carrier cannot be excluded. Furthermore, the variant E2VOa carries a new mutation (Fig. 2), not observed so far among the circulating RIVM-HAV16-090 strains in Germany, highlighting the genetic variability of HAV and the ensuing difficulties in resolving HAV outbreaks.

Post-exposure vaccination was recommended to all contacts during the outbreak investigation. However, only 32% of unprotected contacts (16/51) received vaccinations. Two major obstacles were identified that might have influenced this low vaccination uptake: during that time, there was a considerate scarcity of the monovalent hepatitis A vaccine in Germany; furthermore, the cost takeover of the available post-exposure vaccines was unclear. Hepatitis A vaccine is not part of the routine immunization schedule in Germany; therefore, the costs are not routinely covered by insurance companies. Although there is a recommendation for post-exposure vaccination with hepatitis A monovalent vaccine, the LHA had to substantiate and elaborate the need for it in detail, on a case-by-case basis and in the light of the scarcity of the monovalent hepatitis A vaccine in order to receive reimbursement for the vaccination by insurance companies.

Although washing opportunities, soap, and disinfectant were present and in conformity with the required law, it might be beneficial to consider adjustment and inclusion of contactless faucets, soap and disinfectant dispensers in premises selling ready-to-eat food.

Careful and proper personal hygiene measures need to be facilitated and awareness of hepatitis A as a considerable foodborne pathogen increased, especially among food handlers serving ready-to-eat food. Cooperation and complementation between local health, veterinary health, and food inspection authorities is a key factor in foodborne outbreaks investigations. Another discussion point might be the recommendation of the hepatitis A vaccination for occupations in the food sector. A similar foodborne outbreak in Austria (Schmid et al. 2009) resulted in the uptake of HAV vaccination as an occupational-indicated vaccination in the Austrian Vaccination schedule, and has to be financially supported by the employer (Bundesministerium für Arbeit 2018). Whether occupational-indicated vaccination of HAV in the food sector is cost-effective, remains to be elucidated.

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