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Habilitationsschrift

Epidemiologische Untersuchungen von Infektionskrankheiten, die durch Lebensmittel übertragen werden

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Abkürzungen

95%-KI	95%-Konfidenzintervall
BfR	Bundesinstitut für Risikobewertung
BMG	Bundesministerium für Gesundheit
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
CDI	*Clostridium difficile*-Infektion
CDC	Centers for Disease Control and Prevention, Atlanta, USA
DALY	Disability-adjusted life years
DEGS	Studie zur Gesundheit Erwachsener in Deutschland
ECDC	European Centre for Disease Prevention and Control
EHEC	Enterohämorrhagische *Escherichia coli*
ESBL	Extended-Spectrum-Beta-Laktamase
EU	Europäische Union
GEDA	Studie „Gesundheit in Deutschland aktuell“
HUS	Hämolytisch-urämisches Syndrom
IfSG	Infektionsschutzgesetz
IGeL	Individuelle Gesundheitsleistung
KiGGS	Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland
MDS	Medizinischer Dienst des Spitzenverbandes Bund der Krankenkassen
ÖGD	Öffentlicher Gesundheitsdienst
OR	Odds ratio
RNA	ribonucleic acid, Ribonukleinsäure
RKI	Robert Koch-Institut
RR	Relatives Risiko
RT-PCR	Reverse Transkriptase-Polymerase-Kettenreaktion
STEC	Shigatoxin produzierende *Escherichia coli*
T. gondii	*Toxoplasma gondii*
USA	Vereinigte Staaten von Amerika
WGS	Whole Genome Sequencing
WHO	Weltgesundheitsorganisation

1. Einleitung

1.1. Vorkommen von lebensmittelübertragene Erkrankungen

Eine Vielzahl von Infektionskrankheiten werden durch Lebensmittel übertragen. Die Ursachen für lebensmittelbedingte Erkrankungen können Viren, Bakterien, Parasiten, Toxine oder Prionen sein. Die klinischen Manifestationen reichen von leichtem Durchfall über schwereren gastroenteritischen Symptomen oder Hepatitiszeichen bis zu lebensbedrohlichen septikämischen, neurologischen oder renalen Symptomen.

Um das Vorkommen von lebensmittelübertragenen Erkrankungen in der Bevölkerung zu kontrollieren, werden sie innerhalb des öffentlichen Gesundheitsschutzes epidemiologisch überwacht. Die Überwachung wird durch mehrere Faktoren erschwert. Ein bedeutender Faktor ist Untererfassung (1, 2). Lebensmittelbedingte Erkrankungen können zwar schwer oder sogar tödlich sein, mildere Fälle werden jedoch häufig nicht durch Routineüberwachung erkannt. Zweitens werden viele Krankheitserreger, die durch die Nahrung übertragen werden, auch von Mensch zu Mensch übertragen. Dies kann die Rolle der Übertragung durch Lebensmittel unkenntlich machen. Schließlich wird ein Teil der lebensmittelbedingten Erkrankungen durch Krankheitserreger oder Wirkstoffe verursacht, die noch nicht identifiziert wurden und daher nicht diagnostiziert werden können (3).

Auf der Seite des Infektionsschutzes, also der Überwachung von Krankheiten beim Menschen (Surveillance), werden traditionell Daten genutzt, die im Rahmen der klinisch-mikrobiologischen Diagnostik generiert worden sind und durch gesetzlich geregelte Meldevorschriften vor allem für Labore, aber auch für behandelnde Ärzte, gesammelt werden. In Deutschland wird dafür das Infektionsschutzgesetz (IfSG) und die vom Robert Koch-Institut (RKI) entwickelte Software SurvNet genutzt (4). Diese Surveillance basiert somit auf routinemäßigen Berichten oder Meldungen über eine bestimmte Krankheit an Einrichtungen des Gesundheitswesens. Es dient für die Krankheitsüberwachung. Es ist relativ kostengünstig und nützlich für die Erkennung von Ausbrüchen, Trendanalysen und der Abschätzung von Langzeiteffekten von Präventionsmaßnahmen. Aber dieses System erfasst nur einen Teil der in Wirklichkeit vorhandenen Infektionsfälle und unterliegt in einigen Situationen geringer Sensitivität und Spezifität (5). Zusätzlich zu diesem Meldesystem können im Rahmen einer Surveillance regelmäßige, aktive Abfragen bei Kliniken oder Laboren durchgeführt werden. Dies geschieht durch das RKI in Deutschland z. B. bei pädiatrisch-nephrologischen Abteilungen bezüglich des Vorkommens von HUS (6).

Das Robert Koch-Institut (RKI) erhält im Rahmen des IfSG erregerbasiert Informationen zu Erkrankungsfällen übermittelt. Folgende Erkrankungen werden durch den Konsum von kontaminierten Lebensmitteln verursacht und daher von den Gesundheitsbehörden in Deutschland überwacht: Botulismus, Brucellose, Campylobacter-Enteritis, Cholera EHEC-Enteritis, Giardiasis, Hämolytisch-urämisches Syndrom, Hepatitis A, Hepatitis E, Kryptosporidiose, Listeriose,

Norovirus-Gastroenteritis, Paratyphus, Salmonellose, Shigellose, Trichinellose, Tularämie, Typhus abdominalis, und Yersiniose. Insgesamt sind 35% der überwachten Erreger potentiell lebensmittelübertragen.

Im Meldesystem für Infektionskrankheiten in Deutschland werden auch Daten zu Ausbrüchen erfasst. Dabei wird unterschieden zwischen Ausbrüchen, bei denen aufgrund des Erregers eine lebensmittelbedingte Übertragung angenommen werden kann (potenziell lebensmittelbedingte Ausbrüche) und Ausbrüchen, bei denen in der Ausbruchsdokumentation Lebensmittel explizit genannt werden (7). Letztere werden als explizit lebensmittelbedingte Ausbrüche bezeichnet (7). Lebensmittelbedingte Ausbrüche von Infektionskrankheiten hatten in Deutschland in den letzten Jahren erhebliche Folgen für die Gesundheit der Bevölkerung und die Lebensmittel-Wirtschaft. Die Ausbrüche mit der höchsten öffentlichen Aufmerksamkeit waren 2011 ein Ausbruch von Erkrankungen durch enterohämorrhagische *Escherichia coli* (EHEC) und hämolytisch-urämischem Syndrom (HUS) (8). In 2012 besonders prominent ein Ausbruch von Gastroenteritis an einer Vielzahl von Schulen und Kindertagesstätten in Ostdeutschland (9).

Externe Faktoren weisen darauf hin, dass lebensmittelübertragene Erkrankungen in Häufigkeit und vor allem Diversität zunehmen könnten. Zum einen nimmt die Komplexität der Lebensmittelwarenströme durch die Globalisierung des Handels mit Lebensmitteln und Vorstufen zu und damit auch das Risiko Infektionserreger zu verbreiten, wie auch die Abhängigkeit von anderen Standards der Lebensmittelproduktion. Ein weiterer Faktor ist der grenzüberschreitende Handel mit lebenden Tieren. Darüber hinaus ist das Lebensmittelangebot im Lebensmitteleinzelhandel zunehmend diverser und laufenden Veränderungen unterworfen.

In Deutschland wird ein zunehmender ökonomischer Druck auf landwirtschaftliche Familienbetriebe und mittelständische Hersteller von Lebensmitteln beobachtet (10). Besonders deutlich wurde dies während der Milchkrise 2017 (11). Kostendruck beeinflusst auch die Qualität der Nahrungsmittelproduktion und damit das Erkrankungsrisiko.

Viele Erreger wurden erst in den letzten Jahrzenten als lebensmittelübertragen erkannt und durch die Verfügbarkeit neuer Überwachungsdaten (z.B. mikrobieller genomischer Daten) werden in den nächsten Jahren weitere erkannt werden. Die Diversität und kontinuierliche Anpassung bekannter und neu aufkommender Infektionserreger trägt zur Problemlage bei, was der bedeutende Ausbruch durch den neuartigen EHEC O104 im Jahr 2011 gezeigt hat.

Durch die oben genannten Einflussfaktoren sind regelmäßige, auf valide Daten bestehende Schätzungen zum Vorkommen dieser Erkrankungen erforderlich, um Präventionsanstrengungen zu steuern und zu bewerten.

1.2. Epidemiologie der Gastroenteritis

Die häufigste durch den Konsum von kontaminierten Lebensmitteln verursachte Erkrankung ist Gastroenteritis. Es handelt sich um eine entzündliche Erkrankung des Magen-Darm-Traktes, die in der Regel mit Erbrechen und Durchfall einhergeht. Durchfall (Diarrhoe) ist ein Symptomenkomplex, der durch Stühle mit verminderter Konsistenz und erhöhter Anzahl (drei oder mehr während eines 24-Stunden-Zeitraums) gekennzeichnet ist (12). Problematisch an Durchfallerkrankungen sind der Flüssigkeits- und Elektrolytverlust in Folge des Durchfalls. Obwohl einige nicht-infektiöse Erkrankungen Durchfälle verursachen, werden die allermeisten Durchfall-Episoden von Viren, Bakterien und Parasiten verursacht, wobei bei Erwachsenen ambulant erworbene Norovirus-Enteritis und Campylobacter-Enteritis den größten Anteil ausmachen (13, 14). Bei Kindern ist dies der Rotavirus (15). Die meisten Gastroenteritis-Erkrankten erholen sich innerhalb einer Woche. Bei einem kleineren Teil hält dies zwei Wochen oder länger an. Die Weltgesundheitsorganisation (WHO) definiert persistent anhaltenden Durchfall als eine Episode, die mindestens 14 Tage andauert. Blutige Durchfälle zeigen gastrointestinale Blutungen in Folge von invasiven Infektionen an und reichen von Blutspuren in lockerem Stuhl bis zu massiven Blutungen als Folge von großflächigen Schleimhautläsionen. Blutige Durchfälle sind typisch für EHEC-Enteritis bei Erwachsenen.

Im stationären Bereich sind bezüglich der Häufigkeit die viralen Erreger von Bedeutung. Betrachtet man die Zahl und Schwere der Infektionen ist die *Clostridium difficile*-Infektion (CDI) von hoher medizinischer Relevanz. Diese ist weltweit bei hospitalisierten Patienten im Ansteigen begriffen, aber auch vermehrt bei ambulanten Patienten (16).

Global gesehen stellen akute Gastroenteritiden vor allem ein Problem in Entwicklungsländern dar, da schlechte Wasserhygiene und mangelhafte Sanitäreinrichtungen das Vorkommen von Durchfallerkrankungen stark fördern. Die Sterblichkeit ist besonders hoch bei Kindern unter 5 Jahren (15, 17, 18). Sie ist durch gezielte Bekämpfungsmaßnahmen aber in den meisten Ländern weltweit im Rückgang begriffen. In entwickelten Ländern ist die Sterblichkeit gering. In den letzten Jahren haben gastrointestinale Infektionen aufgrund nosokomialer Ausbrüche und auch aufgrund prominenter lebensmittelbedingter Ausbrüche eine erhöhte medizinischer Bedeutung und öffentliche Aufmerksamkeit erhalten (8, 9, 19).

Die Daten zur Beschreibung der Epidemiologie von Durchfallerkrankungen stammen aus unterschiedlichen Studien-Aufbau. Es sind (i) Risikofaktorstudien als Fall-Kontroll-Studien oder als Kohorten-Studien, letztere in Haushalten und Gemeinschaftseinrichtungen, (ii) Auswertung von Surveillance-Daten, (iii) Ausbruchsuntersuchungen und (iv) Querschnittsstudien. Querschnittserhebungen wie sie auch in dieser Arbeit durchgeführt wurden (Kap. 2.1.1.), können Informationen zur Häufigkeit und Schwere liefern. Sie werden auch durchgeführt, um

Informationen zur medizinischen Versorgung zu erhalten. Schwierigkeiten bestehen hier bezüglich der Saisonalität von bestimmten Durchfallerregern (z.B. Norovirus, Campylobacter) und dem korrekten Erinnerungsvermögen der Befragten (12, 20). In den letzten Jahren wurden bevölkerungsrepräsentative Querschnittserhebungen zum Vorkommen von Gastroenteritis in vielen Ländern durchgeführt, um international Vergleiche anstellen zu können.

Noroviren sind die häufigste Ursache für Gastroenteritis-Ausbrüche. Sie sind auch eine der Hauptursachen für lebensmittelbedingte Krankheiten (21-23). Ein großer lebensmittelbedingter Norovirusausbruch in 2012 assoziiert mit dem Verzehr von Tiefkühlerdbeeren ist der Hintergrund für die Arbeit in Kap. 2.2.3. in dieser Arbeit.

Laut Schätzungen des Centers for Disease Control and Prevention (CDC) sind Noroviren für 50% der sporadischen Gastroenteritis-Erkrankungen, aber auch mindestens 50% aller Gastroenteritis-Ausbrüche verantwortlich. Noroviren wurden 1968 in Norwalk, Ohio, mittels Elektronenmikroskopie als Ursache für Gastroenteritis identifiziert. Noroviren sind kleine, nicht umhüllte, einzelsträngige RNA-Viren, die in die Gattung Caliciviridae eingeordnet werden. Diese Gattung enthält mindestens 130 verschiedene Genotypen, die in 5 Genogruppen gruppiert sind. Sie wachsen schlecht in der Kultur und bis zur Entwicklung der RT-PCR war ihre Detektion schwierig. Humane Norovirus-Infektionen sind meistens durch Genogruppen 1, 2 und 4 verursacht und werden meistens Mensch-zu-Mensch übertragen. Es gibt saisonale Muster mit erhöhter Aktivität während der Wintermonate (24). Noroviren sind extrem ansteckend für alle Altersgruppen. Stuhlproben von einem infizierten Individuum können mehrere Milliarden virale Partikel pro Gramm enthalten, und die infektiöse Dosis beträgt ungefähr 18 virale Partikel. Die Krankheit beginnt typischerweise nach einer Inkubationszeit von 12-48 Stunden. Die Symptome beginnen akut und umfassen Durchfall, Erbrechen, Übelkeit und Bauchkrämpfe. Es wird nur eine kurzzeitige (6-24 Monate) Immunität ausgebildet. Es gibt Hinweise darauf, dass einige Menschen eine natürliche Immunität (basierend auf einer Gen-Variante) besitzen. Sie machen ungefähr 21% der Bevölkerung aus, die damit resistent gegen Norovirus-Infektionen sind. Diese Tatsache erschwert Ermittlungen in Ausbrüchen, da dieser Anteil kein Erkrankungsrisiko trägt (25).

Um die globale und regionale Belastung durch lebensmittelbedingte Krankheiten zu messen, hat die Weltgesundheitsorganisation (WHO) Schätzungen der Inzidenz, Mortalität und Krankheitslast durch 31 lebensmittelbedingte Gefahren in Auftrag gegeben (26-29). Die globale Belastung durch lebensmittelbedingte Krankheiten, die durch Erreger im Jahr 2010 verursacht wurde, betrug 33 Millionen disability adjusted lifeyears (DALYs). Kinder unter fünf Jahren tragen geschätzt 40% der Belastung. Die häufigsten Ursachen für lebensmittelbedingte Erkrankungen waren Durchfallerkrankungen, insbesondere verursacht durch Noroviren und *Campylobacter*. Durchfallerkrankungen waren auch verantwortlich für die Mehrheit der Todesfälle, insbesondere verursacht durch *Salmonella* (nicht-typhoidal). Andere

Hauptursachen für Todesfälle waren *Salmonella Typhi*, *Taenia solium* und Hepatitis A-Virus.

Es gibt Schätzung darüber welche Lebensmittelkategorien am häufigsten für Krankheiten verantwortlich sind und welche spezifischen Krankheitserreger diese verursachen (Pathogen-Lebensmittel-Kombinationen) (30). Zur Berechnung solcher Zuordnungen von Infektionsquellen (food source attribution) werden Informationen unterschiedlicher Herkunft benötigt, darunter Daten zu Ausbruchsuntersuchungen, sporadischen Infektionen, Querschnittsstudien und Erregermonitoring bei Tieren und Lebensmitteln. Die häufigste Kombination in Nordamerika ist *Campylobacter* in Geflügel gefolgt von *Toxoplasma* im Schweinefleisch und *Listeria* in Delikatesswaren und Aufschnitt.

1.3. *Toxoplasma* und Toxoplasmose

Die Toxoplasmose ist die in Deutschland am häufigsten vorkommende parasitäre lebensmittelübertragene Erkrankung beim Menschen. Im Vergleich zu den bakteriellen Erregern muss man aber davon ausgehen, dass das medizinische Problem unterschätzt wird. Dies ist der Hintergrund für die Arbeit in Kap. 2.1.2. *Toxoplasma* (*T.*) *gondii* ist ein obligat intrazelluläres Protozoon, das unterschiedliche Formen annehmen kann: Oozyst, Tachyzoit und Zyste. Oozysten werden von Katzen ausgeschieden und stellen einen Infektionsweg für den Menschen dar. Eine zweiter Infektionsweg geht über die Aufnahme von Zysten, die in Nahrungsmitteln enthalten sind, die von *Toxoplasma*-infizierten Tieren (v.a.: Fleisch vom Schwein und Schaf) stammen und roh oder ohne genügende Erhitzung verzehrt werden. Die Toxoplasmose ist somit eine durch Lebensmittel oder Katzenkontakt übertragene parasitäre Zoonose.

Eine Infektion mit *T. gondii* bleibt in den meisten Fällen klinisch unbemerkt. Sie ist eine für die meisten Menschen lebenslang persistierende Infektion, die bei gegebenen Umständen reaktivieren kann und zu Erkrankungen führt. *T. gondii* führt zu Krankheitsmanifestationen, die je nach Immunstatus des Patienten und der klinischen Situation variieren (i) Kongenitale Toxoplasmose, (ii) Okuläre Toxoplasmose (iii) Toxoplasmose bei immunsupprimierten Patienten (mit und ohne AIDS) und (iv) Toxoplasmose beim immunkompetenten Erwachsenen und Jugendlichen (31).

Die kongenitale Toxoplasmose wird durch die primäre Infektion während der Schwangerschaft ausgelöst. Sie führt bei ungefähr 25% der Infektionen zur materno-foetalen Übertragung und davon bei 25% zur Erkrankung des Ungeborenen, die sich bei Geburt klinisch manifest äußert (Dunn et al., 1999, Li et al., 2014). Die Symptome der kongenitalen Toxoplasmose bei Neonaten sind sehr unterschiedlich und umfassen Hydrozephalus, Mikrozephalie, intrakraniale Verkalkungen, Chorioretinitis,

Strabismus, Blindheit, Epilepsie, psychomotorische oder mentale Retardierung, Petechien aufgrund von Thrombozytopenie und Anämie. In Deutschland besteht eine Meldepflicht für kongenitale Erkrankungen (7). Aufgrund der bei Aborten und foetalen Infektionen selten durchgeführten Labordiagnostik und Schwierigkeiten bei der technischen Umsetzung der Meldepflicht muss man davon ausgehen, dass die Zahlen zur Inzidenz sehr stark untererfasst sind.

Toxoplasmose bei immunkompetenten Patienten ist eine selbstlimitierende und unspezifische Krankheit, die in den meisten Fällen mit einer mehrere Wochen andauernden zervikalen Lymphadenopathie einhergeht, die selten einer Behandlung bedarf. Im Gegensatz zu dem günstigen Verlauf der Infektion bei fast allen immunkompetenten Individuen kann die Krankheit bei immungeschwächten Personen lebensbedrohlich sein. Diese Erkrankungsfälle sind häufig keine Primärinfektionen, sondern Reaktivierungen der persistenten Infektion, die in dem schweren Krankheitsbild einer *Toxoplasma*-Enzephalitis assoziiert ist (31). Sie nimmt durch die Zunahme von Immunsupprimierten in der Bevölkerung stark zu und ist mit einer hohen Letalität assoziiert (32). Trotz der anzunehmenden Bedeutung ist über das Vorkommen und assoziierten Faktoren wenig bekannt. Ein Screening von Frauen zu Beginn der Schwangerschaft auf *T. gondii* Antikörper, wie es in einigen Mitgliedstaaten der EU durchgeführt wird, ist in Deutschland keine Krankenkassenleistung. Es wurde bis 2017 vom Medizinischen Dienst des Spitzenverbandes Bund der Krankenkassen e.V. (MDS) innerhalb der Bewertung als individuelle Gesundheitsleistung ausdrücklich von einer Testung abgeraten (33). Dies obwohl in Studien aus Deutschland beschrieben wurde, dass bei frühzeitiger Erkennung einer akuten Infektion der Schwangeren und rechtzeitiger Gabe von Spiramycin gegen *T. gondii* Häufigkeit und individuelle Schwere der Erkrankung beim Neugeborenen gesenkt werden konnte (34).

Die okuläre Toxoplasmose ist eine Chorioretinitis, die kongenital oder postnatal erworben sein kann. Sie ist Folge einer akuten Infektion oder einer Reaktivierung. Sie kann sporadisch oder im Zusammenhang mit einem Ausbruch auftreten. Letztere wird verursacht durch besondere *Toxoplasma*-Genotypen, die vor allem in Südamerika auftreten. Typische Befunde der *Toxoplasma*-Chorioretinitis sind fokal nekrotisierende Entzündungen von Netz- und Aderhaut, die sich in weißen fokalen Läsionen manifestieren. Die okuläre Toxoplasmose bei Erwachsenen wurde bis vor 20 Jahren als eine Reaktivierung einer kongenitalen Infektion angesehen. Sie wird jedoch mit zunehmender Häufigkeit auch in Verbindung mit einer akuten primären Infektion bei Erwachsenen gesehen.

Die Diagnose wird durch indirekten Antikörpernachweis oder direkten Nachweis mittels PCR gestellt (35). Epidemiologische Daten zum Vorkommen der okulären Toxoplasmose und Toxoplasmose bei immunsupprimierten Patienten in der Bevölkerung in Deutschland werden nicht über das Meldesystem erfasst. Daher ist über das Vorkommen in Deutschland nahezu nichts bekannt. Burden of Disease-

Modellierungen zeigen auf, dass die Toxoplasmose die lebensmittelübertragene Zoonose mit der höchsten Krankheitslast für die Bevölkerung in Europa sein könnte, ohne dass genaue Zahlen zu ihrem Vorkommen vorliegen (36). In vielen Public Health-Programmen ist die Toxoplasmose nicht integriert. In der Tiermedizin sollte das Bewusstsein für die Bedeutung von *T. gondii*-freie Schweine- und andere Tierbestände erhöht werden. Es ist auch nicht Teil des Monitorings von Lebensmitteln auf zoonotische Erreger in Deutschland und der EU (37). Veterinärbehörden brauchen eine verbesserte empirische Basis, um die Standards bei der Tierhygiene und Fleischhygiene hochzuhalten.

1.4. Die Untersuchung von lebensmittelübertragenen Ausbrüchen

Untersuchungen zu Ausbrüchen von Infektionskrankheiten werden als Teil des Infektionsschutzes in Deutschland durchgeführt. Die im Rahmen der Untersuchung gewonnenen Erkenntnisse und Ergebnisse ermöglicht es Epidemiologinnen/ Epidemiologen Risikofaktoren zu identifizieren und präventive Maßnahmen festzulegen, die die Ausbreitung von Krankheiten begrenzen und kontrollieren. Darüber hinaus verbessern Untersuchungen von Ausbrüchen die Evidenzlage zu wichtigen epidemiologischen Basiskennzahlen, Risikofaktoren, Übertragungsmustern und die Wirksamkeit von Kontrollmaßnahmen.

Ausbrüche können aus einer Vielzahl von Gründen auftreten. Sie lassen sich aufgrund ihres primären Übertragungsmechanismus in lebensmittel-trinkwasserübertragene, respiratorische, sexuell-übertragene, nosokomiale und vektorübertragene Ausbrüche unterteilen (38). Bei einigen Ausbrüchen ist der Übertragungsmechanismus auch durch direkten Mensch-zu-Mensch oder Tier-zu-Mensch-Kontakt möglich. Ausbrüche von Erkrankungen werden mit dem Ziel untersucht, weitere Erkrankungen in einer Population zu kontrollieren oder zu verhindern. In der Menschheitsgeschichte entwickelten sich einige Ausbrüche zu gesundheitlichen Krisen oder gar Pandemien (39).

Über die tatsächlichen Verfahren bei Ausbruchsuntersuchungen wurden einige wenige Zusammenfassungen geschrieben (40, 41). In speziellen Trainingsprogrammen werden für Epidemiologinnen/Epidemiologen die Verfahren zur Durchführung von Ausbruchsuntersuchungen gelehrt und von erfahrenen Personen angeleitet (42, 43).

Ausbrüche werden definiert als das Vorkommen von mehr Erkrankungsfällen der gleichen Krankheit innerhalb eines Zeitraumes, eines Ortes oder einer Bevölkerungsgruppe als normalerweise zu erwarten wären. Darüber hinaus werden Ausbrüche im Infektionsschutzgesetz als zwei oder mehr gleichartige Erkrankungen, bei denen ein epidemiologischer Zusammenhang wahrscheinlich ist, definiert (44).

Für die Durchführung einer Ausbruchuntersuchung haben sich international und auch in Deutschland allgemeine wissenschaftliche Ansätze etabliert (45). Das ECDC (46), das CDC (47) und das RKI (48) stellen für die Untersuchung von lebensmittelbedingten Ausbrüchen Übersichten für grundlegende Definitionen und strategische Vorgehensweise bereit, die in ihrem Vorgehen sich sehr ähnlich sind und auf die im Folgenden eingegangen wird.

Idealerweise findet die Bearbeitung innerhalb von interdisziplinären Teams statt, die Expertise aus den Bereichen der klinischen Medizin, Mikrobiologie, Epidemiologie, Lebensmittelsicherheit und ggf. auch der Gesundheitskommunikation Veterinärmedizin, und Umweltmedizin einbringen.

In allen Übersichten wird empfohlen frühzeitig Falldefinitionen zu formulieren und die vorhandenen Daten werden deskriptiv nach Ort, Zeit und betroffenen Personengruppen ausgewertet. Die Interpretation dieser Analysen ist häufig der erste Schritt bei der Generierung von Hypothesen zum Lebensmittelvehikel und dem Übertragungsmechanismus. In den allermeisten Ausbrüchen müssen aber darüber hinaus gezielt epidemiologische Daten zur Lebensmittel-Exposition erhoben werden. Eine erste nicht-hypothesengesteuerte Befragung (Synonym: explorative Befragung oder Trawling Interview) von Erkrankten bezüglich ihres Lebensmittelkonsums und Einkaufsverhalten ist klassische Vorgehensweise. Von großer Bedeutung ist die richtige Zuordnung des Expositionsstatus des Patienten bezüglich des Lebensmittelverzehrs, was die Rationale für die Studie in Kapitel 2.2.4. darstellt. Die Testung dieser Hypothesen findet durch analytische Studien statt. Dies sind bei Gruppenereignissen retrospektive Kohortenstudien, eine Besonderheit der Infektionsepidemiologie, und bei bevölkerungsverteilten Ereignissen Fall-Kontroll-Studien. Durch gezielte Testungen von Lebensmitteln und dem Auffinden des spezifischen Erregers mit molekularen Methoden kann auch ein hoher Grad an Sicherheit gewonnen werden. Kontrollmaßnahmen für die Bevölkerung müssen bei Vorliegen von ausreichend Evidenz durchgeführt werden. Eine offene Kommunikation innerhalb der interdisziplinären Teams und auf unterschiedlichen Ebenen der föderalen Strukturen des Infektionsschutzes und der Lebensmittelsicherheit ist wichtig, damit alle relevanten Informationen einfließen können, jede Gruppe ihre Expertise einbringen kann und nachfolgend die richtigen und ausgewogenen Entscheidungen getroffen werden können. Dies schließt ab einem gewissen Grad auch die wissenschaftliche Gemeinschaft und die Bevölkerung mit ein.

In den letzten Jahren hat die Bestimmung von Erregertypen eine hohe Bedeutung erlangt, dies unter Nutzung der enorm gesteigerten DNA-Sequenziermöglichkeiten im Sinne einer Ganzgenomsequenzierung (Whole Genome Sequencing, WGS). Diese kann verwendet werden, um Ausbrüche zu detektieren und Erkrankungsfälle in Ausbruchsuntersuchungen einzubeziehen oder auszuschließen. Falls dies bei Lebensmittelisolaten angewendet wird, können verdächtige Ausbruchsvehikel und

Quellen detektiert und bestätigt werden. Darüber hinaus kann es mit geeigneten bioinformatischen Analysen auch verwendet werden, um die Existenz von nicht diagnostizierten Erkrankungsfällen und Zwischenstufen in Transmissionsketten vorherzusagen und die Direktionalität der Übertragung abzuleiten. In Zukunft müssen die Bestimmung von Erregertypen zusammen mit Patienteninformationen durch eine Integration in das Meldesystem für den Infektionsschutz (integrierte molekulare Surveillance)in Deutschland routinemäßig für alle Ebenen des Infektionsschutz nutzbar gemacht werden.

Die grundlegenden strategischen Vorgehensweisen und Methoden bei der Untersuchung von Ausbrüchen durch Lebensmittel können international weitestgehend als Konsensus angesehen werden. Unabhängig von der Ätiologie der Krankheit sind die Techniken zur Untersuchung und Bekämpfung von Ausbrüchen im Allgemeinen ähnlich und auch für nosokomiale und respiratorische Ausbrüche, wie auch für Ausbrüche aufgrund nichtinfektiöser Ursachen anwendbar.

1.5. Hintergrund zu EHEC-Erkrankungen und dem HUS/EHEC O104-Ausbruch in 2011

In den frühen 1980er-Jahren wurde *E. coli* vom Typ O157:H7 als eine wichtige Ursache für hämorrhagische Kolitis identifiziert. Dieser Typ und weitere Kolitis-verursachende *E. coli*-Typen wurden enterohämorrhagische *E. coli* (EHEC) genannt. Sie produzieren Exotoxine, die dem Shigatoxin von *Shigella dysenteriae* Typ 1 ähneln (Shigatoxin produzierende *E. coli*, STEC). Sie verursachen blutigen Durchfall, der bei 5-15% der Kinder durch hämolytisches und urämisches Syndrom (HUS) kompliziert wird. HUS wird durch die Trias von mechanischer hämolytischer Anämie, Thrombozytopenie und akuter Nierenschädigung definiert (49). STEC-bedingtes HUS ist die Hauptursache für akutes Nierenversagen bei kleinen Kindern. Die antibiotische Behandlung von Patienten mit EHEC ist kontraindiziert, da das Abtöten der Organismen eine erhöhte Freisetzung des Shigatoxins verursachen kann. Stattdessen umfasst die Behandlung von Schwerkranken Hämodialyse, Plasmaaustausch und andere Strategien zur Entfernung des zirkulierenden Toxins. Diese Erreger haben sich zu einem wichtigen Problem für die öffentliche Gesundheit in den USA und Europa entwickelt, scheinen jedoch in Entwicklungsländern nur sehr selten anzutreffen zu sein.

Aufgrund des Ausbruchspotentials und der schweren Krankheitszustände im Zusammenhang mit EHEC HUS stellt die STEC-Infektion ein wichtiges Problem für die öffentliche Gesundheit dar. Es gab große Ausbrüche von EHEC-Infektionen mit einer großen Public Health-Bedeutung (50-54). Wiederkäuer, insbesondere Rinder, sind die Hauptreservoire für den hoch virulenten Stamm EHEC O157 sowie andere HUS-assoziierte Nicht-O157-Stämme. Die Übertragung auf den Menschen wird am häufigsten mit dem Verzehr von roh verzehrtem Rindfleisch oder nicht pasteurisierter

Milch in Verbindung gebracht. Weitere Infektionsrouten sind Kontakt mit einem Tier oder einer durch EHEC kontaminierten Umwelt (wie Badewasser oder Boden in der Nähe von Wiederkäuern) oder -aufgrund der geringen infektiösen Dosis- von EHEC die Übertragung von Person zu Person vor allem in Kindertagesstätten und anderen Arten von Institutionen.

In Deutschland ereignete sich 2011 der weltweit größte bekannte Ausbruch von HUS verursacht durch EHEC des Serotyps O104:H4. Der HUS/EHEC O104-Ausbruch wurde am 19. Mai 2011 von Nephrologen in Hamburg detektiert und dem RKI kenntlich gemacht und ging bis Juli 2011 (55). Die ersten hypothesengenerierenden Befragungen und Fall-Kontroll-Studien in Hamburg nahmen Fleisch- und Milchprodukte, wie sie eigentlich bei einem EHEC-Ausbruch zu erwarten wären, aus dem Fokus. Sie zeigten eine Assoziation zum Verzehr von Tomate, Gurke und Blattsalat, vor dessen Verzehr von den Bundesbehörden in Norddeutschland gewarnt wurde (56). Der Konsum von Sprossen wurde von den Patienten nicht in erhöhtem Maße erinnert. Am 26. Mai 2011 wurden von einer missverständlichen Presseerklärung der Behörden aus Hamburg Gurken aus Spanien mit dem Ausbruch in Verbindung gebracht (57). Dieses wurde am 31. Mai 2011 klargestellt (58). Es kam zu einer starken öffentlichen Kritik an den Untersuchungen der deutschen Behörden. Ein Kritikpunkt war, dass EHEC aufgrund seines bekannten Reservoirs eigentlich in Fleisch- oder Milchprodukten zu vermuten sei und nicht in pflanzlichen Produkten. Am 03. Juni 2011 wurden die Ergebnisse einer Fall-Kontroll-Studie des Gesundheitsamts Frankfurt in Zusammenarbeit mit dem RKI veröffentlicht (siehe Kapitel 2.2.1.), die den Einkauf an den Salatbars zweier Kantinen in den Fokus rücken konnte. Am 09. Juni 2011 wurden die Ergebnisse einer Kohortenstudie des RKI (siehe Kapitel 2.2.2.) veröffentlicht und zusammen mit der parallel erhobenen Evidenz aus Produktrückverfolgungen der Öffentlichkeit Sprossen als Verursacher des Ausbruchs dargestellt (59). Am 26. Juli 2011 wurde der Ausbruch für beendet erklärt (60). Dem RKI wurden insgesamt 2.987 EHEC-Enteritis-Erkrankungen und 855 HUS-Erkrankungen übermittelt, darunter waren 35 verstorbene HUS-Patienten 18 verstorbene EHEC-Enteritis-Patienten. Durch den Ausbruch sind in der Europäischen Union außerhalb Deutschlands 76 EHEC-Enteritis-Erkrankungen, darunter ein Todesfall und 49 HUS-Erkrankungsfälle aufgetreten (61).

Am Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) wurde eine Task Force EHEC gebildet. Diese bestand aus Experten für Lebensmittelsicherheit und Warenstromanalysen mehrerer Bundesländer, dem Bundesinstitut für Risikobewertung (BfR), dem RKI, dem BVL und der European Food Safety Agency (EFSA). Insgesamt 41 vom RKI und den Ländern in Deutschland übermittelte Ausbruchscluster konnten in Warenstromanalysen einbezogen werden und dadurch war es möglich, diese auf Sprossen aus einen niedersächsischen Produktionsbetrieb zurückzuführen (62). In Bordeaux gab es Ende Juni einen weiteren Ausbruch mit EHEC O104 (63). Es ließ sich feststellen, dass diese Erkrankungsfälle

Bockshornklee-Samen derselben Charge verwendete wie der Gartenbaubetrieb in Niedersachsen und somit eine Verbindung hatte. Diese Samen wurden in 2009 in Ägypten produziert und über mehrere Zwischenhändler bezogen (64).

Dieser Ausbruch unterschied sich von früheren Ausbrüchen von HUS/EHEC gleich in mehrfacher Hinsicht. Eine wesentliche Besonderheit war, dass die Inkubationszeit deutlich länger war, als in vorherigen HUS/EHEC-Ausbrüchen. Die mediane Inkubationszeit von der Exposition bis zu den ersten Erkrankungszeichen betrug acht Tage. Der Zeitraum von Beginn der Diarrhoe bis zur Entwicklung von HUS betrug im Median fünf Tage. Daher war der zurückliegende Zeitraum bei der Expositionsermittlung durch Befragungen auch ungewöhnlich lang, was zu einer deutlich erschwerten Datenerhebung führte (65). Der Ausbruchsstamm wurde als enteroaggregativer *E. coli* klassifiziert, der die Fähigkeit zur ein Shigatoxin-Bildung enthielt und die Bildung von Extended-Spectrum-Beta- Laktamase (ESBL), was die Resistenz gegen Beta-Lactam-Antikörper und Cephalosporine der dritten Generation verleiht (66). Der Ausbruch betraf vor allem erwachsene Frauen, während bei sporadischen HUS-Fällen in Deutschland und in anderen Ausbrüchen ein starker Überhang bei Kindern dokumentiert wurde. Der Anteil der Patienten mit EHEC-Erkrankung, die bei diesem Ausbruch HUS entwickelten, war (~25%) deutlich höher (8).

1.6. Ziele der Arbeiten

Das übergeordnete Ziel aller Arbeiten in dieser Schrift war die Bestimmung der Häufigkeit von lebensmittelübertragenen Infektionen und die Erkennung der Krankheitsursachen, um Prävention dieser Erkrankungen zu verbessern. In den ersten beiden Arbeiten wurden bevölkerungsrepräsentative, epidemiologische Angaben zur Inzidenz von Durchfall-Erkrankungen (Kapitel 2.1.1.) und *Toxoplasma*-Infektionen (Kapitel 2.1.2.) erhoben, die für die Planung und Steuerung von gesundheitspolitischen Maßnahmen von hoher Bedeutung sind. Das Ziel der Arbeiten in Kapitel 2.2.1., 2.2.2. und 2.2.3. war die Identifikation eines Lebensmittels als Auslöser von Erkrankungen in einem Ausbruch, um weitere Erkrankungsfälle unmittelbar zu verhindern. Die letzte Arbeit (Kapitel 2.1.4.) ist ein Beitrag, um das methodische Vorgehen bei Ausbrüchen zu verbessern und konzentriert auf die Erhebung des Anteils von Fehlklassifikation von Ausbruchsfällen bezüglich der Exposition.

2. Eigene Arbeiten

2.1. Bevölkerungsrepräsentative Studien zum Vorkommen von durch Lebensmittel übertragende Infektionskrankheiten

2.1.1. Akute Gastroenteritis bei Erwachsenen in Deutschland

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Infektiöse Magen-Darm-Erkrankungen sind die mit Abstand am häufigsten gemeldeten Infektionserkrankungen in Deutschland (7). Deren Verhinderung gehört zu den Hauptaufgaben des Infektionsschutzes des Öffentlichen Gesundheitsdienstes in Deutschland. In den Vereinigten Staaten gibt es etwa 179 Millionen Fälle von infektionsbedingtem akutem Durchfall pro Jahr (67). Bei älteren Erwachsenen besteht ein erhöhtes Risiko für schwerwiegende Komplikationen und Tod infolge einer Gastroenteritis, speziell bei jenen mit relevanten Grunderkrankungen. Zusätzlich haben Durchfallerkrankungen aufgrund der Behandlungskosten und der Arbeitsausfallzeiten starke sozioökonomische Auswirkungen. Das Robert Koch-Institut (RKI) erhält im Rahmen des Infektionsschutzgesetz (IfSG) erregerbasiert Informationen zu Erkrankungsfällen übermittelt. Man muss aber davon ausgehen, dass die meisten Erkrankungen nicht zur Meldung kommen (z.B.: nicht jeder Erkrankte kommt in Behandlung, häufig wird keine Stuhlprobe veranlasst). Daher ist die tatsächliche Krankheitslast durch infektiöse Durchfallerkrankungen wahrscheinlich wesentlich höher als die Anzahl der erfassten Infektionserkrankungen. Es besteht deshalb ein hoher Forschungsbedarf, insbesondere an bevölkerungsbezogenen, repräsentativen Studien.

Die Studie Gesundheit in Deutschland aktuell (GEDA) wurde 2008 bis 2009 durchgeführt. Die Methoden und allgemeinen Ergebnisse wurden publiziert (68, 69). Die Häufigkeit von Durchfallepisoden nach einem international festgelegtem Standard sowie der Verlauf derselben erfasst (12, 70). Insgesamt wurden 0,95 Episoden / Person pro Jahr (95%-KI 0,90-0,99) geschätzt, was 64,9 Millionen Episoden von akuter Gastroenteritis jährlich bei Erwachsenen entspricht, was mit 24,5 Millionen Arztbesuchen, 19,9 Millionen Krankenhaustagen und 63,2 Millionen verlorenen Arbeitstagen einhergeht (70). Die mittlere Krankheitsdauer betrug 3,8 Tage und unterschied sich nicht zwischen den Altersgruppen. Insgesamt 10,6% der Teilnehmer berichteten von einer Einnahme von Antibiotika aufgrund ihrer Durchfallerkrankung (70).

Die Ergebnisse dieser Studie konnten einen wichtigen Beitrag zur Abschätzung der tatsächlichen Krankheitslast durch infektiöse Durchfallerkrankungen liefern. Sie lieferte Basisdaten zur Situationseinschätzung für den praktischen Infektionsschutz. Die Studie ermöglicht erstmals internationale Vergleiche und trägt zur Abschätzung

der Krankheitslast durch akuten Gastroenteritis im Allgemeinen, als auch bei lebensmittelübertragenen Infektionen und einzelnen Infektionskrankheiten bei. Beispielhaft sei diesbezüglich eine Studie zur Krankheitslast von EHEC in Deutschland (71). Darüber hinaus gab die Studie weitere Hinweise auf eine in den meisten Fällen nicht indizierte Einnahme von Antibiotika zur Therapie einer Gastroenteritis (70).

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Acute gastrointestinal illness in adults in Germany: a population-based telephone survey

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SUMMARY

Population-based estimates of incidence and risk factors for acute gastrointestinal illness (AGI) are important for infectious disease surveillance and healthcare planning. We conducted a nationwide representative cross-sectional telephone survey of 21 262 adults over a 12-month period during 2008–2009 in Germany. Participants were asked if they had either AGI-related diarrhoea or vomiting in a 4-week recall period. We estimated 0·95 episodes/person per year (95% confidence interval 0·90–0·99), corresponding to 64·9 million episodes of AGI annually in adults, which results in 24·5 million outpatient visits, 19·9 million hospital days and 63·2 million days of work lost. We observed an overall declining trend of AGI with increasing age. Diarrhoea was more often reported than vomiting. The mean duration of illness was 3·8 days and did not differ between age groups. Social factors seemed to be weak predictors compared to state of health and health behaviour characteristics. This study allows international comparisons and contributes to the estimation of the global burden of AGI.

Key words: Estimating, gastrointestinal infections, infectious disease, prevalence of disease, virology (human) and epidemiology, zoonotic foodborne diseases.

INTRODUCTION

Acute gastrointestinal illness (AGI) has a huge public health impact in terms of disease incidence, prevention issues and healthcare costs. The majority of AGI is caused by infectious agents.

Over the last years the burden of AGI has been a field of research in many countries. The methodology for community surveys has improved over the years and a common case definition was established to ensure international comparability [1–3]. These efforts resulted in cross-sectional studies for estimates for the burden of AGI from various countries [4–18] and additionally a few population-based cohort studies [19–21].

In Germany, epidemiological analyses of infectious diseases often rely on data from the national notifiable infectious disease surveillance system. Laboratory-confirmed cases are notified to the local public health office. Clearly, these cases represent only the tip of the surveillance pyramid [3]. Many infections go unnoticed because not all patients seek medical care. Moreover, although medically indicated, a stool

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sample is not investigated from all patients for the causative agent, which is prerequisite for notification. Varying degrees of under-ascertainment by age group, sex, socioeconomic status and geographical region can result in biased comparisons of incidence estimates. Despite the fact that many of the gastrointestinal pathogens are notifiable, there is no syndromic surveillance of AGI in Germany. In this situation conducting cross-sectional surveys using a standardized syndromic case definition for AGI is a necessary alternative. These surveys provide representative population estimates of the true burden of acute gastrointestinal disease. A cross-sectional study in North Germany in 2004 verified that in a large proportion of AGI patients an infectious agent could have been detected, indicating that the majority of these are caused by infectious agents [22]. This is why cross-sectional data on the incidence of AGI can complement the system of notifiable disease surveillance while helping to unravel true differences of disease burden from under-ascertainment and reporting artefacts. Furthermore, data on baseline incidence can help to interpret the data of syndromic surveillance implemented on an *ad hoc* basis (e.g. in the case of large and widely dispersed outbreaks) [23]. Additionally incidence estimates may help to formulate precise case definitions differentiating between baseline disease incidence and outbreak case excess during outbreak analyses of gastrointestinal diseases [24–26]. Information on determinants of socioeconomic status, personal health behaviour and self-perceived health status might help to formulate hypotheses on risk factors for related diseases. The data on healthcare utilization of AGI cases is useful for cost-effective analysis of specific interventions.

The main objective of this study was to estimate the incidence of self-reported AGI in the German adult population and investigate sociodemographic and epidemiological factors as determinants of AGI. Furthermore, we also assessed clinical manifestations and utilization of medical services in cases of AGI.

METHODS

Survey methodology

The population-based telephone survey GEDA [Gesundheit in Deutschland Aktuell (Current Health in Germany)] is part of the German health monitoring programme. The GEDA methodology has been described in detail elsewhere [27, 28]. The sampling population consisted of the resident German-speaking adult population living in private households with a fixed telephone line (landline). The proportion of households having landline access was 89% in 2009. Landlines are more prevalent in households with two or more persons and single households of persons aged >60 years [29]. Landline access is slightly more prevalent in rural villages and more prevalent in the elderly. The targeted number of respondents was 21 000. The telephone-number sample was created using the Gabler-Häder design [30]; it was based on phone numbers taken from public telephone directories. In order to include people with unlisted numbers, random numbers were produced based on German area codes, thereby allowing selection of numbers not registered in directories. Altogether, a number pool consisting of both published and unpublished phone numbers was created. In order to give each element of the population the same theoretical likelihood of being interviewed, an additional selection of target persons was conducted at the household level, using the 'last-birthday method'. The computer-assisted telephone interview (CATI) method was applied. Interviews were conducted by 205 interviewers in 385 working shifts which were planned to be equally distributed over the study period. To allow the maximum number of people to be contacted the shifts were worked Mondays to Fridays between 16:00 and 20:00 hours and on Saturdays from 14:00 to 18:00 hours. Interviewers were balanced regarding age and sex to avoid interviewer bias. Selected telephone numbers were attempted to be contacted up to 15 attempts. If telephone contact was made with the household, the interviewer determined if the household contained two or more adults and then asked to speak with the adult household member with the most recent birthday. Study participants were enrolled from July 2008 to June 2009, the response was 29·1% and showed little variation by place and time. In order to improve representativeness, survey weights were generated to adjust for deviation of the target population to the German adult population based on estimates of the German Federal Statistical Office of Germany for 2009. This included a design weighting to (i) number of telephone numbers in the household and (ii) number of persons in the household, and additionally a weighting post-stratification to (iii) age, (iv) sex, (v) region and (vi) education (standard classification: ISCED) [27, 28]. Unless otherwise stated, all statistical analyses account for the weights.

Case definition

Our case definition was based on that proposed by International Collaboration on Enteric Disease 'Burden of Illness' Studies [2]. It deviates from this as vomiting was defined as having had at least three episodes. Diarrhoea was defined as $\geqslant 3$ loose stools in a 24-h period. Persons who reported having diarrhoea but then reported having fewer than three loose stools in a 24-h period were considered not to have had diarrhoea. Vomiting was defined as having had at least three episodes on one day. We defined cases of AGI as respondents who either self-reported diarrhoea ($n=1{\cdot}501$) or vomiting ($n=379$) in the 4 weeks preceding the interview. All others were defined as non-cases.

We excluded all subjects from the analysis who had chronic gastrointestinal diseases, i.e. Crohn's disease ($n=60$), ulcerative colitis ($n=68$), stomach cancer and intestinal tumours ($n=31$), irritable bowel ($n=138$) or coeliac disease ($n=22$) or who were pregnant ($n=147$). We excluded missing values regarding chronic gastrointestinal diseases ($n=28$). Due to data privacy exclusion criteria for alcohol and drugs, related diarrhoea or vomiting could not be included in the study's case definition.

Data analysis

Four-week incidence ($I_{4\,\mathrm{wk}}$) as incidence proportion (expressed in %) was calculated as

$$I_{4\,\mathrm{wk}} = \frac{\sum w_k x_k}{\sum w_k},$$

where x_k is a binary variable indicating whether a person k was a case or not and where w_k is the weight of x_k. 95% confidence intervals (CIs) were used as interval estimates. For reasons corresponding to other studies the annual incidence (I_{annual}) was calculated as $I_{\mathrm{annual}} = I_{4\,\mathrm{wk}} \times (365/28)$ and expressed in terms of episodes/person per year. Odds ratios (ORs) as measures of the association between disease determinants as explanatory variables and the defined AGI cases as outcome variable were obtained using logistic regression controlled for age, sex and age × sex interaction. For categorical variables the category with the highest number of participants was chosen as reference but never one of the two extreme categories. Spatial reference is the administrative system of districts in Germany and time reference is the day of the telephone interview. For comparison of the proportion between age groups and sex, the two-tailed P value for the z test from logistic regression was used. For comparison of average means between age groups and sex, the two-tailed P value from linear regression was used. The analyses were performed using Stata v. 12 (Stata Corp., USA). All statistical tests and regression analyses account for the study weights using the 'svy' command in Stata v. 12.0 [31].

RESULTS

The response rate was 29·1% and a total of 21 262 interviewees responded to the survey, of which 20 800 were eligible for case definition. Of these 11 761 (58·2%) were female. Median age was 46 years (range 18–100, interquartile range: 35–60 years). Altogether 1562 (7·5%) persons reported an AGI. Incorporating the study weights the $I_{4\,\mathrm{wk}}$ of AGI in adults was 7·3% (95% CI 6·9–7·8) corresponding to an I_{annual} of 0·95 episodes/person per year (95% CI 0·90–0·99). Extrapolated to the 2009 overall adult population of 68·3 million this resulted in an estimated 64·9 (95% CI 62·0–67·8) million episodes of AGI per year in adults in Germany.

Effects of age, gender, seasonality, and geographical region

Statistically significant differences of I_{annual} were found between age groups and a borderline significant age × sex interaction ($P=0{\cdot}055$) (Fig. 1). Incidence was highest in young males ($I_{\mathrm{annual}}=1{\cdot}29$ episodes/person per year, $I_{4\,\mathrm{wk}}=9{\cdot}9\%$), and lowest in older males ($I_{\mathrm{annual}}=0{\cdot}54$ episodes/person per year, $I_{4\,\mathrm{wk}}=4{\cdot}2\%$). We observed an overall declining trend of AGI with increasing age both in males ($P<0{\cdot}001$) and females ($P=0{\cdot}003$). Overall, female adults had a higher odds of reporting AGI than males, but this did not reach statistical significance ($P=0{\cdot}081$). However, in the older age groups incidence was higher in females than in males (most pronounced in the 50–59 years age group). Incidence in the Eastern German federal states (former German Democratic Republic plus West Berlin) was slightly lower ($I_{\mathrm{annual}}=0{\cdot}86$ episodes/person per year) but this difference in comparison with Western states failed to achieve significance ($P=0{\cdot}169$). The variation of AGI during the survey period is shown in Figure 2. Incidence ranged from 0·79 in May 2008 to 1·17 in February 2009. A peak was observed from January 2009 to March 2009.

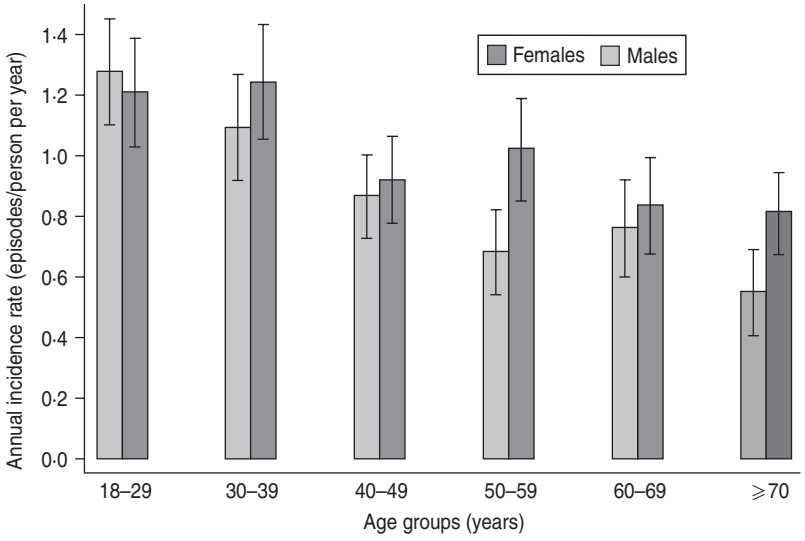

Fig. 1. Distribution of annual incidence of acute gastrointestinal illness in Germany in 2008–2009 by age and sex ($n = 20\,800$).

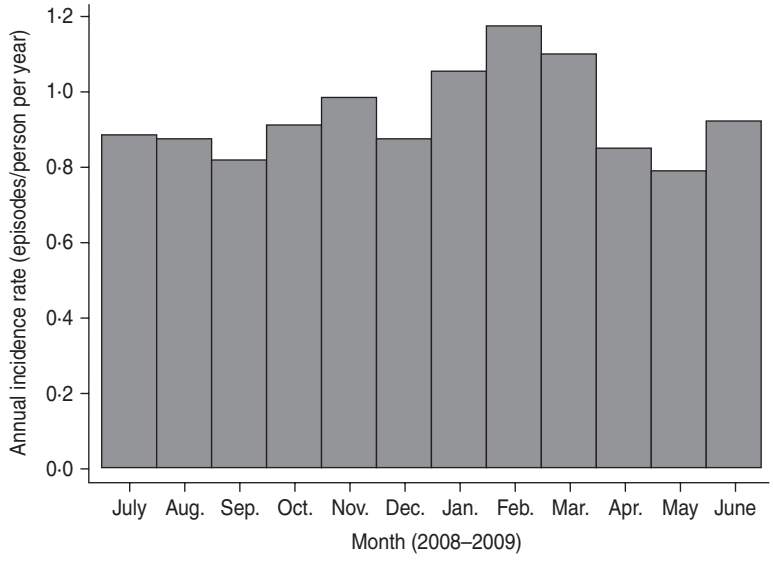

Fig. 2. Timely distribution of annual incidence of acute gastrointestinal illness by month in Germany ($n = 20\,800$). Time reference is the day of telephone interview.

Other risk factors

Due to the influence of age and sex of the respondents on the incidence and the odds ratios, all estimates for risk factors were adjusted for these factors and their interaction (Table 1). Generally, social characteristics seemed to be weak predictors of AGI in this study. Migrant status or migration background was not associated with AGI. Compared to a two-person household as a reference category, people living alone were more likely to report AGI (OR 1·29, 95% CI 1·07–1·55), whereas larger households of four or more people (OR 0·96, 95% CI 0·79–1·17) or the number of infants in the household (OR 1·12, 95% CI 0·97–1·29) were not a risk factor.

The overall health and health behaviour were significantly associated with AGI. Based on a self-reported scale from 1 to 5 (where 1 is very good and 5 is very bad), the respondents were asked for their general state of health. An association of AGI and perceived poor general health status was apparent. The question regarding personal health awareness revealed that in comparison to respondents who strongly cared for their health, people with

Table 1. *Incidence estimates and determinants for acute gastrointestinal illness (AGI) in adults in Germany, 2009 (weighted) (n = 20 800)*

Determinants (*n* = unweighted sample size)	No. of AGI cases (unweighted)	4-week incidence	Annual incidence	(95% CI)	OR	(95% CI)	*P* value
Female* (*n* = 11 761)	915	7·66	0·99	(0·93–1·06)	1·13	(0·99–1·29)	0·081
Male (*n* = 9039)	647	6·85	0·89	(0·83–0·96)	—	—	—
Age (interaction with sex)†							
Female		—	—	—	0·99	(0·99–1·00)	**0·003**
Male		—	—	—	0·98	(0·98–0·99)	**< 0·001**
Social determinants							
Provenance‡§							
East German (*n* = 4001)	278	6·63	0·86	(0·77–0·96)	0·89	(0·75–1·05)	0·169
West German (*n* = 16 799)	1284	7·43	0·97	(0·92–1·02)	Ref.	Ref.	Ref.
Migrant background‡							
No (*n* = 17 741)	1327	7·16	0·93	(0·88–0·98)	Ref.	Ref.	Ref.
One-sided (*n* = 782)	72	9·24	1·20	(0·92–1·49)	1·26	(0·93–1·72)	0·138
Two-sided (*n* = 2275)	163	7·43	0·97	(0·84–1·10)	0·95	(0·75–1·20)	0·649
Net household income (€)‡‖							
<1500 (*n* = 3622)	326	7·99	1·04	(0·92–1·15)	1·15	(0·92–1·44)	0·206
1500–2125 (*n* = 2914)	224	7·20	0·94	(0·82–1·06)	1·02	(0·80–1·30)	0·898
2126–2750 (*n* = 2996)	200	6·86	0·89	(0·78–1·01)	0·90	(0·70–1·15)	0·395
2751–3750 (*n* = 3075)	226	7·71	1·00	(0·88–1·13)	Ref.	Ref.	Ref.
> 3750 (*n* = 2992)	207	7·17	0·93	(0·80–1·07)	0·92	(0·72–1·16)	0·471
No. of persons in household‡							
1 (*n* = 4730)	390	8·00	1·04	(0·92–1·16)	1·29	(1·07–1·55)	**0·007**
2 (*n* = 7421)	483	6·30	0·82	(0·75–0·89)	Ref.	Ref.	Ref.
3 (*n* = 3829)	304	8·11	1·05	(0·95–1·16)	1·09	(0·89–1·33)	0·405
⩾ 4 (*n* = 5245)	384	7·56	0·99	(0·89–1·08)	0·96	(0·79–1·17)	0·703
Health and health behaviour determinants							
Care for health‡							
Very strong (*n* = 2684)	187	6·82	0·89	(0·76–1·01)	1·18	(0·94–1·47)	0·158
Strong (*n* = 8738)	567	6·06	0·79	(0·72–0·86)	Ref.	Ref.	Ref.
Moderate (*n* = 7722)	622	7·90	1·03	(0·95–1·11)	1·30	(1·12–1·52)	**0·001**
Less strong (*n* = 1256)	147	10·99	1·42	(1·20–1·65)	1·85	(1·44–2·37)	**<0·001**
Not at all (*n* = 362)	32	10·07	1·31	(0·95–1·68)	1·70	(1·06–2·75)	**0·029**
General state of health‡							
Very good (*n* = 5071)	214	4·00	0·52	(0·45–0·60)	0·52	(0·43–0·63)	**<0·001**
Good (*n* = 10 613)	723	6·52	0·85	0·79–0·91	Ref.	Ref.	Ref.
Moderate (*n* = 4024)	459	10·38	1·35	1·24–1·46	2·12	1·79–2·51	**<0·001**
Bad (*n* = 841)	125	12·45	1·62	1·36–1·88	2·83	2·16–3·72	**<0·001**
Very bad (*n* = 219)	38	14·22	1·85	1·32–2·39	3·28	2·08–5·18	**<0·001**
Consumption of fruits‡							
Daily (*n* = 13 040)	892	6·62	0·86	0·81–0·92	0·82	0·72–0·95	**0·010**
Less (*n* = 7745)	668	8·29	1·08	1·00–1·16	Ref.	Ref.	Ref.
Consumption of vegetables‡							
Daily (*n* = 10 151)	770	7·19	0·94	0·87–1·00	0·96	0·84–1·10	0·578
Less (*n* = 10 638)	792	7·33	0·95	0·89–1·02	Ref.	Ref.	Ref.
Consumption of fruit juice‡							
Daily (*n* = 3915)	274	6·61	0·86	0·76–0·96	0·91	0·76–1·08	0·276
Less (*n* = 16 840)	1286	7·42	0·96	0·92–1·02	Ref.	Ref.	Ref.
Alcohol consumption‡							
Never (*n* = 3220)	303	9·09	1·18	1·06–1·30	1·72	1·41–2·10	**<0·001**
⩽ 1 time a month (*n* = 4773)	364	7·61	0·99	0·89–1·09	1·31	1·09–1·59	**0·005**
2–4 times a month (*n* = 6234)	405	6·04	0·79	0·71–0·87	Ref.	Ref.	Ref.
2–3 times a week (*n* = 4183)	280	6·14	0·80	0·70–0·90	1·14	0·93–1·39	0·200
⩾ 4 times a week (*n* = 2346)	205	8·33	1·08	0·94–1·23	1·83	1·44–2·33	**<0·001**

Table 1 (*cont.*)

Determinants (*n* = unweighted sample size)	No. of AGI cases (unweighted)	4-week incidence	Annual incidence	(95% CI)	OR	(95% CI)	*P* value
Body mass index (BMI)‡							
Underweight (BMI <18·5) (*n* = 459)	41	8·60	1·12	0·76–1·48	1·20	0·79–1 > 84	0·396
Normal weight (*n* = 10 584)	694	6·44	0·84	0·77–0·90	Ref.	Ref.	Ref.
Overweight (BMI 25–30) (*n* = 7005)	504	7·01	0·91	0·84–0·99	1·30	1·10–1·52	**0·002**
Obesity (BMI > 30) (*n* = 2790)	283	9·57	1·25	1·11–1·38	1·87	1·54–2·27	**<0·001**
Diabetes‡							
Yes (*n* = 1215)	116	9·67	1·26	1·07–1·45	1·81	1·38–2·38	**<0·001**
No (*n* = 19 585)	1446	7·07	0·92	0·87–0·97	Ref.	Ref.	Ref.
Total (*n* = 20 800)	1562	7·26	0·95	0·90–0·99	—	—	—

OR, Odds ratio; CI, confidence interval.
* OR adjusted for age.
† In years and reference category 18 years.
‡ Adjusted for age, sex, and age × sex interaction.
§ Whole of Berlin as East Germany.
¶ A person was defined as having a one-sided migrant background if at least one of the parents was not born in Germany, a two-sided migrant background if the person had no German citizenship, moved to Germany after birth or both parents were not born in Germany.
‖ In Euro (€), discretized by quintiles.
Bold values indicate significance.

self-reported moderate, poor or very poor personal health awareness more frequently reported AGI. However, respondents who self-reported a very strong care did not benefit. Daily consumption of fruits was inversely associated with AGI (OR 0·82, CI 0·72–0·95), whereas daily consumption of vegetables and fruit juice were not statistically related for AGI.

Alcohol consumption had a two-way association with risk for AGI. Overall, 6234/20 756 (30%) of the interviewees reported drinking alcohol 2–4 times a month. People who reported drinking $\geqslant 4$ times a week had a significantly higher incidence (OR 1·83, 95% CI 1·44–2·33). By contrast, respondents who reported drinking less than the reference category or who reported never drinking alcohol (OR 1·72, 95% CI 1·41–2·10) were also significantly more at risk. In an analysis of the influence of alcohol consumption stratified on diarrhoea and vomiting, the results are qualitatively the same for AGI as the combined outcome.

The body mass index (BMI) was a significant predictor of AGI. With each increase of BMI score the risk of AGI increased by 5%. The risk for obese persons (BMI > 30) was the highest. Diabetes mellitus was reported by 5·8% of the participants and is significantly associated with AGI in our study (OR 1·81, 95% CI 1·38–2·38).

Symptoms, severity and healthcare utilization

Of the 1562 respondents who met the case definition for AGI, 78·0% reported diarrhoea, 11·9% reported vomiting and 10·1% experienced both symptoms (Table 2). Bloody diarrhoea was reported by 3·6%. Mean duration of illness was 3·7 days without significant differences by age and sex.

More than one third (37·8%) of cases sought outpatient medical care and 3·4% were hospitalized. Overall, 13·8% of the cases reported providing a stool sample for microbiological examination. This was significantly more often reported by the elderly (24·4% in those aged > 70 years) and by females (16·3%). There were no significant differences between West Germany and East Germany regarding the proportion of patients reporting an outpatient visit ($P = 0·49$) and providing a stool sample ($P = 0·70$). Altogether 49·8% of the AGI cases reported having taken medication against AGI, 31·2% reported a medical prescription and 10·6% of all AGI cases reported antibiotic therapy. The latter was significantly more often prescribed for elderly patients. Fever was associated with antibiotic therapy prescriptions ($P < 0·001$); however, bloody diarrhoea was not ($P = 0·462$). In our study, 23·2% of the AGI patients had to stay away from work with a mean duration of work absenteeism of 4·2 days.

Table 2. *Proportions and average means for associated factors and medical actions taken of cases of acute gastrointestinal illness by age and sex* (*n* = *1562*)

	Age group (years)										
	Total	18–29	30–39	40–49	50–59	60–69	⩾70	*P* value for age*	Female	Male	*P* value for sex*
Diarrhoea (%)	88·1	81·0	87·6	87·7	92·3	91·5	94·8	**<0·001**	87·8	88·7	0·671
Vomiting (⩾3 times/day) (%)	22·0	30·4	28·2	20·0	16·1	14·4	14·4	**<0·001**	23·6	20·1	0·214
Bloody diarrhoea (%)	3·6	3·8	4·3	3·9	3·3	3·6	3·3	0·904	3·3	4·0	0·598
Fever (>38·5 °C) (%)	10·0	16·4	11·8	10·7	5·5	4·5	6·3	**0·001**	10·4	9·9	0·823
Stool sample (%)	13·8	7·7	8·6	12·4	16·0	22·5	24·4	**<0·001**	16·3	10·9	**0·033**
Travel related (%)	6·6	7·6	7·5	9·5	4·1	6·1	2·7	**0·028**	4·6	9·0	**0·002**
Outpatients (%)†	37·8	38·2	30·2	31·9	36·0	42·9	55·5	**0·002**	37·7	38·0	0·939
Hospitalized (%)	3·4	0·9	4·5	1·5	3·2	5·5	7·6	**0·026**	3·8	3·0	0·557
Work absenteeism (%)	23·2	33·2	32·2	28·6	20·7	n.a.	n.a.	n.a.	20·4	26·5	n.a.
Medication taken (%)	49·8	54·2	40·9	45·9	51·4	52·3	57·3	0·247	52·4	46·7	0·085
Medication prescribed (%)	31·2	30·4	22·9	27·9	30·2	40·4	43·9	**0·002**	30·6	31·9	0·693
Antibiotics prescribed (%)	10·6	10·0	5·6	8·1	11·4	17·3	16·0	**0·011**	11·3	9·8	0·517
Mean number of stools per case of diarrhoea	4·7	4·8	4·7	4·7	5·0	4·6	4·3	0·164	4·7	4·7	0·829
Mean duration of symptoms (days)	3·7	3·9	3·6	3·7	3·6	3·6	4·0	0·863	3·8	3·6	0·564
Mean duration of hospitalisation (days)	9·0	3·6	8·1	5·9	8·8	9·7	13·3	**0·003**	9·0	9·1	0·966
Mean duration of work absenteeism (days)	4·2	3·8	3·9	5·1	4·0	n.a.	n.a.	0·231	4·4	4·1	0·628

n.a., Not applicable.

* Proportions: two-tailed *P* value for the *z* test from logistic regression; means: two-tailed *P* value from linear regression.

† Either doctor in private practice, medical service in hospital without hospitalization.

Bold values indicate significance.

In an extrapolation of these proportions to the total adult German population of 68·3 million, the results of our survey add up to 24·5 million outpatient visits, 9·0 million stool sample analyses, nearly 6·9 million antibiotic prescriptions dispensed, 19·9 million days in hospital and 63·2 million working days lost.

DISCUSSION

This is the first survey conducted in Germany which is able to obtain nationwide representative data on the incidence and distribution of AGI in the population. The study, limited to adults, provides nationally representative estimates for disease burden. The sampling procedure and the statistical weighting ensure unbiased samples regarding age, sex, geographical region and education status. For adults young age was the strongest predictor for AGI in this study. It is possible that older persons have been repeatedly exposed to gastrointestinal pathogens during the course of life and acquired relative immunity. Alternatively risky behaviour of young adults leaving home and beginning to prepare their own food (second weaning) could be a contributing factor.

International comparison

Incidence for AGI in adults in Germany is generally in line with observations in similar studies in other countries. Comparisons between countries have to consider the varying case definitions. We used a more restrictive criterion for the identification of AGI-related vomiting. This could explain differences to other countries, e.g. to the Danish study with a higher proportion of cases with vomiting explaining a higher overall incidence [15]. Additionally, most of the studies, unlike ours, included children and adolescents and the cut points for age groups vary between the analyses. A decreasing trend for age is reported in all similar studies. From neighbouring European Union member-state countries Denmark reported higher incidence in younger adults but the same in the elderly (from $I_{\text{annual}} = 2{\cdot}0$ in the 20–29 years age group to $I_{\text{annual}} = 0{\cdot}75$ in those aged $\geqslant 70$ years) [15], Italy reported lower incidence in the elderly (from $I_{\text{annual}} = 1{\cdot}11$ in the 10–24 years age group to $I_{\text{annual}} = 0{\cdot}33$ in those aged $\geqslant 75$ years) [18] and Poland almost the same incidence ($I_{\text{annual}} = 0{\cdot}9$ in the 15–64 years age group) [6] as in Germany. Furthermore, reported incidence of AGI seem to be generally in the same range as in the USA [11], Canada [16], Hong Kong [12] and Australia [10], lower than in New Zealand [4], Cuba [5] and Norway [14], respectively, but higher than in Great Britain [21], Malta [7], Ireland [17] and Malaysia [9].

Generally, similar proportions of bloody diarrhoea in cases of AGI are reported from Denmark and Canada. Higher levels of bloody diarrhoea are published from New Zealand and Australia and lower levels from studies of the USA, Ireland and Malta.

Seasonality and geography

The distribution of AGI during the 1-year study period can be explained by the seasonal variation in infections with viral enteric pathogens (most prominently norovirus). This assumption is supported by the fact that the peak of AGI in January and February, as observed in the present study, corresponds to the 2009 peak of norovirus activity in Germany in the fourth calendar week which was observed from the available surveillance systems of notifiable disease [32]. By contrast, seasonality of AGI of bacterial origin (most prominently *Campylobacter* and *Salmonella*) peaks in August but the summer season is not prominent for AGI incidence in this study. A similar seasonal AGI pattern was discovered by a study in Northern Germany who also found viral pathogens more frequently detected [22]. In neighbouring countries seasonality is similar [6, 15, 18] while other studies report seasonal peaks in summer [10]. There is no difference in the distribution of AGI between East and West Germany which has important implications for the interpretation of surveillance data of enteric diseases in Germany. Since reunification in 1990 a substantial higher incidence in the notification of infectious diseases was observed for East Germany mainly regarding gastrointestinal pathogens [32, 33]. This increased notification rate is not mirrored by the syndromic level in our survey results which demonstrate no differences between the two parts of the country. Based on our study, higher notification rates in the East are not explainable by different food consumption habits, differing population dynamics or the higher daycare attendance rates in infants as has been hypothesized. Different disease awareness in the population could be an alternative explanation.

Determinants

Social and economic factors are not or only weakly associated with the risk of AGI. Although a differing

lifestyle can be assumed, migrants and people with migrant background have no increased risk for AGI. The respondent's self-reported income groups are statistically related to AGI. Thus, in contrast to developing countries, financial factors seem to have a minor influence in industrialized countries like Germany where sanitary hygiene standards and microbiological quality of food and drinking water are not (in the same degree) dependent on socioeconomic status. Larger household size is not related to disease, not even the number of infants. This is surprising, assuming that a large proportion of the AGI cases in this study became infected with pathogens via the faecal–oral route related to contact frequencies with other persons [22, 32]. However, infants who are more susceptible for faecal–oral transmission were not included in the study. Single-person households with a higher infection rate are a notable exception which could be explained by frequent visits to cafeterias and purchases at fast-food outlets (e.g. takeaways).

Characteristics of health and health behaviour are prominent determinants of AGI. The degree of negative perception of an individual's own health status is linearly correlated with the incidence of AGI. This may reflect the influence of concomitant diseases on gastrointestinal infections. A similar effect could be observed regarding self-reported care for health.

There are conflicting results regarding alcohol consumption in this study. It can be hypothesized that people who frequently drink alcohol, as was asked in our study, also do so excessively and therefore report vomiting and diarrhoea. Additionally, frequent consumption of alcohol could affect the overall immunity of the participants [34, 35]. By contrast, people who never drink alcohol are also more likely to report AGI. The reasons for this could be attributed to confounding factors such as alcohol abstinence due to health grounds which also lead to AGI or the defining conditions of diarrhoea and vomiting.

Eating fruit is preventive, drinking fruit juice and eating vegetables is not. This could be a true protective effect or a proxy for nutritional habits. An increase in BMI is associated with AGI. From the probabilistic point of view, eating more in greater quantities and frequency increases the likelihood of consuming a foodborne pathogen as also does a hypothesized increased consumption of risk food. In addition to this effect, eating fat- and carbohydrate-rich and low-fibre diets could have a harmful effect on the gastrointestinal flora and a high BMI is correlated with a generally impaired immunity [36].

Healthcare utilization

AGI is common in adults in Germany and represents a significant burden of illness. Utilization of healthcare service is high in all age groups. Surveys from other countries that asked about prescription of antibiotics reported less utilization than in our survey in Germany (10·6%). This is despite the fact that we did not include children and adolescents, groups which are known to have higher prescription rates for antibiotic treatment than adults. In Ireland 5·6% of AGI cases self-report antibiotic use [37], 8·3% in the USA [37], 3·8% in Canada [37], 3·6% in Australia [37], 6·5% in Italy [18] and 6·4% in New Zealand [4]. Prescription and consumption practices appear to be considerably different in Germany. It remains to be investigated if this disproportion is caused by disease inherent factors, different diagnostic guidelines or differences in healthcare systems. The higher proportions of faecal sampling for diagnostics in the elderly and in females will presumably result in differences in notification rates of gastrointestinal pathogens. This could explain increased overall incidence of laboratory-confirmed cases of norovirus in females in Germany [32]. Incidence estimates based on notification data of viral pathogens increase in those aged $\geqslant 60$ years which was not seen in our study. In the future, differences in age and sex in the distribution of enteric pathogens should be interpreted against this background.

Limitations and strengths

Our study relies on a large number of individuals which generates precise estimates. Persons in households without a landline phone connection could not participate in the study which presumably resulted in underrepresentation of some social groups. This might have introduced selection bias; however, applying the study weights attempts to correct for basic demographic factors. Additionally, the response rate of 29·1% indicates a possibility for selection bias. The study is limited in a way that a full assessment of all possible underlying chronic disease was not able to be performed. The international comparability of incidence estimates is hampered by different survey methodologies and cases definitions. We decided that the AGI-related vomiting criterion requires at least three episodes as we believe that a single episode of vomiting may not be specific enough for an AGI infection. This differs from other work groups. Missing

exclusion criteria for alcohol and drugs in the cases definition were related to data privacy and considered by the authors as a minor deviation. Many gastrointestinal symptoms occur as a consequence of primary respiratory infections and this aspect could not be assessed. A future study could benefit from including respiratory symptoms as part of the survey process. The study concerned AGI from all aetiological agents and did not distinguish between bacterial or viral origin. Pathogen-specific risk estimates would provide a better insight into the risk of frequent bacterial gastroenteritis [8, 38–40]. The telephone interview did not encompass all assumable risk factors for AGI or even precisely record dietary habits. Instead it focused on some general and partially subjective underlying factors and self-reporting of those might introduce exposure misclassification; this certainly restricts the interpretation of our study.

CONCLUSION

The burden of AGI is high in adults in Germany. Almost 9/10 individuals experience an episode each year. Risk factors are more pronounced on the general state of health and health behaviour than on the social situation. Markedly, high rates of prescribed antibiotics in AGI patients should be further investigated. The health-promoting effect of eating fruits and the prevention of obesity, diabetes and alcohol abuse should be increasingly supported in Germany. This survey should be complemented with children and adolescents in the future.

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DECLARATION OF INTEREST

None.

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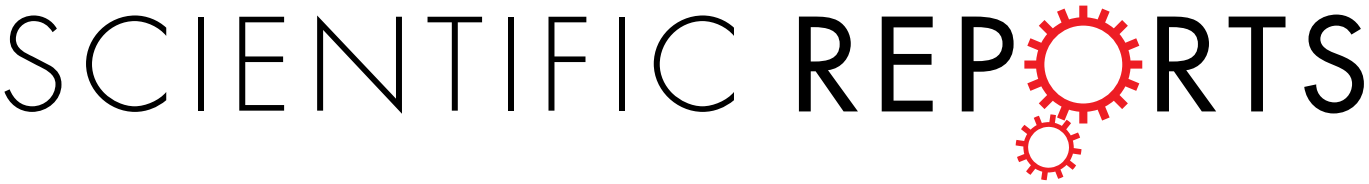
2.1.2. *Toxoplasma-gondii*-Seroprävalenz in Deutschland

Wilking H, Thamm M, Stark K, Aebischer T, Seeber F. Prevalence, incidence estimations, and risk factors of *Toxoplasma gondii* infection in Germany: a representative, cross-sectional, serological study. *Scientific reports*. 2016;6:22551. doi: 10.1038/srep22551

Daten über die Infektionshäufigkeit, die Wahrscheinlichkeit von klinischer Manifestation, sowie der Schwere und Dauer der Symptome der Toxoplasmose fehlen für Deutschland. Sie sind aber von entscheidender Bedeutung, um die bevölkerungsbezogene Krankheitslast zu bestimmen, die zu adäquateren Präventionsstrategien führen kann. Das Ziel dieser Studie war, als ersten Schritt die Seroprävalenz von *T. gondii* in Deutschland zu bestimmen und assoziierte Faktoren zu identifizieren (72, 73). Dafür wurden Seren, die innerhalb der Studie zur Gesundheit Erwachsener in Deutschland (DEGS) gewonnen wurden, genutzt. Da die Infektion mit *T. gondii* eine persistente Infektion ist und eine lebenslange Seropositivität auslöst, wurden aus altersgruppenbezogenen Seroprävalenzänderungen das Vorkommen von Infektionen und Erkrankungen geschätzt (73). Die Studie konnte erstmals aufzeigen, dass die *T. gondii*-Seroprävalenz bei Erwachsenen von 20,0% (95%-KI: 17,1% -23,1%) in der Altersgruppe 18-29 auf 76,8% (95%-KI: 72,7%-80,5%) in der Altersgruppe der 70- bis 79-Jährigen anstieg. In Ostdeutschland ist die Zunahme mit dem Alter stärker. Männliches Geschlecht, Katzenhaltung und BMI≥40 sind assoziiert mit Seropositivität. Vegetarismus ist negativ mit der Seropositivität assoziiert, ebenso wie ein hoher sozioökonomischer Status. Es konnte geschätzt werden, dass 1.325 von 100.000 Frauen im Alter von 18-49 serokonvertieren (73). Extrapoliert auf die deutsche Bevölkerung und die Anzahl der Schwangerschaften bedeutet dies 6.393 jährliche Infektionen während der Schwangerschaft. Unter Annahme von Studienergebnissen zur materno-foetalen Übertragung und Manifestationswahrscheinlichkeit einer Infektion (74, 75) konnten jährlich 1.279 Infektionen bei Ungeborenen und 345 Neugeborene mit klinischen Symptomen geschätzt werden (73).

Die Ergebnisse dieser Studie zeigen, dass annähernd die Hälfte der erwachsenen Bevölkerung bereits eine *T. gondii*-Infektion durchgemacht hat und Dreiviertel der Bevölkerung im Laufe ihres Lebens eine durchmachen wird. Daher muss auch eine hohe Krankheitslast aller Krankheitsmanifestationen in der deutschen Bevölkerung angenommen werden. Ungefähr die Hälfte der gesamten erwachsenen Bevölkerung Deutschland ist infiziert, was deutlich über der Seroprävalenz in den USA (6%) und den Niederlanden (26%) liegt, wo vergleichbare Daten vorliegen (76, 77). Die Ergebnisse deuten darauf hin, dass der hauptsächliche Übertragungsweg über Lebensmittel erfolgt. Die Ergebnisse bieten die empirische Grundlage für Maßnahmen in der Lebensmittelhygiene und Tiergesundheit. Schweine- und andere

Tierbestände sollten in Zukunft *T. gondii*-frei gehalten werden. Die Grundlage für die Bewertung des Screenings von Schwangeren hat sich geändert. Es muss daher re-evaluiert werden. Der Medizinischer Dienst des Spitzenverbandes Bund der Krankenkassen (MDS) überarbeitet momentan seine Bewertung des Toxoplasmose-Test bei Schwangeren als individuelle Gesundheitsleistung (33). Untersuchungen zur *T. gondii*-Seroprävalenz werden zukünftig in bundesweiten Serosurveys untersucht, was die Beobachtung von zeitlichen Trends ermöglichen wird.



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Prevalence, incidence estimations, and risk factors of *Toxoplasma gondii* infection in Germany: a representative, cross-sectional, serological study

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Representative data on the extent of endemicity, burden, and risk of human toxoplasmosis are scarce. We assessed the prevalence and determinants of seropositivity of *Toxoplasma gondii* among adult participants of a nationwide representative cross-sectional survey in Germany. Sera collected from a representative cohort of adults (age 18–79; n = 6,663) in Germany were tested for anti-*T. gondii* IgG antibodies. Interview-derived data were used to evaluate associated factors. Multivariable logistic regression was applied using sampling weights and accounting for survey design cluster effects. Seroprevalence increased from 20% (95%-CI:17–23%) in the 18–29 age group to 77% (95%-CI:73–81%) in the 70–79 age group. Male gender, keeping cats and BMI ≥30 were independent risk factors for seropositivity, while being vegetarian and high socio-economic status were negatively associated. Based on these data, we estimate 1.1% of adults and 1.3% of women aged 18–49 to seroconvert each year. This implies 6,393 seroconversions annually during pregnancies. We conclude that *T. gondii* infection in Germany is highly prevalent and that eating habits (consuming raw meat) appear to be of high epidemiological relevance. High numbers of seroconversions during pregnancies pose substantial risks for unborn children. Efforts to raise awareness of toxoplasmosis in public health programs targeting to *T. gondii* transmission control are therefore strongly advocated.

Infection with the protozoan parasite *Toxoplasma gondii,* the causative agent of toxoplasmosis, is a very common human disease worldwide[1,2]. *T. gondii* persists lifelong in the affected host organism. In cats and other feline obligate hosts, parasites reproduce sexually and shed up to hundreds of millions of oocysts. They resist moderate environmental conditions, and contaminate water and soil where they undergo sporulation. The resulting form is responsible for infection via ingestion of either contaminated food, water or dust[3,4]. Additionally, parasites can be found as tissue cysts in all warm-blooded animals including livestock. Eating raw or undercooked infected meat is thus a second epidemiologically-relevant mode of transmission[5]. Transfer of parasites, e.g. through infected transplants or vertically in utero, is an additional route of transmission[1,2].

In humans, most infections remain asymptomatic or manifest with mild flu-like symptoms; however, severe forms can occur[6,7]. These include congenital toxoplasmosis that can develop when a woman becomes primary infected with *T. gondii* during pregnancy[2,8]. Clinical surveys demonstrate that up to 20% of such maternal infections result in transplacental transmission, and that in 27% of the infected neonates specific symptoms develop[9,10]. Depending on the gestational age of the fetus at infection, predominantly retinochoroiditis, calcifications, hydrocephalus, psychomotoric and neurological disabilities, and fetal death can develop[6,7].

After diagnosis of maternal infection, fast initiation of therapy can efficiently reduce the risk of transplacental transmission and thus lower the disease burden of the newborn[11–14]. However, timely diagnosis can only be achieved through systematic screening of pregnant women. This is currently recommended in France and

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Austria, but in the absence of strong evidence of its benefit, not in the UK and in Germany[15].The reasons are diverse, but it is argued that the cost of the initial as well as follow up tests and the errors that arise are not outweighed by the small number of infections that can be prevented[16] (see also Discussion).

T. gondii infection is also an important cause of visual impairment. Infection at ocular sites (retina and the choroid) causes lesions leading to retinal scarring[6,7]. In a population-based study in Britain, the lifetime risk of symptomatic *T. gondii*-associated ocular disease was determined to be 18 in 100,000 individuals[17]. In Germany, 4.2% of uveitis cases are thought to be due to *T. gondii* infections[6].

Acute and reactivating infections in immunocompromised persons (e.g. AIDS patients or transplant recipients) can affect the central nervous system. An incidence of 3% of cerebral toxoplasmosis, associated with poor prognosis, was found among allogeneic hematopoietic stem cell transplant recipients[18,19]. Collectively, different manifestations of toxoplasmosis lead to a significant amount of years of life lost and many life years lived with sequelae, which constitute an exceptionally high disease burden of public health concern[20–23].

Apart from the well-proven clinical entities, persistent infections with *T. gondii* are suspected to be connected to mood disorders[24]. In the United States National Health and Nutrition Survey, prevalence of serum anti-*T. gondii* IgG was not elevated in unipolar mood disorders, like depression, but was higher in a subset of respondents with a history of bipolar disorder[25]. However, the link between *T. gondii* infection and neurological changes in humans awaits experimental verification and thus remains highly controversial.

Congenital toxoplasmosis is mandatorily notifiable to the Robert Koch Institute, which is responsible for the implementation of data collection and processing of anonymized case data inside the infectious disease notification system of Germany. It is suspected that these data are subject to a high level of underreporting and under-ascertainment of disease. Thus, information on disease incidence is missing. Previous serosurveys for *T. gondii* are limited regarding representativeness and sample size. Available studies are based on convenience sampling (mainly pregnant females) and lack a random selection of participants.

Our objectives were thus to assess the seroprevalence of *T. gondii* IgG antibodies from the population-based German representative health interview and examination survey of adults (DEGS1)[26], to identify and quantify the contribution of factors potentially associated with *T. gondii* seropositivity and to estimate the annual number of infections during pregnancy in Germany.

Results

Seroprevalence and associated factors. Altogether 6,663 participants with available blood samples were included in this study; 99 (1.5%) thereof had equivocal anti-*T. gondii* IgG titers of 4–7 and were thus excluded from the analysis. Out of 6,564 individuals, 3,602 (55%) were seropositive (Table 1). Seroprevalence increased from 20% (95%-confidence interval (95%-CI):17.1–23.1%) in the age group 18–29 to 77% (95%-CI:72.7–80.5%) in the 70–79 age group. Seroprevalence in participants older than 79 years (n = 116), not included in further analyses, was 84%. The observed seroprevalence increased at a rate of 1.09% with each year of age (Fig. 1). A significant interaction was noted between age and sex (p-value = 0.023), since higher seroprevalences were observed among younger males and older females. Male gender, although not associated in univariable analysis, was a significant risk factor in multivariable analysis (odds ratio (OR): 1.8; 95%-CI1.1–2.9). Age-related increase of seroprevalence was stronger amongst residents of East Germany when compared to the West (p-value of interaction term: <0.001) (Fig. 2). Prevalence in East Germany is more than 20% higher than in West Germany in the 40–69 age group.

Factors positively associated with seropositivity included cat contact. 15.8% of the participants indicated keeping a cat in the household, which proved to be a risk factor for seropositivity by multivariable analysis (OR: 1.3; 95%-CI:1.1–1.5), whereas holding a dog or other companion animal had no impact. Being overweight ($25 \leq$ BMI <30) or obese (BMI ≥ 30) was also positively associated with seropositivity (OR of 1.2; 95%-CI:1.0–1.5; and OR: 1.3; 95%-CI:1.0–1.6, respectively). People living in rural areas had an increased risk of infection (OR: 1.41; 95%-CI:1.1–1.8) compared to inhabitants of bigger cities. Factors negatively associated with *T. gondii* infection were being vegetarian (OR: 0.6; 95%-CI:0.4–1.0) and having a high socio-economic status (OR: 0.7; 95%-CI:0.6–0.9). No association could be found with diabetes.

Incidence estimates of seroconversions. Using a regression model we estimated an annual incidence of 1,099 (95% CI:1,016–1,181) seroconversions per 100,000 adult inhabitants of Germany (Table 2). In women aged 18–49 (considered here as main age group potentially bearing children) this corresponds to 1,325 (95% CI: 1,007–1,642) seroconversions. Taking the age-dependent distribution of births among women (data from 2011) and an age-related increase in seroprevalence into account, it can be assumed that 74.1% of births were delivered by mothers susceptible to primary infection ("pregnancies at risk"). The susceptibility decreased from 90.8% in the age group 15–19 years to 52.6% in those 45–49 years old. The incidence of 1,325 in 100,000 women implied 6,393 predicted infections during the annual 662, 485 pregnancies in Germany (Table 3). These are 1.0% of all pregnant women and 1.3% of seronegative pregnant women (pregnancies at risk).

Estimates of incidence of congenital toxoplasmosis. In a recent meta-analysis among patient cohorts, the rate of mother-to-child transmission among seroconverting pregnant women was reported to be 20% (95%-CI:15–26%)[9]. Extrapolated to the numbers derived here this would result in 1,279 (95% CI:959–1,662) annual cases of fetal infections in Germany. Based on estimates that 27% of infected neonates manifest with *T. gondii*-specific symptoms[10], this would result in 345 neonates with clinical symptoms (congenital toxoplasmosis) annually in Germany.

Depression. Participants in the survey who recalled an episode of depression during their lifetime had no association with seropositivity in univariable analysis (OR: 0.94; 95%-CI:0.78–1.13) (Table 4). This is confirmed

Characteristic (total no.)[a]	n (pos)[a]	Prevalence in % (95% CI)	Univariable analysis		Multivariable analysis	
			Odds ratio (95% CI)	p-Value	Odds ratio (95% CI)	p-Value
Sex						
Women (n = 3,443)	1,864	48.85 (46.56–51.14)	ref	ref	ref	ref
Men (n = 3,121)	1,738	49.31 (46.35–52.27)	1.02 (0.90–1.15)	0.767	1.76 (1.08–2.91)	0.023
Interaction term sex*age	–	–	–	–	0.99 (0.98–1.00)	0.024
Age						
Yearly	–	–	1.05 (1.05–1.06)	<0.001	1.06 (1.05–1.06)	<0.001
Age group (years)						
18–29 (n = 994)	214	19.95 (17.10–23.13)	ref	ref	–	–
30–39 (n = 790)	301	35.46 (31.01–40.17)	2.21 (1.70–2.85)	<0.001	–	–
40–49 (n = 1,212)	628	48.10 (44.57–51.64)	3.72 (3.00–4.60)	<0.001	–	–
50–59 (n = 1,298)	796	57.97 (54.25–61.61)	5.54 (4.37–7.01)	<0.001	–	–
60–69 (n = 1,267)	903	69.48 (65.13–73.50)	9.13 (7.01–11.89)	<0.001	–	–
70–79 (n = 1,003)	760	76.82 (72.74–80.45)	13.30 (10.20–17.34)	<0.001	–	–
Residence (East-West)[b]						
West (n = 4,484)	2,067	44.02 (42.10–45.96)	ref	ref	ref	ref
East (n = 2,080)	1,535	68.17 (64.13–71.95)	2.72 (2.24–3.30)	<0.001	0.98 (0.56–1.71)	0.936
Interact term East*age	–	–	–	–	1.03 (1.02–1.04)	<0.001
Residence (north-south)[c]						
North (n = 1,683)	1,029	53.90 49.94–57.81)	1.46 (1.20–1.79)	<0.001	1.17 (0.93–1.46)	0.177
Middle (n = 2,879)	1,650	50.04 (46.12–53.96)	1.26 (1.02–1.53)	0.026	0.98 (0.80–1.20)	0.859
South (n = 2,002)	923	44.38 (41.23–47.58)	ref	ref	ref	ref
Population of municipality						
<5,000 (n = 1,171)	725	56.21 (49.89–62.35)	1.67 (1.27–2.19)	<0.001	1.41 (1.11–1.79)	0.005
5,000–<20,000 (n = 1,598)	904	52.11 (47.50–56.69)	1.41 (1.14–1.76)	0.002	1.18 (0.97–1.44)	0.100
20,000–<100,000 (n = 1,941)	975	43.50 (40.35–46.71)	ref	ref	ref	ref
>100,000 (n = 1,854)	998	48.72 (44.68–52.79)	1.23 (1.01–1.51)	0.043	1.17 (0.96–1.43)	0.118
Pet in household[d]						
No pet (n = 4,360)	2,400	49.00 (46.64–51.36)	ref	ref	–	–
Any pet (n = 2,045)	1,099	48.46 (45.38–51.55)	0.98 (0.87–1.10)	0.718	–	–
Dog[d]						
No (n = 5,588)	3,070	49.19 (46.93–51.46)	ref	ref	–	–
Yes (n = 806)	424	46.66 (41.94–51.45)	0.90 (0.75–1.09)	0.288	–	–
Cat[d]						
No (n = 5,383)	2,936	48.37 (45.99–50.76)	ref	ref	ref	ref
Yes (n = 1,011)	558	51.46 (47.70–55.21)	1.13 (0.97–1.32)	0.127	1.27 (1.06–1.51)	0.009
Other animals[d]						
No (n = 5,679)	3,126	49.29 (47.04–51.54)	ref	ref	–	–
Yes (n = 715)	368	45.73 (40.62–50.93)	0.87 (0.71–1.06)	0.168	–	–
Eating vegetarian[d]						
No (n = 6,148)	3,413	49.74 (47.54–51.93)	ref	ref	ref	ref
Yes (n = 248)	94	35.77 (28.21–44.11)	0.56 (0.40–0.80	0.002	0.62 (0.42–0.99)	0.048
Body mass index (BMI)						
Underweight (BMI < 18.5) (n = 86)	22	23.95 (14.44–37.02)	0.50 (0.27–0.94)	0.031	0.66 (0.34–1.28)	0.212
Normal weight (18.5 ≤ BMI < 25) (n = 2,430)	1,063	38.44 (35.83–41.12)	ref	ref	ref	ref
Overweight (25 ≤ BMI < 30) (n = 2,457)	1,470	54.94 (52.08–57.76)	1.95 (1.70–2.24)	<0.001	1.23 (1.03–1.47)	0.024
Obesity (BMI ≥ 30) (n = 1,552)	1,023	58.99 (55.53–62.39)	2.35 (2.15–2.55)	<0.001	1.28 (1.01–1.55)	0.048
Diabetes (12 month prevalence)						
No (n = 6,072)	3,253	47.92 (45.76–50.10)	ref	ref	ref	ref
Yes (n = 432)	313	67.12 (60.87–72.82)	2.22 (1.70–2.90)	<0.001	0.82 (0.61–1.10)	0.181
Socio-economic status[e]						
Low (n = 1,034)	617	54.93 (50.97–58.83)	1.23 (1.03–1.47)	0.024	1.20 (0.95–1.52)	0.132
Middle (n = 3,933)	2,217	49.77 (47.13–52.43)	ref	ref	ref	ref
High (n = 1,553)	745	41.42 (38.09–44.83)	0.71 (0.61–0.83)	<0.001	0.72 (0.60–0.85)	<0.001
Total (n = 6,564)	3,602	49.08 (46.92–51.23)	–	–	–	–

Table 1. Stratified seroprevalence of IgG antibodies against *T. gondii* detected by ELFA in adults aged 18 to 79 years and results of weighted logistic regression analysis of potential risk factors for seropositivity, 2008–2011. [a]unweighted. [b]Eastern states: Berlin, Brandenburg, Mecklenburg-West Pomerania, Saxony, Saxony-Anhalt, Thuringia. Western states: Baden-Württemberg, Bavaria, Bremen, Hamburg, Hesse, Lower Saxony, Northrhine-Westfalia, Rhineland-Palatinate, Saarland, Schleswig-Holstein. [c]Northern states: Schleswig-Holstein, Hamburg, Lower Saxony, Bremen, Berlin, Brandenburg, Mecklenburg-West Pomerania. Middle states: Nordrhine-Westfalia, Hesse, Saxony, Saxony-Anhalt, Thuringia. Southern states: Rhineland-Palatinate, Baden-Württemberg, Bavaria, Saarland. [d]at day of interview. [e]Socio-economic status is measured using a score composed of income, education and professional status.

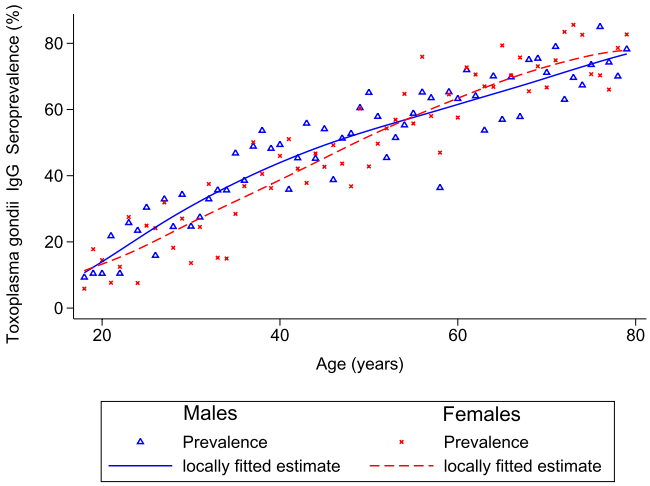

Figure 1. Estimated *T. gondii* seroprevalence, by age (18–79 years) and gender, in Germany, 2008–2011.

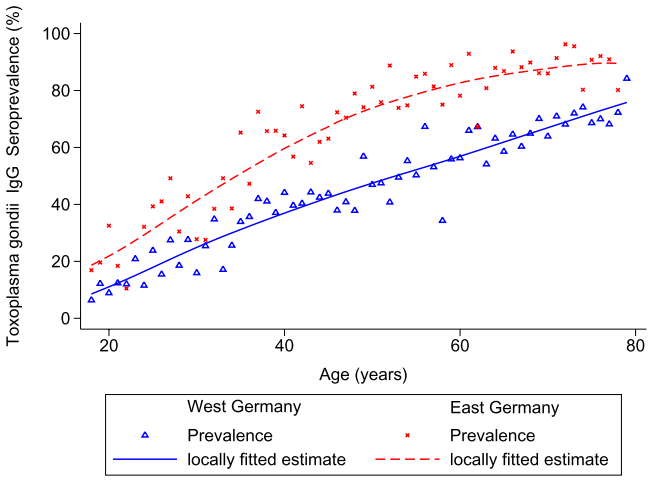

Figure 2. Estimated *T. gondii* seroprevalence, by geographical origin (East-West) and gender, in Germany, 2008–2011. Berlin is considered as East.

by multivariable analysis adjusted for age and gender as well as for associated factors (OR: 0.84; 95%-CI:0.55–1.29) from analysis in Table 1.

Discussion

This first nationwide representative seroprevalence study for *T. gondii* antibodies in Germany finds a high seroprevalence compared to other countries. Comparing our findings to previous data from Germany is hampered by the older studies' lack of representativeness, but higher prevalences were found in a serosurvey among blood donors between 1994 and 1996 in North-East Germany (59%; n = 4,854; age 20–40)[27], as well as in a study among pregnant women from South-West Germany (39%; n = 5,670; age 15–47)[28]. Thus our findings suggest a decrease in *T. gondii* infections in Germany over the last two decades. Similarly, in France, where comprehensive serosurveys reported a higher overall seropositivity in women of childbearing age (36.7%; n = 15,130; age 15–49), it decreased from 54.2% in 1995 to 43.8% in 2003 and to 36.7% in 2010[29]. In The Netherlands in 2006–2007, seroprevalence was significantly lower than in our study (26%; n = 5,541; age 0–79), decreasing from 41% in 1995/1996[30]. In the USA, seroprevalence declined from 14.1%; in 1988–1994 to 9.0% (n = 10,477; age 12–49) in 1999–2004[31]. These differences show that improvements for *T. gondii* infection prevention in Germany are necessary and also possible.

The case numbers from the German notification system are restricted to congenital toxoplasmosis and range between 23 cases in 2008 and 6 cases in 2014, without obvious time trends or regional clustering[32]. This is only a small fraction of cases we calculated in this study. Reasons for this discrepancy could be a high degree of under-ascertainment and under-reporting, as not all cases of congenital toxoplasmosis are laboratory confirmed, and laboratories do not have the clinical or demographic information to carry out the notification. As a result, current notification rates do not reflect the full picture of disease burden in Germany. Our extrapolations used

Age group	Annual incidence in 100,000 persons	95% confidence interval
Women		
18–79 years	1,189	1,077–1,301
18–49 years	1,325	1,007–1,642
50–79 years	944	620–1,268
Men		
18–79 years	1,007	897–1,117
18–49 years	1,436	1,186–1,686
50–79 years	794	454–1,134
Total	1,099	1,016–1,181

Table 2. Annual incidence of seroconversions of IgG antibodies against *T. gondii* stratified by sex and age groups.

Age of the mother at child birth[a]	Number of women in German population[b]	Annual expected number of seroconversions[c]	Number of births in Germany[b]	Proportion of women giving birth (in %)	Proportion of seronegative women (in %)[d]	Annual expected number of seroconversions among pregnancies (% of pregnancies at risk)
15–19	1,985,672	26,310	16,459	0.83	90.8	159 (1.1)
20–24	2,421,962	32,091	88,777	3.67	84.5	857 (1.1)
25–29	2,447,109	32,424	191,010	7.81	78.1	1,843 (1.2)
30–34	2,437,824	32,301	222,218	9.12	71.7	2,144 (1.3)
35–39	2,359,922	31,269	115,634	4.90	65.4	1,116 (1.5)
40–44	3,116,101	41,288	27,131	0.87	59.0	262 (1.6)
45–49	3,490,840	46,254	1,256	0.04	52.6	12 (1.8)
Total	18,259,430	241,937	662,485	–	74.1	6,393 (1.3)

Table 3. Seroprevalence of *T. gondii* infection by age group in women of childbearing age. [a]Estimates for the 15–17 year-old were derived from the study data assuming constant increase as in the age group 18 to 25 years. [b]German Federal statistical office, 2011. [c]at an incidence of 1,325 in 100,000 inhabitants. [d]inverse of prevalence, estimates from the regression model.

Lifetime depression (total no.)[a]	n (pos)[a]	Prevalence (95% CI)	Univariable analysis		Multivariable analysis[b]		Multivariable analysis[c]	
			Odds ratio (95% CI)	p-Value	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
No (n = 5,747)	3,161	49.12 (46.90–51.34)	ref	ref	ref	ref	ref	ref
Yes (n = 768)	409	47.56 (43.05–52.11)	0.94 (0.78–1.13)	0.497	1.05 (0.84–1.19)	0.365	0.84 (0.55–1.29)	0.420

Table 4. Stratified seroprevalence of IgG antibodies against *T. gondii* detected by ELFA in adults aged 18 to 79 years, and results of weighted logistic regression analysis of seropositivity on depression as an outcome, 2008–2011. [a]unweighted, lifetime incidence of depression (self-reported) was assessed by asking the closed question "Have you ever been diagnosed with depression by a physician or a psychotherapist"? [b]adjusted for age, sex and interaction between both. [c]adjusted for variables in multivariable model of Table 1.

manifestation rates from Li *et al.*[9] and Dunn *et al.*[10] for the calculation of incidence of congenital toxoplasmosis,which is independent of the influence of disease surveillance processes and therefore might provide a more accurate picture.

Calculations of the incidence of ocular and cerebral toxoplasmosis based on seroconversion rates are difficult, since information from surveys on manifestation rates is scarce, and the few sources available are outdated. Jones and Holland[33] estimated, based on North American surveys in the 1970s, that 2% of *T. gondii*-infected individuals develop ocular toxoplasmosis. Extrapolated to incidence of seroconversion in Germany, this results in 22 patients of clinical ocular toxoplasmosis per 100,000 inhabitants annually. Due to an increase of immunosuppression in the aging population it can be assumed that incidence of cerebral toxoplasmosis is also increasing, although data on the population level are missing.

We found no evidence for a role for *T. gondii* in depression. This supports previous evidence from the population-based United States National Health and Nutrition Survey (NHANES III)[25]. Altogether, evidence on manifestation rates of all health outcomes of *T. gondii* infections is scarce and therefore studies have to be initiated to eliminate these limitations and to fully understand the disease burden of toxoplasmosis.

Seropositivity strongly increases in all age groups, indicating a strong force of infection also in the elderly. Frequent seroconversion in this group is particularly problematic since immunosuppression becomes more

prominent with age. The observed increase is particularly strong in East Germany, arguing that the risk pattern changed after unification of Germany in 1990. Alternatively, this could be explained by a birth cohort effect of a previous time frame, when a higher risk of seroconversion resulted in higher seroprevalence today in older age groups. Although not associated in univariable analysis, men have a 1.76-times higher chance of being seropositive in multivariable analysis. This can be explained by effect modification of the factors higher socio-economic status, cat holding and male gender included in the final model. Since male Germans eat about twice as much meat and meat products as females[34], the higher seroprevalence we observe in men can be reconciled with these eating habits. In our study, seroprevalences for *T. gondii* vary between regions in Germany. The consumption of freshly prepared raw minced meat (beef and pork), known in Germany as "Hackepeter" or "Mett", is more widespread in East compared to West Germany[35,36]. This may explain the higher seroprevalence in East Germany and additionally confirms that raw meat poses a substantial risk for infection with *T. gondii*[4,37].

Furthermore, our results show that overweight and obese study participants have an increased chance of becoming seropositive, consistent with recent data from a smaller study in Germany[38]. There is good evidence that higher meat consumption is connected to an increase in body weight[39,40],which in turn leads to a higher chance to ingest contaminated meat. Eating vegetarian, on the other hand, is negatively associated with seropositivity, arguing that consumption of oocyst-contaminated vegetables is not a major driver of seroconversion. Altogether, these data are consistent with the majority of infections being food-borne[4,37,41].

Some previous epidemiological studies did not observe an elevated risk for cat owners[37,42], while representative serosurveys in The Netherlands have reported such an association[30,43]. A case-control study in the USA reported a higher risk for cat owners with 3 or more cats[44]. Cats are biologically essential in the life cycle of *T. gondii,* but cat exposure seems to be a less important factor compared to exposure to contaminated food, in particular meat. Nevertheless, prevention of infection through contact with cats is possible since they shed oocysts only for 1–3 periods during lifetime, and sporulation can be avoided by daily removal of cat litter[45].

The strengths of our study include a large sample size with a sampling process leading to a representative study population, with an appropriate adjustment for demographic variables. As the DEGS1 study recruited only the adult population, we have no data on infants and children. This deficiency should be addressed in the future. The analyses in our study are based on seroprevalence data, but seroconversion is not equivalent to clinical manifestation of disease. The associated factors in our study are mostly contextual factors. Specific and proximal factors like lifetime cat exposures or lifetime raw meat consumption were not available from the survey. The same is true for the association to lifetime depression, which is only one mood disorder hypothesized to be connected to *T. gondii* infections.

Toxoplasmosis is hard to combat from the public health point of view and often neglected in programs targeting food-borne diseases[46]. Primary prevention of infections is important and can prevent a significant proportion of disease cases of all clinical manifestations[47]. Since a *T. gondii* infection can reactivate upon immune suppression, it should be avoided in all population groups to prevent subsequent ocular or cerebral disease later in life.

Secondary prevention in the form of a repeated serological screening during pregnancy might be successful to prevent further pregnancy-associated cases. Arguments for serological screening have to consider the positive predictive value of the screening tests and the risk-benefit ratio of medication used to treat infection. The effectiveness of prenatal screening and treatment is debated, largely because of different opinions on cost-benefit[48,49], health consequences of the screening for the mother[50], and the effectiveness of the treatment[16,51]. Nevertheless, early serological screening of pregnant women to detect maternal seroconversion, followed by rapid treatment in utero (as is done in France and Austria) may prevent transplacental transmission and thus neonatal infection and clinical manifestation[11–14]. In these countries, the key for success seems to be frequent re-testing using highly sensitive PCR analysis of appropriate maternal samples and rapid treatment[2,52]. This practice might explain the striking differences in percentages of observed hydrocephalus cases in newborns of congenital infections seen between France and the US (0.3–0.8% vs. 31%)[53–55]. In the latter country, this regimen is not practiced. In Austria, with its systematic prenatal screening program, a 6-fold decrease in transmission compared to untreated women was recently reported[56]. At the rates of congenital infection reported here, screening is considered to be cost-saving[57]. In Germany, screening tests to detect *T. gondii* infection during pregnancy are currently not covered by statutory health insurance, after scientific evaluation of its pros and cons by their medical services[58]. As a result, pregnant women have to self-pay for these tests. This significantly affects the testing rate, especially in women of lower socioeconomic status[59].

Patient numbers and preventable cases are expected to be significantly higher than can be inferred from the notification system. It should be re-evaluated whether serological testing of pregnant women should be offered by physicians and paid for by health insurance in Germany. Moreover, women of childbearing age and those immunocompromised should be more specifically targeted and informed about potential risks, with emphasis on the prevention of food-borne infection pathways. Finally, veterinary services, the meat industry and agriculture should continue their efforts to reduce *T. gondii* in meat, especially pork, as this is frequently eaten raw in Germany.

Methods

The German health interview and examination survey of adults (DEGS1) was a representative, nationwide cross-sectional survey conducted between 2008 and 2011. Detailed study descriptions have been presented previously[26]. Briefly, it followed a stratified two-stage cluster sampling strategy to assess the health status of adults between 18 and 79 years of age in Germany. Three professionally trained teams each visited a total of 180 sample points. The sample points are distributed across Germany according to federal state and municipality size in order to reflect the distribution of the German population. After written informed consent 7,988 persons (age 18–79) participated in DEGS1. These were interviewed with a standardized questionnaire and blood samples were given. The response rate among those approached was 48.4% and the analysis of non-responder questionnaires revealed

high population representativeness among participants. Interview data were used to assess potential risk factors for positive outcome[26]. DEGS1 was approved by the ethical review board of the Charité Medical School, Berlin, Germany (No. EA2/047/08) and all methods were carried out in accordance with the approved guidelines.

Aliquots of sera (thawed twice) from DEGS1 were analyzed for the presence of anti-*T. gondii* IgG antibodies by a commercial automatic and quantitative enzyme-linked fluorescence assay (ELFA; VIDAS TOXO IgG II; Biomérieux SA, France) of the same batch according to the manufacturer's instructions. In comparative studies, sensitivity of the assay was shown to be above 99% and specificity above 98%[60]. Titers of 0–3 were considered negative, 4–7 equivocal, and $\geq$8 positive. We excluded all samples with equivocal test results (1.5%) from the analysis.

Univariable and multivariable logistic regression used sampling weights, accounted for the cluster structure of the survey design and checked for effect modifications. Survey weights based on age, sex, residence in West or East Germany and nationality (German vs. non-German) were calculated to correct for deviations from the German population statistics (December 31, 2010) and used throughout the analysis.

We investigated associations between explanatory variables and seropositivity by univariable logistic regression analyses. The most frequent category was selected as the reference category except for variables where a norm category was available. All variables below p-value of 0.15 were included in a multivariable logistic regression model. As seropositivity regarding *T. gondii* is cumulative and therefore strongly related to age, all two-way interactions involving age were included. Age-related prevalence was smoothed by using the Lowess procedure of the program Stata 12.1 and graphed stratified for sex and East-West geographical origin. The annual incidence of seroconversions was calculated from linear regression using the one-year age group as independent and weighted prevalence as dependent variable. The annual number of seroconversions among pregnant women between 15 and 49 years of age in Germany was calculated by using the number of births in each one-year age group of mothers multiplied by the expected proportion of susceptibles, considering an annual incidence of 1,325 in 100,000 women, and corrected for the mean duration of pregnancies (266 days). For the age group 15 to 17 years, prevalence estimates were derived from the study data, assuming a constant increase as in the age group 18 to 25 years.

For the calculation of seroconversions among pregnancies, data on number of women and number of childbirths by age of the mother for 2011 were retrieved from the German Federal statistical office (https://www.destatis.de/). In the calculations we regarded multiple births by looking at how many children each respondent had.

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Author Contributions

H.W. conceived the study, analyzed data, and generated figures. M.T. coordinated and supervised the samplings and organized sample logistics. T.A. and K.S. co-designed and overlooked the study and interpreted data. F.S. organized on-site sample logistics, supervised the laboratory tests and analyzed data. H.W., T.A. and F.S. wrote the manuscript.

Additional Information

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2.2. Studien zu Ausbrüchen durch Lebensmittelverzehr

2.2.1. Fall-Kontroll-Studie zu EHEC O104 identifiziert Salatbar, Frankfurt (Main) 2011

Wilking H, Götsch U, Meier H, Thiele D, Askar M, Dehnert M, et al. Identifying risk factors for shiga toxin-producing *Escherichia coli* by payment information. *Emerging infectious diseases*. 2012;18(1):169-70. doi: 10.3201/eid1801.111044*

*diese Veröffentlichung ist eine Research Letter als Kurzbericht mit Originaldaten aus einer analytischen Studie (78). Diese wurde vorher und danach nicht in einem wissenschaftlichen Journal mit Gutachterverfahren publiziert. Eine Vorveröffentlichung hat in entsprechenden Sachstands- und Abschlussberichten des RKIs stattgefunden (79, 80). Diese Veröffentlichung unterlag einem Peer-Review-Verfahren, es gab drei Gutachten.

Zu einem frühen Zeitpunkt wurde ein regional isoliertes Cluster unter Mitarbeitern einer Firma mit zwei Standorten eines Frankfurter Unternehmens in Frankfurt (Main) auffällig (78). Zwischen dem 8. Mai und 23. Mai 2011 erkrankten insgesamt 60 Mitarbeiter an blutigem Durchfall; 18 davon entwickelten ein HUS. Für insgesamt 51 von 60 Patienten konnten tagegenaue Daten zum Erkrankungsbeginn zwischen dem 09. Mai und 17. Mai 2011 erhoben werden, die zur Darstellung der zeitlichen Dynamik innerhalb der Untersuchungen genutzt wurde (78). Eine bakteriologische Untersuchung bestätigte in vier Patienten den O-Typ O104 und den Shigatoxin-Typ *stx2*, kompatibel mit dem Ausbruch in Norddeutschland, darüber hinaus noch in fünf Patienten alleine den O-Typ O104 und in zwei Patienten den Shigatoxin-Typ *stx2* der nicht ausschließlich vom *E. coli* Typ O104 produziert wird. Die Patienten mit HUS oder Shigatoxin-Typ *stx2*-Nachweis waren meldepflichtig an das Gesundheitsamt. . Es wurde eine Untersuchung bei Kunden der zwei Betriebs-Kantinen durchgeführt, um Hinweise auf das Infektionsvehikel zu erhalten (78). Die Kantinen boten unterschiedliche Hauptgerichte in ihren zwei Filialen an. Darüber hinaus gab es eine Auswahl an rohen Salaten und Gemüse (30 unterschiedliche Sorten), sowie Früchten (Fruchtsalat, Obst auf die Hand), Desserts und regelmäßig Spargelgerichte. Zu diesem frühen Zeitpunkt war den lokalen Behörden weder der später vermutete Zusammenhang mit Sprossen, noch ein möglicher Zusammenhang mit dem Verzehr von Tomaten, Salatgurken und Blattsalat bekannt. Das RKI wurde im Laufe des Geschehens in Frankfurt (Main) um Unterstützung der Untersuchungen, insbesondere des epidemiologischen Designs und der statistischen Analysen gebeten.

Es wurde recht schnell deutlich, dass es zu Schwierigkeiten bei der Erinnerungsfähigkeit der Patienten gekommen ist. Daher entschieden wir uns für eine Studie unter Verwendung von Daten basierend auf dem obligatorischen, bargeldlosen Kantinen-Bezahlsystem entschieden (78).

Insgesamt wurden 23 frühe Fälle von blutigem Durchfall und eine zufällige Auswahl (nicht gematcht) an nicht erkrankten Personen (n=30) eingeschlossen (eingebettete

Fall-Kontroll-Studie) (78). Todesfälle wurden ausgeschlossen. Mitarbeiter, die an der Salatbar gekauft hatten, hatten eine mehr als fünffach höhere Chance (OR: 5,83; 95%-KI: 1,42-23,88) an blutigem Durchfall zu erkranken im Vergleich zu Kantinenbesuchern, die keinen Salat gekauft hatten. Dies war unabhängig von Alter und Geschlecht. Der Erwerb von Hauptgerichten, Desserts und Früchten war nicht assoziiert.

Somit konnte ein Bestandteil der Salatbar als Vehikel bestätigt werden. Obwohl der Erwerb nicht unbedingt gleichzusetzen ist mit dem Verzehr von Lebensmittel konnten durch dieses Vorgehen schnell aussagekräftige Ergebnisse erzielt werden. Darüber hinaus konnte Misklassifizierung durch schlecht erinnerbare Lebensmittel-Einkäufe reduziert werden. Die Ergebnisse wurden am 02. Juni 2011 den Kolleginnen und Kollegen zur Prüfung übergeben und am 03. Juni 2011 in einer Presseerklärung des RKI veröffentlicht (81). Außerdem gingen sie in den Sachstandbericht des RKI vom 30.06.2011 (80), dem Abschlussbericht des RKI (79) und basierend darauf in mehreren Berichte von Behörden des öffentlichen Gesundheitsschutzes und des Verbraucherschutzes ein.

Die Ergebnisse dieser Studie konnten zeigen, dass die Ursache des HUS/EHEC O104-Ausbruch in 2011, trotz der unklaren Informationslage in den Vortagen, nicht in Fleisch- oder Milchprodukten zu vermuten ist. Das Untersuchungsteam konnte sich weiterhin auf pflanzliche Produkte konzentrieren, was sich in den Folgetagen als richtig herausstellen sollte. Die Ergebnisse der bundesweiten Warenstromanalysen ergaben, dass beide Kantinen über den Betreiber und einem Zwischenhändler mit dem Hersteller von Sprossen in Verbindung standen, der auch für den Ausbruch in Norddeutschland verantwortlich war. Es wurde bestätigt, dass diese Sprossen auch in beiden Kantinen angeboten wurden. Befragung der Kantinenbesucher zu den einzelnen spezifischen Salatbestandteilen, die von der Salatbar genommen wurden, war nicht möglich.

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Identifying Risk Factors for Shiga Toxin–producing *Escherichia coli* by Payment Information

To the Editor: During May and June 2011, a large outbreak of hemolytic uremic syndrome (HUS) and diarrhea caused by Shiga toxin–producing *Escherichia coli* (STEC) occurred, centered on northern Germany (*1*,*2*). Early on, salads and raw vegetables were suspected to be food vehicles (*3*). Also in May, the staff department of a local company informed the Health Protection Authority in Frankfurt in southwestern Germany about the rapidly increasing number of patients with bloody diarrhea and HUS among employees at 2 company office sites. Both sites were served by cafeterias run by the same caterer. Main dishes were prepared in the cafeterias' kitchens and differed between the 2 sites. However, in both cafeterias various fresh foods from a salad bar and fruits, desserts, and daily asparagus dishes originated from the caterer's main kitchen. The salad bar included 30 items. Suspecting that this outbreak was linked to the one in northern Germany, we conducted an outbreak investigation to confirm the epidemiologic link to focus epidemiologic and traceback investigations.

A face-to-face survey among hospitalized employees and by email among all other employees was conducted, which included personal details, symptoms, and information about general food eaten at the cafeterias. We defined outbreak cases as infections in employees of the company at 1 of the 2 sites who by May 23, 2011, were either hospitalized with bloody diarrhea or HUS or who self-reported onset of bloody diarrhea from May 8 through May 23. A total of 320 persons responded to the survey, and 285 (89%) of 320 of the responders stated they used the cafeterias; 60 employees fulfilled our case definition. Case-patients' median age was 33 years (range 22–60 years), and 36 (60%) of 60 were female. Thirty case-patients were hospitalized; HUS developed in 18 (30%) (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/1/11-1044-FA1.htm). Disease onsets occurred over 9 days. Beginning and magnitude of the outbreak were not different between cafeteria locations. Bacteriologic diagnostics for 11 patients yielded results that are compatible with the outbreak strain (*4*).

We used billing data from the cafeterias' obligatory cashless payment system to ascertain risk factors for disease. A nested case–control study design was chosen, limited to a fraction of the cohort to obtain rapid risk estimates. Exposures included were purchases of any fruit, salad bar item, dessert, or asparagus dish in either cafeteria from May 2 through May 13. On the basis of customer identification numbers, the caterer provided billing information for persons with early cases (n = 23). Controls were randomly chosen persons from the caterer's database whose disease status was checked against the survey information (n = 30) and who did not report symptoms of diarrhea (nonbloody), vomiting, or nausea during the same period. Univariable logistic regression was performed.

In univariable analysis, salad bar purchases were highly associated with illness (odds ratio 5.19; 95% CI 1.28–21.03), and desserts, fruit, and asparagus dishes were not (Table). Three (9%) of the case-patients remained unexposed to salad bar items according to the payment system data. The analysis of main courses

Table. Univariable analysis of risk factors for bloody diarrhea among users of 2 cafeterias in Frankfurt, Germany, 2011

Risk factor	No. case-patients exposed/ total no. (%)	No. controls exposed/ total no. (%)	Univariable analysis* Odds ratio (95% CI)	p value
Salad bar	20/23 (87)	16/30 (53)	5.83 (1.42–23.88)	0.014
Dessert	16/23 (70)	18/30 (60)	1.52 (0.48–4.81)	0.473
Fruits	5/23 (22)	10/30 (33)	0.53 (0.15–1.81)	0.312
Asparagus dish	7/23 (30)	11/30 (37)	0.76 (0.24–2.41)	0.635
Female sex	16/23 (70)	15/30 (50)	2.28 (0.73–7.15)	0.155
Age, y				
<30	12/23 (52)	6/30 (20)	2.80 (0.62–12.66)	0.181
30–<40	5/23 (22)	7/30 (23)	Reference	Reference
40–<50	4/23 (17)	13/30 (43)	0.43 (0.09–2.14)	0.303
≥50	2/23 (9)	4/30 (13)	0.70 (0.09–5.43)	0.733

*Estimates in a multivariable model remained virtually unchanged.

purchased in 1 cafeteria revealed that no such meal had been consumed by >5 (22%) of 23 case-patients. Beginning May 23, the cafeterias were closed for 1 week, and salad sales were suspended for a longer period. There were no additional cases.

These results and the identification of the same rare serotype of O104:H4 renders this a satellite outbreak to the larger outbreak in northern Germany, which is the largest outbreak in terms of HUS ever described worldwide. Sprouts are believed to be the food vehicle (*5*). Sprouts available in the Frankfurt cafeteria salad bars were traced back to a producer of fenugreek sprouts, which appear to be the common source of primary cases in the entire outbreak (*5*). Sprout consumption could not be studied directly in Frankfurt because of the intense media attention on the sprout hypothesis once it had been announced. Also, it was thought that too much time had passed to successfully recall actually selected salad bar items consumed a few weeks previous.

Cafeteria billing information allowed for a rapid investigation while avoiding exposure misclassification attributable to ill-remembered food purchases (*6*). Using data sources independent of individual memory is quite useful. In previous studies, similar tools were successfully applied for the detection of outbreak vehicles. Credit card information was used during an investigation on STEC in beef sausages in Denmark (*7*), supermarket purchase records for STEC in Iceland (*8*), and grocery store loyalty card records for cyclosporiasis in Canada (*9*). Shopper card information was used in the United States in an outbreak of *Salmonella enterica* serovar Montevideo (*10*). However, billing information also could have introduced exposure misclassification, e.g., purchased food that was left uneaten or brought for colleagues. Analysis on ingredient level is often not possible. This study emphasizes the need for recall-independent investigation methods. In settings where such methods are available, they should be exploited early and relevant data saved from routine deletion.

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2.2.2. Kohortenstudie zu EHEC O104 bestätigt Sprossen, Lübeck 2011

Buchholz U, Bernard H, Werber D, Böhmer MM, Remschmidt C, Wilking H, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *The New England journal of medicine*. 2011;365(19):1763-70. doi: 10.1056/NEJMoa1106482

In der zweiten und dritten Woche des HUS/EHEC O104-Ausbruch fiel auf, dass Einzelfälle von Erkrankungen häufig mit Lebensmittelkonsum in spezifischen Restaurants und Kantinen zueinander in Verbindung standen. In einem Restaurant in Lübeck waren Erkrankte aus drei Besuchergruppen bekannt. Am 07. Juni 2011 startete vormittags die Datenerhebung in Lübeck (siehe Abbildung 1). Es konnten vor Ort aus der Buchungsdokumentation 7 weitere Gruppen mit Erkrankungsfällen von blutigem Durchfall oder HUS identifiziert werden und Erkrankte und Nicht-Erkrankte für die Studie rekrutiert werden (62). Insgesamt hatten 8 Patienten ein HUS entwickelt und waren meldepflichtig an das Gesundheitsamt. Der Chefkoch des Restaurants wurde bezüglich sämtlicher Zutaten der Rezepte und Garnierungen befragt und anschließend Besuchergruppen nach dem Vorkommen von klinischen Symptomen und dem Namen des gegessenen Gerichtes. Dies wurde im Sinne einer Kohortenstudie ausgewertet.

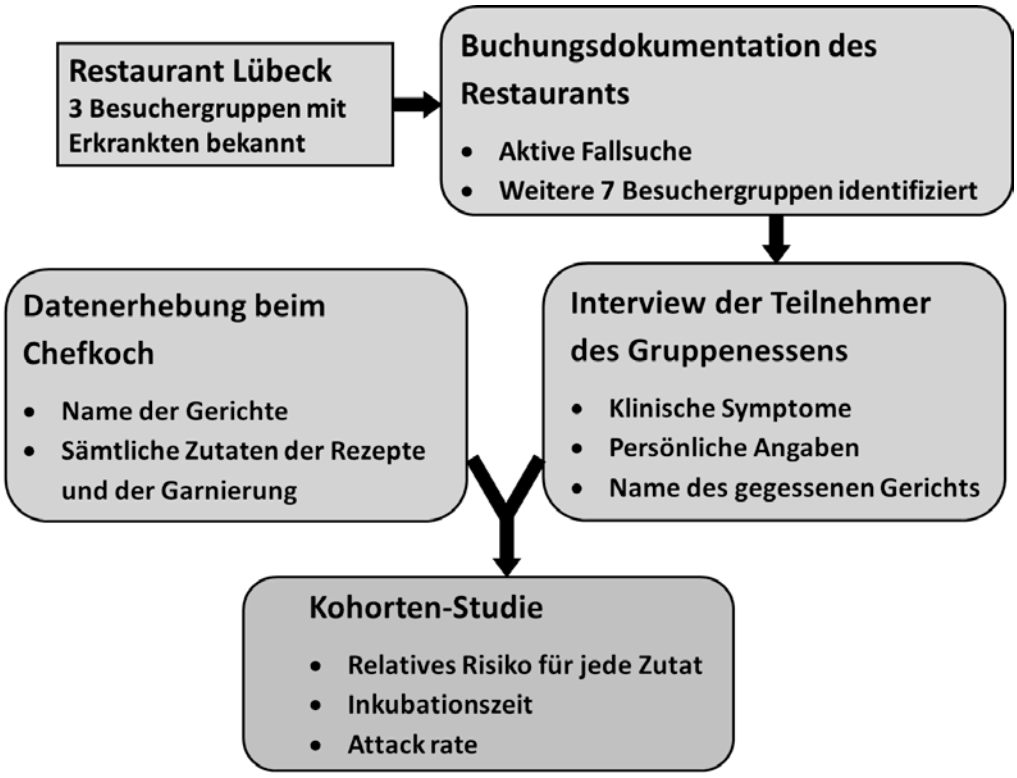

Abbildung 1: Schematische Darstellung des Informationsfluss innerhalb einer Kohortenstudie (rezeptbasierte Studie) zu Risikofaktoren von Erkrankungen mit Nachweisen von EHEC O104, Lübeck 2011. Quelle: Die Abbildung wurde erstellt von Hendrik Wilking.

Die Datenaufbereitung und Auswertung fand in der Nacht vom 08. Juni 2011 auf den 09. Juni 2011 statt. Die Bestellung von Gerichten mit rohen Sprossen wurde als einziger Risikofaktor identifiziert (RR: 14,2; 95%-KI: 2,6-∞) (62). Es gab keinen Patient, der nicht Sprossen bestellt hatte. Andere Zutaten waren nicht assoziiert. Die Ergebnisse wurden am 09. Juni 2011 zusammen mit der parallel erhobenen Evidenz aus Produktrückverfolgungen der Öffentlichkeit präsentiert. Der Zeitraum zwischen Start der Datenaufnahme und Publikation des Risikoschätzers betrug 54 Stunden. Am 10. Juni 2011 gab es eine gemeinsame Presseerklärung der Bundesbehörden, in der die Warnung vor Tomaten, Gurken und Blattsalaten aufgehoben wurde und vom Verzehr von rohen Sprossen abgeraten wurde (59). Die Evidenz von Sprossen als Vehikel wurde wissenschaftlich publiziert (62). Außerdem gingen sie ein in den Abschlussbericht des RKI (79) und basierend darauf in mehrere Berichte von Behörden des öffentlichen Gesundheitsschutzes und des Verbraucherschutzes.

Die Ergebnisse dieser Studie konnten zeigen, dass der Konsum von Sprossen mit sehr hoher Wahrscheinlichkeit die Ursache des größten Ausbruchs von HUS war. In nachfolgenden Untersuchungen konnte eine Charge Sprossensamen, die für den Ausbruch in Deutschland und einen weiteren in Frankreich verantwortlich waren, identifiziert werden. Der größte Anteil war noch nicht in die Lebensmittel-Produktion gegangen und wurde vernichtet. Es wurden mit dieser Maßnahme weitere Ausbrüche mit potentiell vielen Erkrankten und Todesfällen verhindert. Basierend auf den Ergebnissen wurden die Kriterien bezüglich der Lebensmittelsicherheit von Sprossen verbessert.

Das Studiendesign war in seinem Vorgehen bei der Datenintegration zwischen den Patienten und dem Herstellungsprozess (rezeptbasierte Kohortenstudie) sehr innovativ. Es ermöglichte die größtmögliche Unabhängigkeit vom Erinnerungsvermögen der Studienteilnehmer und konnte so Fehlklassifikation von Ausbruchsfällen bezüglich der Exposition (exposure misclassification) reduzieren.

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German Outbreak of *Escherichia coli* O104:H4 Associated with Sprouts

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ABSTRACT

BACKGROUND

A large outbreak of the hemolytic–uremic syndrome caused by Shiga-toxin–producing *Escherichia coli* O104:H4 occurred in Germany in May 2011. The source of infection was undetermined.

METHODS

We conducted a matched case–control study and a recipe-based restaurant cohort study, along with environmental, trace-back, and trace-forward investigations, to determine the source of infection.

RESULTS

The case–control study included 26 case subjects with the hemolytic–uremic syndrome and 81 control subjects. The outbreak of illness was associated with sprout consumption in univariable analysis (matched odds ratio, 5.8; 95% confidence interval [CI], 1.2 to 29) and with sprout and cucumber consumption in multivariable analysis. Among case subjects, 25% reported having eaten sprouts, and 88% reported having eaten cucumbers. The recipe-based study among 10 groups of visitors to restaurant K included 152 persons, among whom bloody diarrhea or diarrhea confirmed to be associated with Shiga-toxin–producing *E. coli* developed in 31 (20%). Visitors who were served sprouts were significantly more likely to become ill (relative risk, 14.2; 95% CI, 2.6 to ∞). Sprout consumption explained 100% of cases. Trace-back investigation of sprouts from the distributor that supplied restaurant K led to producer A. All 41 case clusters with known trading connections could be explained by producer A. The outbreak strain could not be identified on seeds from the implicated lot.

CONCLUSIONS

Our investigations identified sprouts as the most likely outbreak vehicle, underlining the need to take into account food items that may be overlooked during subjects' recall of consumption.

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Members of the Task Force EHEC (Enterohemorrhagic *Escherichia coli*) at the Federal Office of Consumer Protection and Food Safety in Germany, the Danish HUS (Hemolytic–Uremic Syndrome) Investigation Team, the Governmental Institute of Public Health of Lower Saxony HUS Investigation Team, the Lower Saxony State Office for Consumer Protection and Food Safety HUS Investigation Team, the Robert Koch Institute HUS Investigation Team, and the Swedish HUS Investigation Team are listed in the Supplementary Appendix, available at NEJM.org.

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Human infection with Shiga-toxin–producing *Escherichia coli* is a major cause of postdiarrheal hemolytic–uremic syndrome. This life-threatening disorder, which is characterized by acute renal failure, hemolytic anemia, and thrombocytopenia, typically affects children under the age of 5 years. Shiga-toxin–producing *E. coli* O157 is the serogroup that is most frequently isolated from patients with the hemolytic–uremic syndrome worldwide.[1]

In May 2011, a large outbreak of the hemolytic–uremic syndrome associated with the rare *E. coli* serotype O104:H4 occurred in Germany.[2-5] The main epidemiologic features were that the peak of the epidemic was reached on May 21 and May 22[4,5] and that the vast majority of case subjects either resided or had traveled in northern Germany. Almost all patients from other European countries or from North America had recently returned from northern Germany.[2,6,7] Of the affected case subjects, 90% were adults, and more than two thirds of case subjects with the hemolytic–uremic syndrome were female.[4]

Early studies in Hamburg suggested that infections were probably community-acquired and were not related to food consumption in a particular restaurant. A first case–control study that was conducted on May 23 and 24 suggested that raw food items, such as tomatoes, cucumbers, or leaf salad,[3] were the source of infection. The consumption of sprouts, which was previously implicated in outbreaks of Shiga-toxin–producing *E. coli* in the United States[8] and Japan,[9] was mentioned by only 25% of case subjects in exploratory interviews, so consumption of sprouts was not tested analytically.

This report describes the investigations that were conducted by the federal agencies under the auspices of the German Ministry of Health and the Ministry of Food, Agriculture, and Consumer Protection, as well as by the respective state agencies, to identify the vehicle of infection of this international outbreak.

Methods

Study Design

Three types of parallel studies were conducted: one case–control study, one recipe-based restaurant cohort study, and combined trace-back and trace-forward investigations. The main results of all three studies became available between June 2 and June 9, 2011. Results, even if preliminary, were communicated among the investigation groups and the Task Force EHEC (Enterohemorrhagic *Escherichia coli*) at the Federal Office of Consumer Protection and Food Safety in Berlin. If the results were judged to have appropriate validity, they were communicated as soon as possible to the public.

The case–control and cohort studies were conducted within the framework of the Communicable Diseases Law Reform Act of Germany. Mandatory regulations were observed, and review by an ethics committee was not required.

Case–Control Study

From May 29 to June 4, we conducted a case–control study to further specify the type of raw vegetables associated with illness in this outbreak. A case was defined as clinically diagnosed hemolytic–uremic syndrome in an adult who was hospitalized in one of three hospitals in northern Germany, located in the cities of Bremen, Bremerhaven, and Lübeck. Control subjects were individually matched with case subjects on the basis of age group and neighborhood. Case and control subjects were predominantly asked about consumption of fruit and vegetable items, including sprouts, during the 14 days before the onset of illness (for case subjects) or before the interview date (for control subjects) (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org).

Recipe-Based Restaurant Cohort Study

Since the earlier studies had not identified a single source of infection, we conducted a cohort study at restaurant K in Lübeck, Schleswig–Holstein. Preliminary information revealed that several visitor groups with subsequent cases of gastroenteric disease had eaten in restaurant K between May 12 and May 16, 2011, which was defined as the outbreak period in this study. Using the booking notes, we identified cohorts that had eaten in the restaurant during this period and asked all members about the menu items they had consumed. We interviewed the chef of the restaurant about the ingredients and their quantities used to prepare the menu items offered in the restaurant.

A case was defined as an illness in a member of any of the cohorts that was associated with bloody diarrhea, self-reported laboratory-confirmed Shiga-toxin–producing *E. coli* O104 infection, or

the hemolytic–uremic syndrome with an onset of diarrhea within 2 weeks after visiting restaurant K. Non–case subjects were those who remained healthy after visiting restaurant K. We excluded from the analysis all subjects who had diarrhea that was not bloody, who did not have laboratory-confirmed Shiga-toxin–producing *E. coli* O104 infection, or whose disease onset was later than 14 days after visiting restaurant K. For the analysis, we evaluated only data on ingredients that were used in the dishes that were served to the restaurant guests (i.e., data that were solely based on the information obtained from the chef) (for details, see the Supplementary Appendix).

To estimate the total number of cases accrued at restaurant K during the study period, we collected information on the total number of main dishes purchased in this period from billing data. Using the attack rate among persons who were served sprouts from the cohort study, we calculated the number of ill persons as the number of main dishes served containing sprouts times the attack rate among sprout eaters.

ENVIRONMENTAL, TRACE-BACK, AND TRACE-FORWARD INVESTIGATIONS

From physicians, patients, county and regional health departments, and foreign national public health institutes, we received information on clusters or apparently sporadic cases of illness that occurred in the context of the overall epidemic among persons who had probable exposure at only one location or venue (see the Supplementary Appendix). Information was continuously reported to national and local food-safety authorities and to the task force.

On the basis of findings in the early studies, food-safety authorities initially concentrated their investigations on tomatoes, cucumbers, and leaf salads, as well as on other vegetables eaten raw and salad ingredients, including toppings. Local and state food-safety authorities assessed distribution channels of raw food products connected with clusters or single case subjects with single exposures. In addition, both epidemiologically suspected and other raw food items were sampled and, after specific enrichment procedures, were tested by means of immunoassay for Shiga toxin and polymerase-chain-reaction assay for the Shiga-toxin stx2 prophage gene cluster and for genetic markers of the O104:HA strains. The task force initiated a trace-forward investigation for sprouts from suspect producer A to outbreak clusters, a study that was conducted by the food-safety authorities of the respective counties, compiled by the pertinent federal food-safety authorities, and analyzed by the task force. The task force also initiated a trace-back investigation from producer A.

Table 1. Vegetables or Fruits Evaluated in a Case–Control Study in the German Outbreak.*

Food Item	Case Subjects Exposed	Control Subjects Exposed	Matched Odds Ratio (95% CI)	P Value
	no./total no. (%)			
Sprouts	6/24 (25)	7/80 (9)	4.35 (1.05–18.0)	0.04
Cucumbers	22/25 (88)	52/79 (66)	3.53 (0.96–12.9)	0.06
Apples	22/24 (92)	57/81 (70)	3.91 (0.86–17.7)	0.08
Peppers	16/24 (67)	35/80 (44)	2.66 (0.90–7.9)	0.08
Strawberries	19/26 (73)	43/81 (53)	2.33 (0.90–6.0)	0.08

* P>0.10 for raw onions, tomatoes, leaf salad, asparagus, carrots, and basil.

RESULTS

CASE–CONTROL STUDY

We included 26 case subjects (9 male and 17 female) and 81 control subjects in the study. On univariable analysis, the only significant variable was sprouts (Table 1). Other food items, such as raw minced beef and milk and other dairy products, were not significantly associated with illness.

The sequential addition and removal of other variables resulted in a multivariable model containing only sprouts (matched odds ratio, 5.8; 95% confidence interval [CI], 1.2 to 29.0) and cucumbers (matched odds ratio, 6.0; 95% CI, 1.1 to 31.0). Before the date on which the public was advised not to consume sprouts (June 10, 2011), only 6 of 24 case subjects (25%) remembered having consumed them (Table 1, and the Supplementary Appendix). After that date, we wished to ascertain the possible degree of false recall among the case subjects. We tried to recontact all case and control subjects who had not reported sprout consumption previously. Of 8 case subjects who could be reached, 3 (38%) remembered having eaten sprouts in the 14 days before the onset of illness. By contrast, all 37 control subjects who had not reported sprout consumption in previous interviews continued to report that they had not eaten sprouts.

Table 2. Characteristics of 10 Cohorts of Subjects Who Visited Restaurant K between May 12 and May 16, 2011.*

Cohort No.	Size	Subjects with Data	Male Sex	Median Age (IQR)	Subjects with Diarrhea	Subjects with Bloody Diarrhea	Subjects with Laboratory-Confirmed STEC Infection	Subjects with HUS	Subjects Evaluated for Case Definition†	Subjects Fulfilling Case Definition	Attack Rate for Subjects Fulfilling Case Definition
	no.		%	*yr*	*no.*				*no. (%)*	*no.*	%
1	37	37	5	49 (45–57)	10	9	10	4	34 (92)	9	26
2	2	2	50	49 (46–52)	1	1	2	0	2 (100)	1	50
3	31	31	55	57 (45–68)	11	4	5	1	25 (81)	5	20
4	11	10	0	54 (53–55)	2	0	1	0	9 (90)	1	11
5	12	9	0	46 (43–48)	1	1	2	0	9 (100)	1	11
6	19	19	37	32 (15–49)	1	1	1	0	17 (89)	1	6
7	10	10	50	40 (40–42)	0	0	0	0	9 (90)	0	0
8	17	14	57	68 (65–73)	3	3	4	1	14 (100)	3	21
9	25	25	40	74 (69–75)	9	9	6	1	25 (100)	9	36
10	13	11	36	24 (21–48)	3	1	2	1	8 (73)	1	13
Total	177	168	32	53 (42–67)	41	29	33	8	152 (90)	31	20

* HUS denotes hemolytic–uremic syndrome, IQR interquartile range, and STEC Shiga-toxin–producing *Escherichia coli*.

† The case definition was the presence of bloody diarrhea, laboratory-confirmed infection with Shiga-toxin–producing *E. coli*, or the hemolytic–uremic syndrome with an onset of disease within 2 weeks after visiting restaurant K. According to the case definition, 16 subjects were excluded either because they had diarrhea that was not bloody or because the date of the onset of diarrhea was more than 14 days after visiting restaurant K or was unknown.

RECIPE-BASED RESTAURANT COHORT STUDY

We identified 10 cohorts with a total of 177 persons who had eaten at restaurant K (Table 2). Of these persons, we interviewed 168 (95%), including 161 who were interviewed directly and 7 for whom information was obtained from a proxy. Among the 152 persons who could be evaluated for the case definition, 31 (20%) had an illness that fulfilled the case definition. Among these subjects, the hemolytic–uremic syndrome developed in 8 (26%) (see the Supplementary Appendix).

In univariable analysis of all raw food items, only visitors who had been served sprouts were significantly more likely to become ill (Table 3). The P value for the risk ratio for all other items was greater than 0.15. Of 115 persons who had been served sprouts, 31 (27%; 95% CI, 19 to 36) became case subjects, whereas none of 37 persons who had not been served sprouts reported having gastrointestinal symptoms that fulfilled the case definition. Thus, all 31 case subjects had been served menu items containing sprouts. Nearly half the menu items served in the restaurant contained raw sprouts as a garnish or were served with a side salad containing raw sprouts. Side salads contained radicchio, Chinese cabbage, lettuce, cucumber, tomato, and sprouts. No menu item contained cooked sprouts. During this period, the restaurant used only one type of sprout assortment, which was received from a distributor in Schleswig-Holstein and contained four types of sprouts: lentil sprouts, alfalfa sprouts, fenugreek sprouts, and adzuki bean sprouts.

During the outbreak period, 884 main dishes containing sprouts were served to the guests of the restaurant. Applying the attack rate of 27% among sprout eaters, we extrapolated that a total of 239 cases of bloody diarrhea (95% CI, 168 to 318) occurred among customers of this restaurant.

ENVIRONMENTAL, TRACE-BACK, AND TRACE-FORWARD INVESTIGATIONS

The task force identified 41 clusters or cases with single exposures. One of the clusters was a hotel in lower Saxony in which Swedish citizens were affected (cluster 1) (Fig. 1). A "spicy sprout mixture" that was sampled from the distributor (distributor 1) of the hotel's restaurant on June 2, 2011,

Table 3. Relative Risk of Infection Associated with Sprouts and Other Raw Food Items in Univariable Analysis.

Food Item	Total Subjects Evaluated	Subjects Exposed (Percent of Cohort)	Cases among Subjects Exposed (Attack Rate)	Subjects Not Exposed (Percent of Cohort)	Cases among Subjects Not Exposed (Attack Rate)	Relative Risk (95% CI)	P Value
	no.			*no. (%)*			
Sprouts	152	115 (76)	31 (27)	37 (24)	0	14.23 (2.55–∞)	0.001
Tomatoes	152	50 (33)	14 (28)	102 (67)	17 (17)	1.68 (0.77–3.62)	0.18
Cucumbers	152	50 (33)	14 (28)	102 (67)	17 (17)	1.68 (0.77–3.62)	0.18
Chinese cabbage	152	45 (30)	13 (29)	107 (70)	18 (17)	1.72 (0.77–3.71)	0.17
Radicchio	152	45 (30)	13 (29)	107 (70)	18 (17)	1.72 (0.77–3.71)	0.17
Lettuce	152	45 (30)	13 (29)	107 (70)	18 (17)	1.72 (0.77–3.71)	0.17

tested positive for Shiga toxin on a commercial immunoassay. Although confirmatory testing later proved to be negative, immediate trace back led to producer A in Lower Saxony, at which a total of 452 water, seed, sprout, and surface samples tested negative for Shiga-toxin–producing *E. coli* O104:HA. Producer A was licensed as a horticultural farm and produced 18 different sorts of sprouts at the time. Protective measures consisted of regularly instructing employees on the application of proper hygiene necessary for the production of sprouts and the frequent testing of sprouts for salmonella, according to European Union regulations, as well as for coliforms.

All employees of producer A were interviewed, and 5 of 15 had become ill in May 2011 or tested positive for O104:H4. Employees frequently ate sprouts produced at their company. Preferred types were fenugreek, broccoli, and garlic sprouts.

Tracing forward from producer A led to four distributors (Fig. 1). Distributor 1 was connected not only to the restaurant in which cluster 1 had occurred but also to restaurant K. Distributor 4 delivered food to a caterer in Frankfurt that was linked to a cafeteria outbreak that occurred early in the epidemic.[3]

Subsequently, 22 more distributors (for a total of 26) that obtained sprouts from producer A were identified. Distributors were located in 7 of the 16 federal states. Each of the 41 case clusters could be linked with at least one of the identified sprout distributors (Fig. 2).[10] Fenugreek or lentil sprouts were suspected as the outbreak vehicle because these types of sprouts were the common ingredients in two different sprout mixtures that were packaged for distribution by producer A and had been supplied to most of the 41 clusters.

In mid-June, investigations on the origin of sprouts that were consumed by additional case subjects revealed that two case subjects from Lüneburg, Lower Saxony, had eaten a homegrown sprout mix that included fenugreek sprouts. The seeds for these sprouts had been purchased at a retail store that had the same supplier of seeds (supplier X) as producer A (Fig. 1).

DISCUSSION

We report evidence from epidemiologic, microbiologic, and food trace-back and trace-forward investigations that incriminates sprouts as the vehicle of infection in this large outbreak of the hemolytic–uremic syndrome associated with Shiga-toxin–producing *E. coli*. Although definitive molecular evidence is lacking, the argument that sprouts were responsible for this outbreak is strong on the basis of the following five factors: both epidemiologic studies implicated sprouts, the restaurant study showed that 100% of cases of illness could be explained by the consumption of sprouts, no other food ingredient consumed at restaurant K was associated with the risk of illness, all 41 clusters or cases of single exposure could be linked to sprout producer A and its distribution channels, and several employees of sprout producer A who frequently consumed sprouts at the company became symptomatically ill early in the outbreak period or tested positive for Shiga-toxin–producing *E. coli* O104:H4.

Information obtained during the outbreak investigation in Germany already hinted at an outbreak source before producer A in the seed and sprout distribution chain. Seeds that were used by producer A and by the two case subjects from

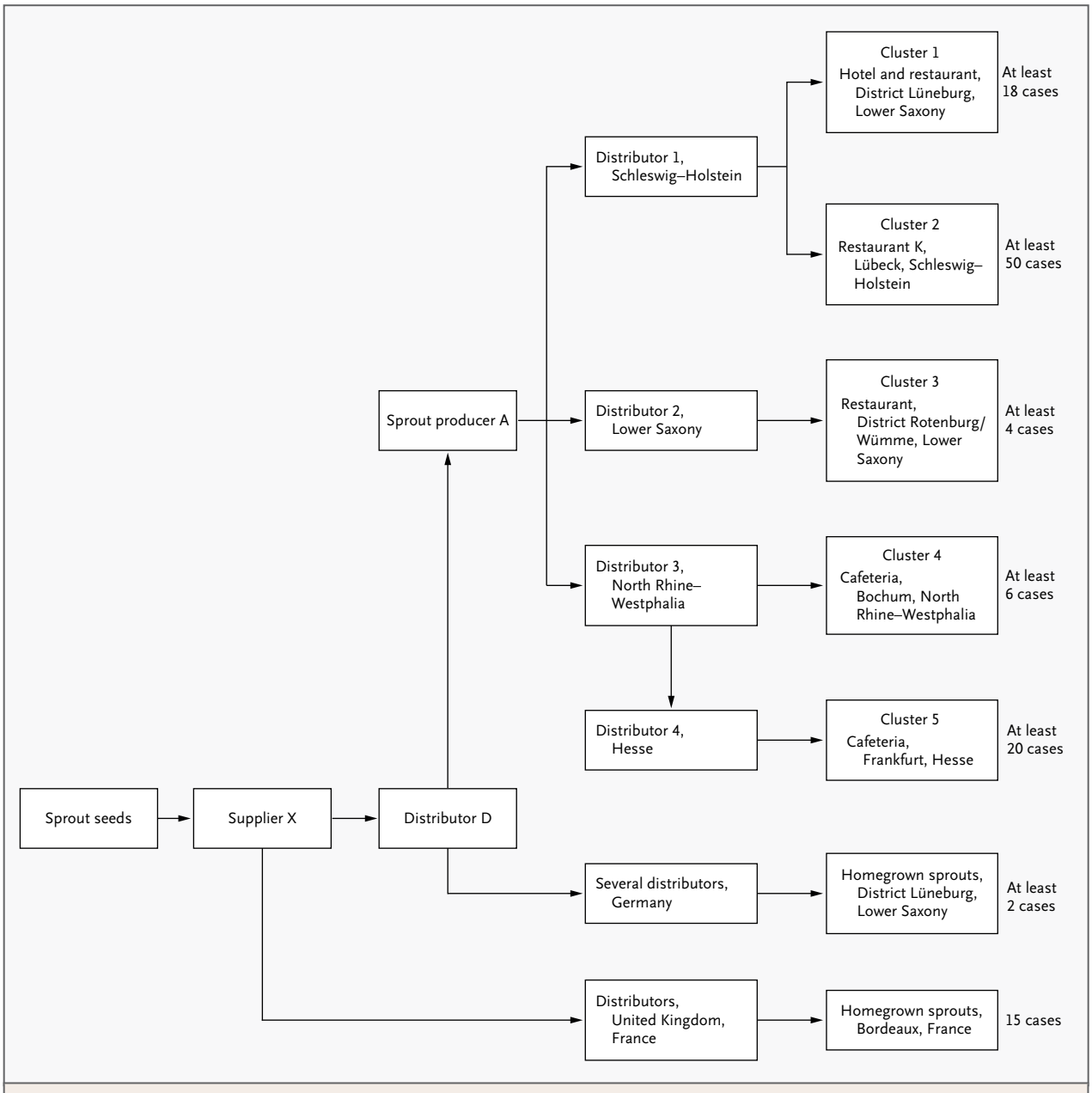

Figure 1. Anatomy of the German Outbreak.

Shown are the trading connections from supplier X to sprout producer A and through four distributors to five outbreak clusters, as well as to two other distributor groups leading to a cluster of illnesses caused by Shiga-toxin–producing *E. coli* in Lüneburg and an outbreak in France unrelated to producer A. Known case subjects at restaurant K included those who were part of the cohort study and others who were not.

Lower Saxony who grew their own sprouts originated from supplier X. In June 2011, an outbreak of the hemolytic–uremic syndrome associated with Shiga-toxin–producing *E. coli* O104:HA occurred in Bordeaux, France.[11] The *E. coli* responsible for the outbreak was genetically related to that in the German outbreak, and there was an epidemiologic association with consumption of homegrown fenugreek sprouts. These findings spurred trace-back investigations by a task force (set up by the European Food Safety Authority (EFSA), which consisted of experts from the European Commission, relevant European Union member states, the European Center for Disease Preven-

tion and Control, the World Health Organization, the Food and Agriculture Organization of the United Nations, and EFSA staff members. It concluded that a certain lot of fenugreek seeds that was imported in late 2009 from Egypt was the most likely common link for the outbreaks in Germany and France.[12] With respect to the point of contamination, it is possible that it occurred at the site where seeds were produced, during transportation, or at the importer. By August 2011, this question had not been resolved.

The account of the outbreak was dramatic: 4321 outbreak cases, including 3469 cases of Shiga-toxin–producing *E. coli* and 852 cases of the hemolytic–uremic syndrome, had been reported by July 26, 2011, when the outbreak was declared to be over.[13] By that time, 50 patients had died. After epidemiologic and food-safety investigations had concurred in identifying sprouts as the outbreak vehicle, the public had been advised on June 10 to abstain from the consumption of raw sprouts, to eliminate raw sprouts in possession at that time, and to remove any sprouts stemming from producer A.[14] In addition, producer A was temporarily closed. Cases of illness still occurred until the end of July 2011, partially as a consequence of secondary transmission,[15] but the number of cases dropped substantially.

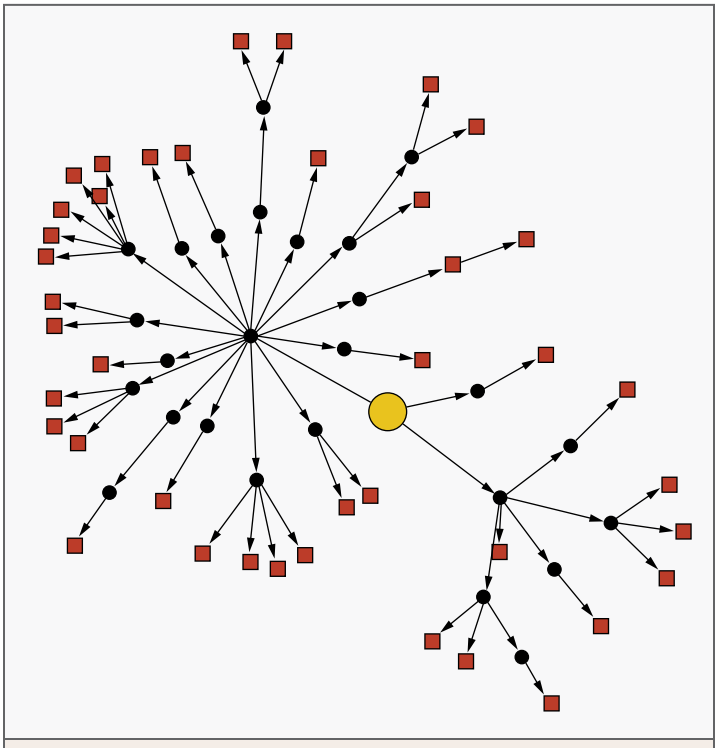

Figure 2. Trading Network Leading to the German Outbreak.

The trading network for the contaminated sprouts led from producer A in Lower Saxony (yellow circle) to 26 sprout distributors (black dots) and 41 identified outbreak clusters (red squares), established by combined back and forward tracing.

Early in the outbreak investigation, raw food products other than sprouts had been suspected as the vehicle. The three studies that we present here built on these findings and complement one another. The early epidemiologic findings helped food-safety authorities to streamline their investigations, which led them to turn their attention to producer A. Since the case–control study was conducted before sprouts came into focus, the significant association between sprouts and illness is therefore revealing and important. Nevertheless, the findings received strong support only through the restaurant study and the food trace-back and trace-forward investigations. The restaurant study provided an idea of why tomatoes, cucumbers, and leaf salad had been suspected early on. The one dish that frequently exposed guests to sprouts was the side salad, which contained tomatoes, cucumbers, three sorts of leaf salads, and sprouts. Sprouts may have been the ingredient that visitors recalled least in such a mixed salad. This hypothesis is also suggested by the results of the repeated interviews in the case–control study. Because the earlier studies had attempted to find a vehicle that explained the majority of cases, sprouts were missed. Although international guidelines[16] generally recommend otherwise, this experience suggests that food items or ingredients that are deemed to be hard to remember should be included in analytical studies, even if such items are mentioned by less than 50% of those surveyed.

Producer A was licensed as a horticultural company. Although hygienic measures were satisfactory and local food-safety authorities had inspected the company routinely under the same conditions as a food-processing company, it became apparent that European legislation has important deficits regarding Shiga-toxin–producing *E. coli*. Production of food that is vulnerable to contamination with this pathogen, such as sprouts or sprout seeds, should be monitored for this organism so that hygienic measures prevent amplification. In addition, both incoming seeds and outgoing food products should be tested for Shiga-toxin–producing *E. coli*.

In general, focused restaurant studies provide a favorable situation to identify the vehicle, even in large, geographically dispersed outbreaks, because place and time of exposure are known and

menu cards can be used to spur visitors' memories. If one or few specific menu items are identified, ingredients can be further analyzed.[17] If not, the reason may be that a common ingredient is contained in many menu items, resulting in the necessity to collect detailed ingredient information of the whole or a large part of the menu,[18,19] information that needs to be obtained from those preparing the food.

In conclusion, we have presented investigative results regarding an outbreak of the hemolytic–uremic syndrome associated with Shiga-toxin–producing *E. coli*. Under favorable circumstances, the recipe-based restaurant cohort study proved to be a quick method for detecting suspected food ingredients with high reliability in a complicated setting of exposures. Recommendations regarding sprout use and consumption may need to be strengthened or adjusted as a consequence of this outbreak.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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2.2.3. Ad Hoc-Surveillance-Implementation und Identifikation des Infektionsvehikels in einem massiven überregionalen Norovirus-Ausbruch, Ostdeutschland 2012

Bernard H, Faber M, Wilking H, Haller S, Höhle M, Schielke A, et al. Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. *Eurosurveillance - European communicable disease bulletin*. 2014;19(8):20719. https://doi.org/10.2807/1560-7917.ES2014.19.8.20719

In 2012 kam es in sechs ostdeutschen Bundesländern zu einem ungewöhnlich großen Ausbruch von Gastroenteritis in einer Vielzahl von Gemeinschaftseinrichtungen wie Kindertagesstätten und Schulen (9). Die meisten Patienten erkrankten zwischen dem 25. September und 28. September 2012. Das RKI wurde am Morgen des 27. September 2012 zunächst aus Brandenburg, später auch von anderen Bundesländern telefonisch unterrichtet. Zu diesem Zeitpunkt lagen keine aussagekräftigen Daten zum Ausmaß des Problems vor.

Es besteht eine Meldepflicht für Gastroenteritis-Ausbrüche in Einrichtungen, aber keine Pflicht zur Übermittlung der Informationen über die Gesundheitsämter hinaus. Außerdem gibt es auch keine Meldepflicht von Gastroenteritis-Einzelfällen in Deutschland. Es gab somit für einen so großen bundeslandübergreifenden Ausbruchs in ungewöhnlich vielen Einrichtungen auch keine klare technische Umsetzung der Datenübermittlung innerhalb des Meldewesen, die routinemäßig vorher entwickelt wurde und in diesem Fall ad Hoc eingesetzt werden konnte. Daher musste kurzfristig nach alternativen Möglichkeiten gesucht werden, um dieses Geschehen zu erfassen und zu überwachen. Es konnte noch am selben Tag eine Arbeitsgruppe mit den betroffenen Bundesländern hergestellt werden, tägliche Telefonkonferenzen durchgeführt und ein Erfassungs-Formular entwickelt werden, das eine einheitliche Datenerfassung vom Gesundheitsamt bis zum Robert Koch-Institut ermöglichte.

Durch das plötzliche Auftreten von Erkrankungen sowohl innerhalb der Einrichtungen, als auch zwischen Einrichtungen wurde früh die Hypothese eines lebensmittelbedingten Ausbruchs verfolgt. Kolleginnen und Kollegen des RKI führten vier analytisch-epidemiologische Studien bei Kindern durch, um das Lebensmittel als Ursache des Ausbruchs zu bestimmen (9). Durch diese Studien konnten Tiefkühlerdbeeren identifiziert werden, die in der Gemeinschaftsverpflegung in den betroffenen Schulen verzehrt wurden (82). Die anschließenden Warenstromanalysen der Lebensmittelsicherheitsbehörden konnten aufzeigen, dass die Tiefkühlerdbeeren aus einer einzigen aus China importierten Charge stammten (9). In der Folgewoche wurde Norovirus unterschiedlicher Genotypen in Patienten und Erdbeeren gefunden.

Die Ergebnisse dieser ad Hoc-Datenerfassung und deskriptiven Analysen konnten die Dimension des Ausbruchs zeigen. Er war mit 11.000 Fällen in 390 Gemeinschaftseinrichtungen der bisher mit Abstand größte aufgedeckte

lebensmittelübertragende Ausbruch in Deutschland (9). Der Verlauf des Geschehens konnte überwacht werden. Durch die Erfassung der Daten konnten Schulen in Berlin, Sachsen und Thüringen identifiziert werden, die gute Voraussetzungen für Fall-Kontroll-Studien boten. Dies führte letztendlich zur Identifikation der Tiefkühlerdbeeren als verursachendes Vehikel. Darüber hinaus wurden die Daten zu den Einrichtungen den Lebensmittelbehörden zur Verfügung gestellt und dienten der Rückverfolgung der Erdbeeren (83). Ein Großteil der Charge dieser Tiefkühlerdbeeren war noch nicht verzehrt, konnte sichergestellt und vernichtet werden. So wurden weitere Erkrankungsfälle verhindert.

Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012

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From 20 September through 5 October 2012, the largest recorded food-borne outbreak in Germany occurred. Norovirus was identified as the causative agent. We conducted four analytical epidemiological studies, two case–control studies and two surveys (in total 150 cases) in secondary schools in three different federal states. Overall, 390 institutions in five federal states reported nearly 11,000 cases of gastroenteritis. They were predominantly schools and childcare facilities and were supplied almost exclusively by one large catering company. The analytical epidemiological studies consistently identified dishes containing strawberries as the most likely vehicle, with estimated odds ratios ranging from 2.6 to 45.4. The dishes had been prepared in different regional kitchens of the catering company and were served in the schools two days before the peaks of the respective outbreaks. All affected institutions had received strawberries of one lot, imported frozen from China. The outbreak vehicle was identified within a week, which led to a timely recall and prevented more than half of the lot from reaching the consumer. This outbreak exemplifies the risk of large outbreaks in the era of global food trade. It underlines the importance of timely surveillance and epidemiological outbreak investigations for food safety.

Introduction

Infection with norovirus is the most common cause of acute infectious gastroenteritis in European countries [1,2], usually manifesting with self-limiting symptoms of vomiting and diarrhoea, with sudden onset and short duration [3]. Large protracted outbreaks of norovirus gastroenteritis are often recognised in institutions such as hospitals and homes for the elderly [4], with person-to-person transmission predominating. Food-borne norovirus outbreaks are common, but still under-recognised [5,6].

In Germany, outbreaks of acute infectious gastroenteritis are notifiable to the local public health departments according to the Protection Against Infection Act of 2001. The health departments conduct epidemiological investigations and take control measures. They also transmit outbreak information electronically to the public health authority of the respective federal state and, subsequently, to the Robert Koch Institute (RKI) on the national level [7]. On request of the state health authorities, the RKI assists in outbreak investigations, including analytical epidemiological studies.

On mid-day of 27 September 2012, the public health authority of the federal state of Brandenburg informed the RKI about several outbreaks of gastroenteritis in schools and childcare facilities in Brandenburg amassing to at least 500 cases. Diarrhoea or vomiting in affected individuals had started on the evening before. All affected institutions offered lunch provided by Caterer X, a company operating across Germany, and a food-borne outbreak was suspected. According to the public health department of one of the affected counties, Caterer X was already aware of gastroenteritis cases in four other German federal states (Berlin, Saxony-Anhalt, Saxony and Thuringia). The RKI informed the two national food safety authorities, the Federal Institute for Risk Assessment (BfR) and the Federal Office of Consumer Protection and Food Safety (BVL), and the public health authorities of the other 15 federal states about the situation and requested information on similar outbreaks. By the evening of the same day, the RKI had knowledge of more than 4,000 cases relating to outbreaks in schools and childcare

facilities supplied by Caterer X in four neighbouring federal states in the east of Germany.

This report focusses on the epidemiological investigations to identify the outbreak vehicle and to prevent further cases. Details of the laboratory investigations are presented elsewhere [8].

Methods

Descriptive analysis

In daily teleconferences, the public health authorities of the affected federal states and the RKI exchanged information on the number of affected institutions (including aggregated case numbers) and on laboratory results from human samples taken in the context of the outbreak. For the descriptive analysis, we defined a case as a person with diarrhoea or vomiting from 19 September through 7 October 2012, who did not test positive for any pathogen other than norovirus and who attended an affected institution. An institution was considered to be affected if it offered meals by any external caterer and if at least 10 cases had occurred in that institution (or, in small institutions, if 10% of persons were cases). We did not restrict affected institutions to those supplied by Caterer X to remain sensitive to the potential involvement of other caterers in this outbreak.

Analytical studies

On the individual level we conducted two case–control studies (CCS) and two surveys in affected secondary schools in three federal states. For the CCS, we interviewed pupils directly at their schools, for the surveys, a web-based (Survey 1) and an email (Survey 2) questionnaire were used. Exposure histories were recorded for menu items offered in these schools for lunch (as listed on weekly menu plans) and other food items available in the school, e.g. in the cafeteria. The canteens of all four schools had been supplied by different regional kitchens of Caterer X.

The causative agent was unknown at the start of all four studies, but was suspected to be norovirus or bacterial toxins, based on reported symptoms and the sudden and almost simultaneous occurrence of disease within the institutions. Thus, the relevant period of exposure was considered to be the three days before the start of the outbreaks in these institutions (the dates were not identical at the four study sites). In all four studies, we investigated whether eating at the school canteen was associated with illness. We restricted the calculation of food-specific associations to individuals who reported having had lunch at the school canteen on any of the days of the exposure period. Pupils who reported gastroenteric illness in the family in the week before the outbreak period were excluded from the analysis because they could have been secondary cases of illness in their household. We compared cases and controls regarding their food exposures, calculated odds ratios (ORs) and 95% confidence intervals (CI), and assessed statistical significance using Fisher's exact or other appropriate tests. If several food items were associated with disease in univariable analyses with an OR>1, a p value <0.2 and an exposure reported by at least 25% of cases, multivariable logistic regression analysis (exact method for the surveys) was performed with a manual forward selection of variables (cut-off: p<0.2). Statistical analyses were conducted in R [9] for the CCS and Stata [10] for the surveys.

Case–control study 1

CCS1 was conducted on 1 and 2 October in School A in a city in Saxony. The school had experienced a sudden surge of gastroenteritis cases during calendar week 39 (24–30 September) with a peak on Wednesday, 26 September, and had been closed on 28 September due to the outbreak.

We restricted the study to pupils from grades 5 to 7 (10–13 year-olds) because these age groups were predominantly affected in this school. We defined a case as a pupil with onset of vomiting or diarrhoea from 24 to 30 September (outbreak period for CCS1). Of the approximately 70 cases, we selected two thirds for the study using systematic random sampling. Eligible controls were all pupils from three school classes who did not report vomiting or diarrhoea during the outbreak period. The classes were arbitrarily chosen by the deputy head of the school. Assuming an exposure prevalence of 70% among cases, a case-to-control ratio of 1 would have allowed us to detect an OR of 4 with a power of 86% at a significance level of 5%.

We collected information on the participants' age and sex, symptoms, date of symptom onset and on food exposures at the school's canteen during calendar week 39, as indicated by the canteen's menu plan, which listed four meal choices daily. For data entry and immediate univariable analysis on site, we used the Linelist tool, a spreadsheet file developed at the RKI, to assist in the epidemiologic investigation of local outbreaks [11].

On 2 October, we conducted a sub-study restricting the study population to those who had reported eating at the school canteen on Monday 24 September. Cases with symptom onset after 28 September were excluded from the analysis. In this sub-study, participants were explicitly asked for the consumption of strawberry compote because it had not only been part of one main meal but also been offered as a dessert with two of the other three meal choices. The information was collected in an aggregate fashion during a congregation of cases and controls in the school auditorium, by sending each pupil to one of four corners depending on the pupils' outcome and exposure status (a live 2x2 table). The children were first split into two groups based on presence of symptoms, and these groups were divided further based on their recollection of having eaten strawberries. Pupils were explicitly told not

to walk with their friends, but according to their recollection of symptoms and food consumption.

Case–control study 2

CCS2 was conducted on 4 October in School B in the state of Thuringia. The school had not been closed in response to the outbreak. The aim of the study was to investigate whether the results of CCS1 were reproducible in a different geographical region. The methodology was identical to CCS1 with the following exceptions: Pupils from grade 8 (14 years-old) were additionally included, the outbreak period was from 24 through 27 September, and eligible controls came from five arbitrarily chosen classes. Because strawberry compote was offered as dessert with several meal choices, even on the same day, we asked for this food item in an additional question.

Survey 1 (web-based questionnaire)

This study was conducted at School C located in Saxony using an online questionnaire. The school had experienced a sudden surge of gastroenteritis cases during the last two weeks of September and, in response to the outbreak, had been closed on Friday 28 September. Lunches consisted of a main component (e.g. chicken wings), pre-ordered by the pupils and dispensed by canteen staff, and side dishes, salads and desserts for a self-service buffet cart.

Study participants were recruited through a letter, distributed by teachers and addressed to the parents of all pupils of grades 5 to 8 (n=451) present at the school on 5 October. It informed about the aims of the study and invited participation in an online survey. The questionnaire was accessible (password-protected) from 5 October through 8 October. It contained questions on demography (age, sex, grade), potential disease history (symptoms, time course) and food exposure history from 20 through 27 September (choice of three main components and around 10 sides and desserts daily). We defined a case as a pupil with onset of vomiting or diarrhoea from 20 through 29 September. Cases with an onset date 20–23, 24–26 and 27–29 September were defined as first-, second- and third-wave cases, respectively.

Survey 2 (email questionnaire)

This study was conducted at School D in Berlin. On 5 October, 38 cases of gastroenteritis had been notified to the local health department by the head of the school. Because school holidays had just begun at the start of the study, face-to-face interviews were not feasible. We therefore developed a questionnaire covering, in addition to demographic and symptom information, meals served between 24 and 28 September (choice of four dishes daily plus salad buffet). This questionnaire was emailed to the parents of all pupils under the age of 18 years (approximately n=900). Questionnaires could be returned to the RKI via electronic or regular mail between 1 and 5 October. Cases were defined as pupils with onset of diarrhoea and/or vomiting from 24 through 28 September.

Food trace-back investigations

The German Task Force on Food and Feed safety, consisting of food safety authorities of affected states and at the national level, convened on 29 September. The task force coordinated food safety investigations, which also included epidemiological product tracing investigations.

Results

Descriptive analysis

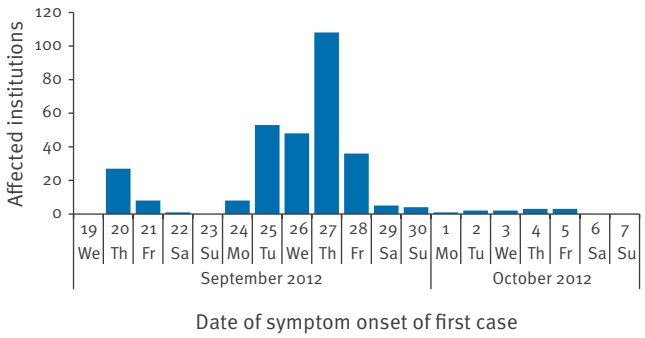
A total of 390 institutions in five federal states in East Germany were reported as affected during the outbreak period. The earliest outbreak in an institution started on 20 September, the latest on 5 October, and most started between 25 and 28 September with a peak on 27 September (n=108 institutions, 28%) (Figure 1). A median of 21 children were affected per institution (inter-quartile range (IQR): 12–37).

The majority of affected institutions were schools (244/390, 63%) and childcare facilities (140/390, 36%), three were facilities for disability care, two were homes for the elderly and one was a rehabilitation clinic.

A total of 10,950 persons, mostly children and teenagers but also staff members, were reported ill in the affected institutions. The median proportion of cases among regular attendees was 14% (IQR: 10–22) across all affected institutions, and 18% (IQR: 12–27) in childcare facilities. At least 38 (0.3%) people required hospitalisation; the majority of illnesses were of short duration and self-limiting. Figure 2 shows the incidence of illnesses among persons under the age of 18 years by district. The federal states of Saxony, Brandenburg and Berlin were predominantly affected, which also reflects the distribution of affected institutions (n=130, 129 and 88, respectively).

Figure 1

Number of affected institutions by date of onset of first case in the respective institution, multistate outbreak of norovirus gastroenteritis, Germany, 2012 (n=309[a])



[a] Date of onset of first case was available for 309 institutions.

As of 8 October 2012, 555 human specimens (339 from ill persons, and a convenience sample of 216 staff members of Caterer X with unknown disease status) were reported by the health authorities of four of the five affected federal states. Of those, 32% were positive for norovirus (40% of ill persons, 20% of staff members). No other viral or bacterial pathogens or bacterial toxins were reported from the respective health departments in connection with the outbreak.

Analytical studies

All four analytical studies, comprising 150 cases and 274 controls, identified dishes containing strawberries as vehicles of infection (either strawberry compote or strawberry fruit quark) (Table).

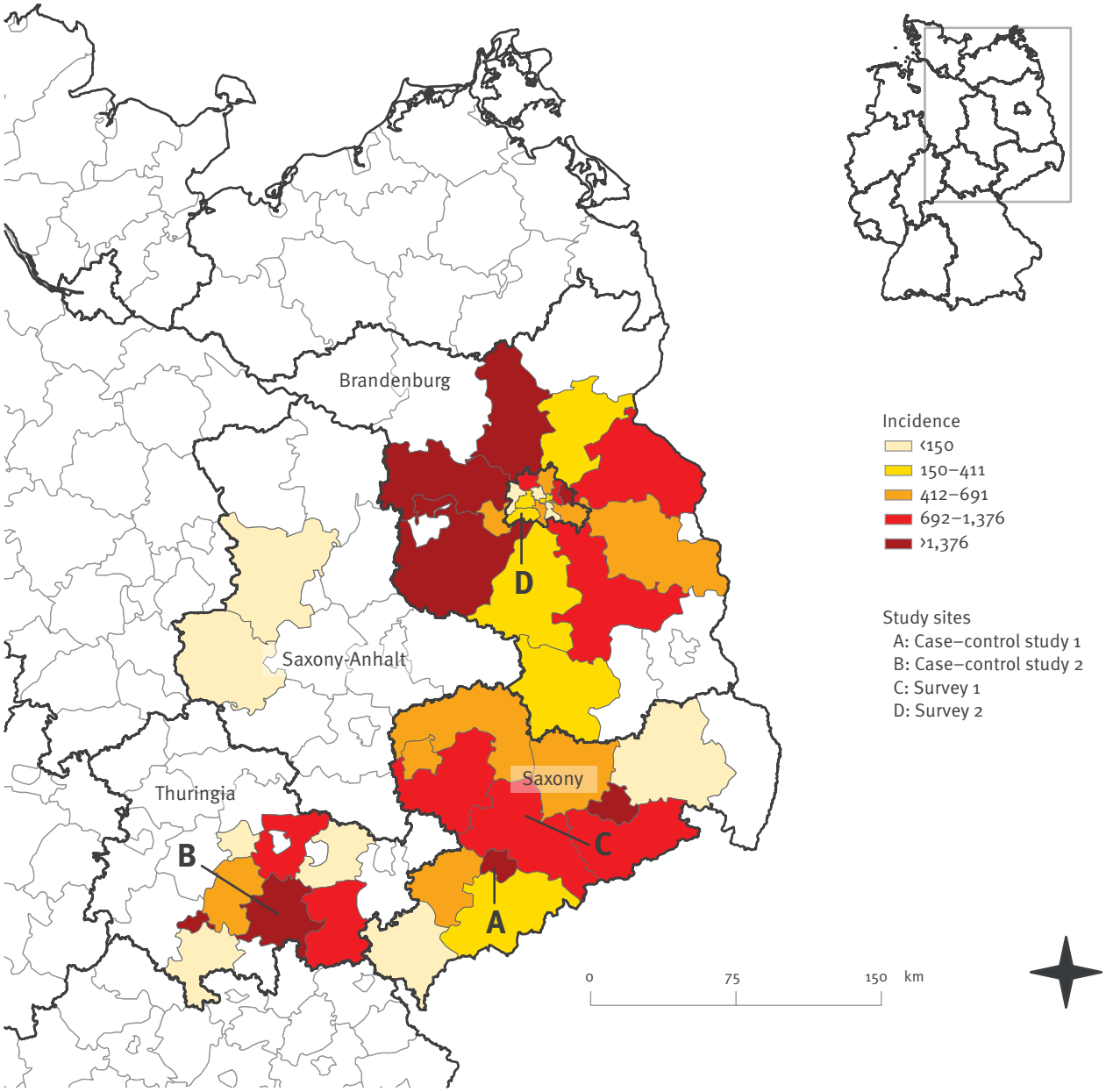
Case–control study 1

We included 43 cases and 54 controls (median age for each: 11 years), three potential secondary household cases were excluded. Symptom onset was from 24 through 30 September, with a steep increase and a peak of the epidemic curve on Wednesday 26 September (n=16 cases) suggesting a point source (Figure 3A).

Most cases, and a higher proportion of cases than controls, had eaten at the school's canteen on Monday 24 and Tuesday 25 September (see Table), but not on the following two days (when a substantial proportion of cases were already sick). The only dish offered on one of these two days that was positively associated

Figure 2

Cases per 100,000 population under the age of 18 years by districts, and locations of analytical study sites, multistate outbreak of norovirus gastroenteritis, Germany, 2012 (n=10,950)



Table

Results of univariable and multivariable risk factor analyses, multistate outbreak of norovirus gastroenteritis, Germany, 2012 (n=424)

School lunch exposure	Cases		Controls		Univariable analysis			Multivariable analysis		
	Total	Exposed (%)	Total	Exposed (%)	OR	95% CI	p value	OR	95% CI	p value
Case–control study 1										
Mon 24 Sep	43	36 (84)	54	41 (76)	1.63	0.59–4.53	0.45	Not performed		
Semolina pudding and strawberry compote	36	26 (72)	40	20 (50)	2.60	1.00–6.77	0.06			
Any strawberry containing dish	36	32 (89)	40	27 (68)	3.85	1.12–13.21	0.03			
Strawberry compote[c]	37	28 (76)	40	11 (28)	8.20	2.66–26.03	<0.0001			
Tue 25 Sep	42	34 (81)	53	37 (70)	1.84	0.70–4.84	0.24			
Wed 26 Sep	43	24 (56)	53	38 (72)	0.50	0.21–1.16	0.13			
Thu 27 Sep	43	17 (40)	53	37 (70)	0.28	0.12–0.66	<0.01			
Case–control study 2										
Mon 24 Sep	39	37 (95)	73	40 (55)	15.26	3.42–68.11	<0.01	[a]		
Semolina pudding and strawberry compote	37	30 (81)	40	17 (43)	5.80	2.06–16.30	<0.01	[a]		
Strawberry compote	37	32 (86)	40	11 (28)	16.87	5.23–54.40	<0.01	16.87	5.23–54.40	<0.01
Tue 25 Sep	39	33 (85)	73	42 (58)	4.06	1.52–10.88	0.01	[a]		
Pasta Bolognese	33	27 (82)	42	25 (60)	3.06	1.04–9.00	0.05	[b]		
Wed 26 Sep	39	3 (8)	73	30 (41)	0.12	0.03–0.42	<0.01	[a]		
Thu 27 Sep	39	9 (23)	73	27 (37)	0.51	0.21–1.24	0.14	[a]		
Survey 1										
First wave										
Thu 20 Sep: Strawberry fruit quark	25	22 (88)	60	15 (25)	22.00	5.28–125.13	<0.001	16.87	5.23–54.40	<0.01
Thu 20 Sep: Fresh plums	20	7 (35)	53	10 (19)	2.32	0.61–8.34	0.15	[b]		
Second wave										
Mon 24 Sep: Strawberry compote	16	12 (75)	27	3 (11)	24.00	3.76–177.14	<0.001	33.80	3.41–∞	<0.01
Tue 25 Sep: Peas and carrots	15	9 (60)	27	2 (7)	18.75	2.63–202.28	<0.001	23.66	2.22–∞	<0.01
Tue 25 Sep: Red cabbage	15	7 (47)	30	5 (17)	4.38	0.88–22.35	0.03	[b]		
Tue 25 Sep: Sauce	18	14 (78)	26	13 (50)	3.50	0.78–18.12	0.06	[b]		
Mon 24 Sep: Semolina pudding	18	13 (72)	32	17 (53)	2.29	0.58–10.08	0.19	[b]		
Tue 25 Sep: Boiled potatoes	14	8 (57)	28	10 (36)	2.40	0.54–10.96	0.19	6.10	0.55–∞	0.14
Third wave										
Wed 26 Sep: Strawberry quark dessert	7	5 (71)	25	1 (4)	60.00	3.31–2,944.34	<0.001	45.42	3.31–2,944.92	<0.01
Mon 24 Sep: Fruit yoghurt	7	5 (71)	26	4 (15)	13.75	1.43–172.24	<0.01	[b]		
Tue 25 Sep: Plain yoghurt	8	4 (50)	24	4 (17)	5.00	0.61–39.84	0.06	[b]		
Survey 2										
Any school lunch 24–28 Sep	14	14 (100)	72	25 (35)	35.00	5.51–∞	<0.0001	Not performed		
Wed 26 Sep: Semolina pudding and strawberry compote	14	10 (71)	25	3 (12)	16.45	2.77–139.30	<0.001			
Wed 26 Sep: Strawberry compote (with any dish)	12	11 (92)	21	5 (24)	30.61	3.19–1,605.80	<0.001			

CI: confidence interval; OR: odds ratio.

Only exposures positively associated with disease occurrence with p<0.2 and explaining at least 25% of cases are shown.

[a] Not considered for multivariable analysis.

[b] Not part of the final multivariable model, i.e. p≥0.2.

[c] Results from a sub-study ('live 2x2 table').

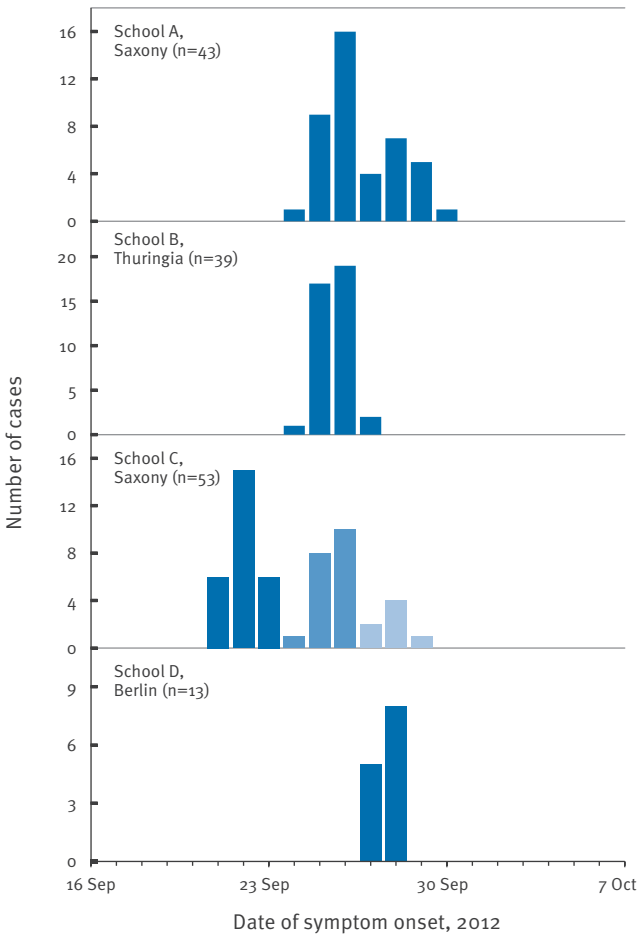
with illness with a p value ‹0.1, was semolina pudding, which had been served on Monday with a choice of cherries, sugar and cinnamon, or cold strawberry compote (Table).

Of the 36 cases, 26 reported to have chosen the semolina pudding. However, on that day strawberry compote had been offered with three of the four meal choices. We therefore compared cases and controls regarding the choice of any meal containing strawberry compote. Cases had chosen significantly (p‹0.05) more often a strawberry compote-containing dish than controls. In the sub-study including those who had eaten at the canteen on Monday 24 September, 28 of 37 cases but only 11 of 40 controls reported to have eaten strawberry compote (OR=8.20; 95% CI: 2.66–26.03; p‹0.01).

Having identified a dish served on Monday 24 September as the likely vehicle of infection, we estimated a median incubation period of two days (IQR: 2–6 days; onset data only available by full days).

Figure 3

Time course of the norovirus outbreak: number of cases included in the four analytical studies, by date of symptom onset, Germany, 2012 (n=148)



The columns for School C are filled with different colours for the three waves of disease (see case definition).

Case–control study 2

We included 39 cases and 73 controls in the analysis (median age: 11 and 12 years, respectively), excluding again three potential household secondary cases. The epidemic curve showed an even steeper increase in case numbers with 36 of 39 of cases with symptom onset on Tuesday 25 (n=17) or Wednesday 26 (n=19) September (Figure 3B).

Most cases, and a higher proportion of cases than controls, had eaten at the school's canteen on Monday 24 and Tuesday 25 September (see Table), but not on the following two days (when a substantial proportion of cases were already sick). On both days, the consumption of one dish was significantly associated with illness: semolina pudding with strawberry compote, sugar and cinnamon on Monday, and pasta with Bolognese sauce on Tuesday (Table). Again, the strawberry compote had been offered with two of the four dishes on Monday, and the association between consumption and illness was even stronger when analysing compote as a separate variable. In multivariable analyses including the exposure variables strawberry compote on Monday and pasta on Tuesday, only the consumption of strawberry compote remained significantly associated with illness (OR=16.87; 95% CI: 5.23–54.4; p‹0.01).

Survey 1 (web-based questionnaire)

We included 54 cases and 75 controls (median age of each: 12 years) in the analysis (participation rate: 29%). The epidemic curve showed three peaks of dates of symptom onset (Figure 3C). Overall, the proportion of persons having had lunch at the school canteen from 20 through 27 September was significantly higher in cases than in controls (98% vs 76%, OR=16.7; 95% CI: 2.4–710.1; p‹0.01).

In univariable analyses (Table) we found an association between being a case in the first wave and the consumption of strawberry quark and fresh plums both served on 20 September. In the multivariable analysis, only the former remained statistically significant (OR=27.13; 95% CI: 5.24–276.40; p‹0.01).

For the cases in the second wave, we found a significant association with having eaten at the school canteen on 24 September (multivariable analysis: OR=11.1; 95% CI: 1.38–88.4; p‹0.05). Of the 35 items served on that and the following day, six were associated with disease and included in the multivariable analysis, in which strawberry compote (OR=33.80; 95% CI: 3.41–∞; p‹0.01) and carrots and peas (OR=23.66; 95% CI: 2.22–∞; p‹0.01) remained significant.

For cases in the third wave, the univariable analysis showed three different food items to be associated with occurrence of disease. In the multivariable analysis only strawberry quark remained statistically significant (OR=45.42; 95% CI: 3.31–2,944.92; p‹0.01).

Survey 2 (email questionnaire)

We received 86 completed questionnaires (response 10%). 14 participants were classified as cases, 72 as controls. Median age of cases and controls was 12 years (range: 9–16 and 9–17, respectively). Onset times peaked on the afternoon of 27 September and the following morning (Figure 3D).

The proportion of persons having eaten at the school's canteen from 24 through 28 September was significantly higher in cases than in controls. Of all dishes on offer during that period, only semolina pudding with strawberry compote resulted in a statistically significant positive association with disease in the univariable analysis (Table). Strawberry compote was also offered as a dessert alongside China vegetables, but only one pupil reported to have chosen this dish. The strongest association was found when asking specifically for the consumption of strawberry compote (independent of main course). The median incubation period, calculated from the most likely time of exposure to strawberries (13:00 on 26 September) and the individual times of symptom onset, was 35 hours (range: 12–40 hours).

Food trace-back investigations

Frozen strawberries had been used in regional kitchens of Caterer X. They were part of a lot of 22 tonnes imported by Company Y in Saxony from a company in China, packaged in 2,201 boxes of 10 kg each. Of the institutions with available information, 98% (368/377) were supplied by regional kitchens of Caterer X, the remainder were supplied by two smaller catering companies. All three caterers were supplied by the same company (Company Y). All affected institutions had received products containing the implicated frozen strawberries. Starting on 5 October, the date of a joint press release by RKI, BfR and BVL, Company Y began withdrawing the lot of frozen strawberries from their customers (the company had already stopped further delivery of the strawberries before that date). Overall, delivery stop and recall ensured that at least 1,136 boxes (more than 11 tons) of strawberries from the incriminated lot did not reach the consumer. The remaining 1,065 boxes (ca 10.7 tons) had either already been used or were destroyed under the supervision of the local food safety authorities after the recall. On Oct 8, Saxony's State Health Laboratory detected norovirus in a sample obtained from an unopened box of the incriminated lot of frozen strawberries [12].

Discussion

We report here the largest recorded food-borne outbreak in Germany. It affected several hundreds of institutions supplied almost exclusively by one large caterer and was associated with strawberries imported frozen in a large lot from China. Norovirus was identified as the causative agent. Although the individual clinical courses of disease were mild, the overall disease burden was considerable. The high number of cases caused substantial distress and impairment of the daily routine in affected institutions and families, considerable concerns about food safety in canteens for children, and nationwide media interest.

The epidemiological studies provided strong evidence for strawberries as the vehicle of infection. Conducted in different geographical regions and using various designs, they consistently and exclusively showed statistically significant associations between illness and the consumption of strawberry dishes. Furthermore, in all studies, affected institutions offered strawberry dishes two calendar days before the peak of illnesses, and in one instance, several strawberry-containing dishes, served on different days, caused several waves of illnesses.

Epidemiological evidence guided food safety investigations on the local, state and national level [12]. Early identification of the vehicle of infection led to a timely withdrawal of more than half of the lot. Assuming a similar level of contamination in the part of the lot that was withdrawn, at least 11,000 cases were averted by the withdrawal, probably even more, seeing as only a fraction of the delivered strawberries had been prepared for consumption. According to the report of the German Task Force on Food and Feed Safety, some of the involved regional kitchens of Caterer X reported not to have heated the strawberries during preparation of the implicated dishes whereas others stated that they had, which may in part explain that not all institutions supplied by the involved regional kitchens reported cases of gastroenteritis [12].In response to this outbreak, recommendations in Germany for institutions catering for vulnerable populations (including schools and child care facilities) have been amended and now specifically include the advice to heat frozen berries [13]. Furthermore, from 1 January 2013, a European Union (EU) regulation requires 5% of consignments of frozen strawberries imported from China into the EU to be tested for norovirus [14].

Infectious disease outbreaks due to contaminated produce have gained importance in the recent past [15], including norovirus outbreaks in Europe linked to frozen raspberries [16-22] or blackberries [23] and the multistate outbreak of hepatitis A due to mixed frozen berries [24]. Also strawberries have repeatedly been incriminated in large hepatitis A outbreaks in the United States [25,26] and in Europe [27]. Germany has recently faced a number of outbreaks caused by contaminated vegetables or fruits including sprouts and watermelons [28-30]. The original contamination of the food vehicles or relevant ingredients occurred in countries that were not known to be affected by outbreaks, which complicated or even prevented thorough source investigations. In none of these outbreaks, including this one, was the mode of contamination elucidated. Undoubtedly, transnational source investigations pose particular challenges [31]; political and economic issues may sometimes hamper effective collaboration. A better understanding of how the berries

became contaminated is crucial for developing long-term prevention measures upstream of the retailer. Several different norovirus genotypes of genogroups I and II were detected in the strawberries [8] (and also in human samples) [32]. Together with the large scale of the outbreak, this lends support to the hypothesis that the use of contaminated water in the production of the strawberries was responsible for the outbreak.

This report exemplifies the risk of large outbreaks in the era of global food trade. Today, unprecedented volumes of produce (here 22 tonnes) are distributed to a large number of markets throughout the world [33], thereby increasing the risk for food safety. Public health surveillance needs to adapt to these challenges, e.g. be able to detect outbreaks caused by widely disseminated foods. Surveillance using molecular subtyping information allows establishing links between disease occurrences in different regions, usually seemingly sporadic cases or small clusters [34]. The outbreak described here appeared as an accumulation of concurrent local outbreaks in several adjacent states. Thus, in addition to molecular surveillance, rapid communication of local outbreaks to the state level, as it happened in the outbreak-detecting state of Brandenburg, enables rapid recognition and investigation of supra-regional events even before the aetiology is known, and should be implemented in routine infectious disease surveillance.

Outbreak Investigation Team

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Conflict of interest

None declared.

Authors' contributions

HB, MF, HW and DW drafted the manuscript. HB and DW were the principle investigators of CCS 1, DW of CCS 2, MF of Survey 1, and SH of Survey 2 (all substantially contributed to conception and design, data collection, analysis and interpretation). HB designed the tool used for data collection and analysis in CCS 1 and 2 and represented the RKI at the Task Force Gastroenteritis collaborating with the food safety authorities. HW was responsible for the collection, analysis and interpretation of descriptive data. MH performed the data analysis of CCS 1 and 2. AS substantially contributed to data interpretation and represented the RKI at the Task Force Gastroenteritis. TD participated in data collection of CCS 1 and was a principle investigator of CCS 2 (data collection and interpretation). CS was a principle investigator for the State Health Authority Brandenburg (collection, analysis and interpretation of descriptive data). SSM was a principle investigator for the State Health Authority Saxony (collection, analysis and interpretation of descriptive data) and contributed to the conception of CCS 1. GF coordinated the German Task Force on Food and Feed Safety. OH and KS substantially contributed to the interpretation of descriptive and study data.
All authors critically revised the manuscript and approved of the final version.

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2.2.4. Beurteilung der Validität der Datenerhebungen zum Nahrungsmittelkonsum bei ad Hoc-Studien in Ausbruchssituationen

Gertler M, Czogiel I, Stark K, Wilking H. Assessment of recall error in self-reported food consumption histories among adults-Particularly delay of interviews decrease completeness of food histories-Germany, 2013. *PLoS One*. 2017 Jun 22;12(6):e0179121. doi: 10.1371/journal.pone.0179121

Die Befragung von Patienten und Nicht-Erkrankten bezüglich ihres Lebensmittelkonsums ist bis heute das klassische Vorgehen zur Generierung von Hypothesen bei Untersuchungen von lebensmittelbedingten Infektionskrankheiten (45-47, 84). Falls aber Patienten und Nicht-Erkrankte sich bei Befragungen schlecht erinnern und wenn das jeweilige Lebensmittel nicht bewusst als verzehrt wahrgenommen wird („Tarnkappen"-Essen), kann dies innerhalb von lebensmittelbedingten Ausbrüchen zu Fehlklassifikation von Ausbruchsfällen bezüglich der Exposition (exposure misclassification) führen (84).

Als besonders erfolgreiche Strategien beim HUS/EHEC O104-Ausbruch in 2011 stellte sich die Nutzung von Datenquellen aus dem Herstellungsprozess oder der persönlichen Abrechnung von Lebensmitteln heraus. Diese konnten in Frankfurt und Lübeck in analytisch-epidemiologische Studien integriert werden (siehe Kapitel 2.2.1. und Kapitel 2.2.2.) (62, 78).

Um den Einfluss von exposure misclassification zu quantifizieren und die Durchführung nicht-gedächtnisabhängiger Untersuchungsmethoden weiter zu testen, wurde eine Simulationsstudie durchgeführt, um die Häufigkeit und die Determinanten von Erinnerungsfehlern zu bewerten.

Besucher der Kantine einer Bank in Berlin-Mitte, in der ausschließlich bargeldlos bezahlt werden konnte, wurden an einem Tag nach ihrem Essen bezüglich ihrer täglichen Auswahl unter 13 Lebensmitteln in den drei vorangegangenen Wochen befragt. Die Antworten wurden mit den elektronischen Zahlungsinformationen (Goldstandard) verglichen. Der Anteil von falsch-positiver und falsch-negativer Erinnerung wurde berechnet. Dabei wurden der Einfluss der Länge der Zeit seit dem Verzehr (3-21 Tage), sowie Alter, Geschlecht, Bildungsniveau, Ernährungsgewohnheiten und Nahrungsmittelgruppen berücksichtigt.

Die Ergebnisse dieser Studie konnten erstmals den Anteil von Fehlklassifikation der Exposition in analytischen Studien bei lebensmittelbedingten Ausbrüchen aufzeigen. Das Vergessen wird durch die Verzögerung von Interviews deutlich verstärkt. In Ausbruchsuntersuchungen kann dies sowohl die Generierung von Hypothesen als auch die Ergebnisse analytisch-epidemiologischer Studien beeinträchtigen. Diese Simulationsstudie zeigt die Notwendigkeit nicht-gedächtnisabhängiger Untersuchungsmethoden. Wenn verfügbar, können Daten aus Bezahlsystemen die Informationen zur Lebensmittelkonsum verbessern. In Situationen in denen dies

möglich scheint, sollten Gesundheitsämter und Veterinärämter relevante Daten sichern und eine Auswertung in Erwägung ziehen.

PLOS ONE

RESEARCH ARTICLE

Assessment of recall error in self-reported food consumption histories among adults—Particularly delay of interviews decrease completeness of food histories—Germany, 2013

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Abstract

Introduction

Poor recall during investigations of foodborne outbreaks may lead to misclassifications in exposure ascertainment. We conducted a simulation study to assess the frequency and determinants of recall errors.

Methods

Lunch visitors in a cafeteria using exclusively cashless payment reported their consumption of 13 food servings available daily in the three preceding weeks using a self-administered paper-questionnaire. We validated this information using electronic payment information. We calculated associated factors on misclassification of recall according to time, age, sex, education level, dietary habits and type of servings.

Results

We included 145/226 (64%) respondents who reported 27,095 consumed food items. Sensitivity of recall was 73%, specificity 96%. In multivariable analysis, for each additional day of recall period, the adjusted chance for false-negative recall increased by 8% (OR: 1.1;95%-CI: 1.06, 1.1), for false-positive recall by 3% (OR: 1.03;95%-CI: 1.02, 1.05), for indecisive recall by 12% (OR: 1.1;95%-CI: 1.08, 1.15). Sex and education-level had minor effects.

Discussion

Forgetting to report consumed foods is more frequent than reporting food-items actually not consumed. Bad recall is strongly enhanced by delay of interviews and may make hypothesis generation and testing very challenging. Side dishes are more easily missed than main courses. If available, electronic payment data can improve food-history information.

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Introduction

Interviewing sick persons concerning their food history is probably the oldest and most important method used for hypothesis generation in investigations of outbreaks of foodborne infectious disease. In a next step, analytical studies comparing interview data from sick and healthy people (case control or cohort design) allows for hypothesis testing. This strategy is recommended by international guidelines [1–4]. Interviewees´ poor recall can lead to exposure misclassification of food items which is a frequent experience of any public health epidemiologist which can lead to problems in identifying and testing hypotheses. Misclassification may hinder identification of contaminated vehicles in food-borne outbreaks [5]. If a vehicle is poorly remembered it can hardly be detected. At the same time, uncontaminated food items which are associated with the actual vehicle but better recalled could be wrongly suspected. This is especially problematic for outbreaks of diseases with long incubation periods including listeriosis, Hepatitis A or during the outbreak of Shiga toxin–producing Escherichia coli (STEC) O104:H4 infection in Germany 2011 [6]. Additionally, interview-based investigations are even more difficult when the disease sets patients into a state in which they cannot be interviewed.

During the STEC outbreak in 2011 in Germany, studies designed independently from the human recall capability have been particularly successful [7]. In one of the case-control studies, the cashless payment system of a company cafeteria used by the investigators provided food histories of patients and controls in a short time [8]. Other similar experiences of use of electronic payment data to investigate foodborne outbreaks were reported [9–11].

Little information is available about the actual frequency and determinants of recall error and misclassification of food items. In a study from 1986 epidemiologists investigated food recall during a luncheon in their institute. The investigators videotaped 32 attendees at the buffet table and interviewed them afterwards concerning their food selection. Consumers failed more often to report selection of desserts and bread compared to other servings, but influence of recall period could not be studied [12]. Similarly, Mann et al. observed attendees of a luncheon documenting their selection. Then, they compared the observed food choice with reported food history of the attendees from questionnaire-based interviews five days after the meal. They estimated sensitivity of recall of 88% and specificity between 73% and 93% [5].

To better understand determinants of food history recall, we simulated an outbreak investigation and used electronic data from personal payment cards as gold standard for food history in a cafeteria in Berlin, Germany, to check the recall of the consumers as ascertained using a paper-based questionnaire.

Material and methods

Visitors of a company cafeteria in a bank in Berlin were approached and interviewed during the regular opening hours at lunchtime (11:45 AM to 2:30 PM). In the morning of the same day, all employees with access to the cafeteria received an information letter via email, informing them about the interviews, the simulative and anonymous nature of the study. In the cafeteria, employees of the Robert Koch Institute (RKI), the responsible public health agency for the control of infectious diseases in Germany, approached the cafeteria guests to further inform them about the study and invite them to participate.

Participants were asked to fill in a standardized questionnaire about daily cafeteria visits and their food consumption of 13 different regularly served items in the cafeteria during the preceding three weeks (15 opening days). Additionally, personal characteristics (year of birth, sex, education degree), information on dietary habits (eating vegetarian, low-calorie-diet and having any food intolerance) and the personal customer identifier code number (ID) displayed

on the card of the cashless payment system were retrieved. The questionnaire was designed using the same layout as the normal weekly menu of the cafeteria to increase ability to remember as might have been done by the field epidemiology team in a real outbreak scenario. Every day, the canteen offers three different main courses which, like four of the five offered side dishes and like two of the three desserts, vary every day. In addition, consumers may choose from a salad bar and may choose to take bakery (a roll or a bread) with their lunch. For analysis, we grouped the varying categories together, into 8 food item categories: main courses, side dishes, boiled potatoes (the non-varying third side dish), vegetable side dishes, desserts, fruit-salad (the non-varying third dessert), salad-bar (available every day) and bakery (available every day). To visualise the questionnaire, it is provided in supportive information files "S1 Questionnaire German" and "S1 Questionnaire English".

The management of the cafeteria provided printed copies of the canteen payment of each participant's IDs. All paper records were digitalised with software EpiData Entry (http://www.epidata.dk/). Double data entry and checks were performed for all data to reduce data entry errors.

For analysis the electronic payment information was used as standard and misclassifications were categorised as false-positive (reported eaten, not paid), false-negative (reported not eaten, but paid) and indecisive (Don't know-answer). We used multivariable logistic regression separately for each misclassification category as dependent variable. We used recall period, sex, age group, degree of education, dietary habits and food item categories in each model as independent variables, without selection of variables. Statistical analysis was performed with STATA version 12.1C.

In this study, anonymous data on food histories and demographic characteristics were retrieved. No information on disease, disease-related states or disease-relevant exposures were collected. In detail, we asked healthy volunteers to report anonymously about their food intake in their canteen—there was no outbreak, nobody was asked for symptoms or about his/her medical condition, nobody was treated or underwent biomedical diagnostic tests or similar.

Participants were informed before and at the beginning of the survey about the simulation character of the study and were only included after written informed consent. We compared the reported food histories with those registered by the electronic payment system (identification by canteen card ID number).

To guarantee the highest possible level of anonymity, we requested and received approval of the data safety office at the Robert Koch Institute (the German National Public Health Institute). Therefore we consider this study to be in accordance with the Declaration of Helsinki without having applied for a review of an institutional ethics committee prior to the interviews.

Results

Study population

Altogether, 241 visitors responded to our survey. We excluded 18 of whom payment information could not be read or ID was ambiguous, 39 who declared to have used another person´s payment card at least once and39 who did not respond to one third or more of the inquired food items. Overall, we analysed data from 145 participants. None-responders did not differ from participants regarding age ($p = 0.142$) and gender ($p = 0.472$). Altogether 84/145 (58%) participants were female. Median age was 41 years, range: 22–64 years; 80/145 (55%) stated they hold a university degree. Of 28,275 (13x15x145) possible food recalls 1,180 (0.04%) were excluded because of no response or single purchase data could not been read out from the database.

Overall sensitivity and specificity of recall

Altogether 27,095 recalls were analysed. Of 3,523 purchased items, participants reported eating 2,268 (overall sensitivity of 72.8%), denied 846 and indecisively (Don't know-answer) answered for 409. Of 23,572 items, actually not purchased, participants reported 20,931 as not eaten, 872 as eaten (overall specificity of 96.0%) and indecisively answered for 1,769. Altogether, participants indecisively recalled 2,178 (8.0%) food items. Median number of errors per participant was 11 with a range of 1–46. There was no significant association between the number of foods selected from the 13 investigated items and the number of reporting errors ($p = 0.429$). To allow better interpretation and adjustment of the results of other investigations, measures of performance of interviews are provided for each associated variable in detail in supportive information Tables A-C in S1 File.

Influence of recall period

All participants together paid for between 155 (day 18) and 255 (day 13) food items per day. False-negative recall increased with recall period (Fig 1). There were remarkably few bad recalls on day 14 and day 17 interrupting a continuous decline. The chance of false negative recall was twice as high after 21 days compared to 7 days (OR: 2.04; 95%-CI: 1.21, 3.45), while differences in false-positive recall are less pronounced (Table 1). In multivariable analysis, for each additional day, the chance for false-negative recall increased by 8% (OR: 1.08; 95%-CI: 1.06, 1.1), for false-positive recall by 3% (OR: 1.03; 95%-CI: 1.02, 1.05), for indecisive food recall by 12% (OR: 1.12; 95%-CI: 1.08, 1.15).

Influence of type of food

Compared to the main courses, other food items were generally less accurately recalled. The use of the salad bar in the cafeteria was especially prone to false-negative recall (OR: 2.29; 95%-CI: 1.41, 3.71) as well as false-positive recall (OR: 2.23; 95%-CI: 1.49, 3.33) and indecisive recall

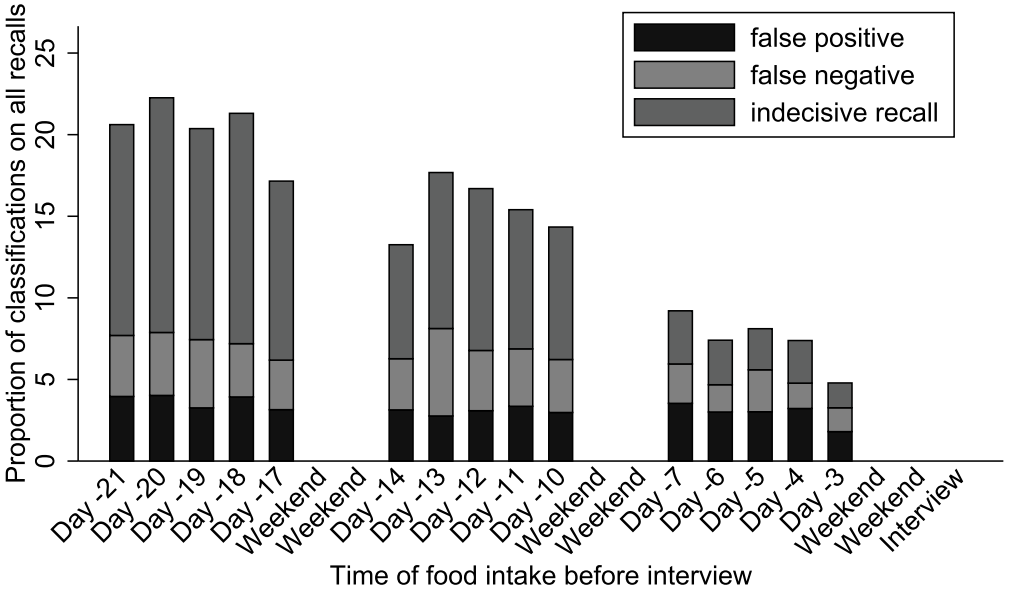

Fig 1. Distribution of the proportion of misclassifications of food recalls by recall period, Berlin, Germany, 2013.

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Table 1. Results of multivariable logistic regression of associated variables on different categories of misclassification of reported food selections, Berlin, Germany, 2013.

Associated factors	False-negative recall		False-positive recall		Indecisive recall	
(No. study participants)	Odds ratio	95%-CI	Odds ratio	95%-CI	Odds ratio	95%-CI
Recall period						
3 days	0.53	0.30, 0.95	0.50	0.31, 0.82	0.45	0.22, 0.92
4 days	0.51	0.28, 0.94	0.97	0.59, 1.57	0.81	0.60, 1.08
5 days	0.94	0.57, 1.55	0.93	0.61, 1.41	0.80	0.51, 1.24
6 days	0.53	0.30, 0.95	0.87	0.54, 1.40	0.85	0.65, 1.11
7 days	Ref	Ref	Ref	Ref	Ref	Ref
10 days	1.43	0.88, 2.32	0.84	0.50, 1.41	2.67	1.50, 4.75
11 days	1.55	0.94, 2.57	1.03	0.67, 1.60	2.89	1.59, 5.24
12 days	2.22	1.42, 3.45	0.90	0.57, 1.41	3.42	1.85, 6.33
13 days	2.29	1.43, 3.65	0.95	0.61, 1.46	3.37	1.72, 6.59
14 days	1.48	0.91, 2.41	0.98	0.63, 1.50	2.26	1.17, 4.36
17 days	1.82	1.19, 2.78	1.02	0.63, 1.65	3.80	2.04, 7.07
18 days	1.92	1.20, 3.08	1.41	0.92, 2.17	5.26	2.92, 9.46
19 days	2.35	1.47, 3.77	1.08	0.66, 1.79	4.77	2.52, 8.99
20 days	2.31	1.41, 3.78	1.38	0.87, 2.19	5.39	2.96, 9.82
21 days	2.04	1.21, 3.45	1.36	0.87, 2.11	4.75	2.53, 8.91
Sex						
Female (n = 84)	Ref	Ref	Ref	Ref	Ref	Ref
Male (n = 59)	0.89	0.62, 1.28	1.46	1.11, 1.91	1.79	0.86, 3.74
Age group						
20–29 (n = 32)	0.63	0.38, 1.06	0.77	0.52, 1.13	3.67	1.34, 10.00
30–39 (n = 36)	0.67	0.40, 1.14	0.80	0.52, 1.23	2.10	0.85, 5.15
40–49 (n = 37)	0.87	0.53, 1.43	1.14	0.82, 1.59	1.79	0.82, 3.90
50–65 (n = 37)	Ref	Ref	Ref	Ref	Ref	Ref
University graduate						
Yes (n = 80)	1.16	0.79, 1.69	1.02	0.77, 1.35	1.03	0.49, 2.17
No (n = 65)	Ref	Ref	Ref	Ref	Ref	Ref
Eating vegetarian						
Yes (n = 9)	0.77	0.40, 1.47	1.66	1.09, 2.52	4.30	1.16, 15.92
No (n = 136)	Ref	Ref	Ref	Ref	Ref	Ref
Eating low-calorie						
Yes (n = 10)	1.80	0.71, 4.59	1.36	0.79, 2.34	1.08	0.42, 2.76
No (n = 135)	Ref	Ref	Ref	Ref	Ref	Ref
Food intolerance						
Yes (n = 2)	0.31	0.14, 0.69	0.49	0.12, 2.12	22.49	4.44, 113.94
No (n = 143)	Ref	Ref	Ref	Ref	Ref	Ref
Food item categories						
Bakery	17.72	9.03, 34.76	0.32	0.18, 0.56	0.85	0.58, 1.23
Side dish	2.50	1.90, 3.29	1.09	0.85, 1.40	1.43	1.20, 1.70
Dessert	2.82	2.13, 3.72	1.10	0.80, 1.51	1.02	0.76, 1.35
Vegetables	2.95	2.20, 3.96	1.53	1.20, 1.95	1.25	1.06, 1.46
Main courses	Ref	Ref	Ref	Ref	Ref	Ref
Fruit Salad	32.76	10.25, 104.74	0.21	0.08, 0.51	0.76	0.55, 1.04
Salad bar	2.29	1.41, 3.71	2.23	1.49, 3.33	1.82	1.26, 2.62
Potatoes	2.74	1.65, 4.54	1.67	1.16, 2.41	1.68	1.35, 2.08

(*Continued*)

Table 1. (*Continued*)

Associated factors	False-negative recall		False-positive recall		Indecisive recall	
(No. study participants)	Odds ratio	95%-CI	Odds ratio	95%-CI	Odds ratio	95%-CI
Recall period						
Total (n = 145)						

Multivariable, Odds ratio and 95% confidence interval derived from logistic regression; Recall period defined as the interval from the day of food consumption to the day of the interview in days

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(OR: 1.82; 95%-CI: 1.26, 2.62). Similarly, vegetables and potatoes, although less likely as food vehicles of outbreaks, were poorly recalled comparing to main courses in all three categories. False-positive recall was less likely in bakery products and fruit salad.

Influence of demographic characteristics

The 59 males paid for 1,420 food items (24 per person) while the 84 females paid for 1,668 items (20 per person). While false-negative recall did not differ between males and females, the chance for false-positive recall was higher in males (OR 1.46; 95%-CI 1.11, 1.91). False-positive recall was also higher in vegetarians (OR 1.66; 95%-CI 1.09 2.52). False-negative recall did not vary by age or education. However, indecisive recall was more likely in vegetarians (OR: 4.30; 95%-CI: 1.16, 15.92) and in 20–29 year old participants compared to those aged 50–65 years (OR: 3.67; 95%-CI: 1.34, 10.00). Level of education of participants was not associated significantly with false-negative (OR: 1.16; 95%-CI: 0.79, 1.69), false-positive (OR: 1.02; 95%-CI: 0.77, 1.35) and indecisive recall (OR: 1.03; 95%-CI: 0.49, 2.17).

Effort for data acquisition

Data collection based on the questionnaire required presence of 10 persons in the cafeteria for 3 hours to contact and inform visitors, receive interviewees´ informed consent, to distribute and receive the questionnaires. In comparison, to extract the data from the payment system required one staff for 2 hours.

Discussion

This study shows that exposure misclassification can be a significant problem in the investigation of foodborne infectious disease outbreaks using data from food history interviews. The misclassification can be differential regarding the inquired food items, leading to an underestimation of measures of association of the true outbreak vehicle and false incrimination of other vehicles. For example, this scenario happened during investigation of large outbreaks of STEC in Germany caused by sprouts [7,13] and Salmonella Saintpaul in the USA caused by jalapeño and serrano peppers [14,15]. In both outbreaks epidemiological association from early studies initially identified different products. We found that the proportion of false-negative recalls is higher than false-positive, indicating that forgetting to report consumed foods is more likely than reporting food-items actually not consumed. Higher specificity and lower sensitivity of recall were reported before in a similar experiment [12].

Influence of recall period

While false-negative recall and indecisive recall strongly increases with time, false-positive recall does not. After recall periods of two weeks or more, around 20% of all items do not get

reported correctly which means lower power in epidemiological studies to detect outbreak vehicles. The high chance for false-negative recall is particularly problematic for hypothesis generation. Outbreak vehicles may be underestimated or overseen only because the exposure lies two weeks or more in the past.

Influence of type of food

Decker et al. reported more accurate recall of more complex or distinctive dishes compared to a range of relatively similar vegetable side dishes. This is supported by our findings suggesting better recall of main courses compared to all other dishes, particularly compared to unvarying daily offerings like fruit salad and bakery. Contrarily to Decker et al., we did not find indication of significant misclassification of desserts [12]. However, better recall of main courses needs to be taken into account when evaluating explorative findings, to avoid missing vehicles in side dishes. Particularly consumption at the salad bar is poorly recalled which is in accordance with observations from an outbreak in Germany [7,8]. Unfortunately, we could not obtain information on different salad bar items as this was not included in the billing data.

Influence of demographic characteristics

Altogether, respondent-related variables have a smaller impact than recall time and food item variables. Our findings confirm a higher chance for false-positive recall in men. This is in accordance with findings of Decker et al. [12]. Increasing age does not lead to poor recall in our study. Participants who declare being vegetarian have a higher chance for false-positive or indecisive recall despite the assumption that sensible diet leads to better recall of food consumption. However, this finding is based only on small numbers: only few study participants indicated being vegetarian (n = 9) or eating low-calorie food (n = 10).

Effort for data acquisition

The interviews of participants required 15-times more work compared to the extraction of the electronic information from the billing system. Therefore the latter provides potential to make data collection quicker, more accurate and allows for larger study populations. However, it's only applicable if a large proportion of cases and non-diseased persons pay cashless. An electronic interface between billing systems and databases of public health agencies might accelerate investigations.

Limitations

Unfortunately, in our simulation study only printouts were available, demanding manual data entry. The bank as employer and the cafeteria allowed us only limited interview time. In a real scenario, such would be much longer and provide more detailed information especially regarding the different main courses and regarding individual food choices. Furthermore, the data from the payment system was only specific on the menu level and not on the choice of the visitor. Therefore, participants were not asked if they had eaten anything containing a specific ingredient and they did not have the possibility to report items which were not on the questionnaire. In a real-life scenario investigations on ingredients level might be complemented by interviews with the chefs and the kitchen staff.

One main limitation of recall-independent electronic data is that it cannot tell if paid food items were actually eaten by the participant. But we think that this misclassification is of minor importance compared with misclassification due to incorrect recall.

Conclusion

Our results show that earliness of interviews of patients during foodborne outbreaks is essential, particularly when the pathogen and disease have long incubation periods. At least, hypothesis generating exploratory interviews should be performed before failure of recall. If available, electronic payment data for food history collection can facilitate and accelerate investigations, especially if patients are very sick or even dead. Data from our study can be used for better interpretation and adjustment of the results of surveys, case-control studies and cohort studies in outbreaks.

Supporting information

S1 File. Table A: False-negative food recalls by different groups for reported food selections, Berlin, Germany, 2013. Table caption: Univariable, Odds ratio and 95% confidence interval derived from logistic regression; CI, confidence interval; Recall period defined as the interval from the day of food consumption to the day of the interview in days. Table B: False-positive food recalls by different groups for reported food selections, Berlin, Germany, 2013.
Table caption: Univariable, Odds ratio and 95% confidence interval derived from logistic regression; CI, confidence interval; Recall period defined as the interval from the day of food consumption to the day of the interview in days. Table C: Indecisive (Don't know-answer) food recalls by different groups for reported food selections, Berlin, Germany, 2013.
Table caption: Univariable, Odds ratio and 95% confidence interval derived from logistic regression; CI, confidence interval; Recall period defined as the interval from the day of food consumption to the day of the interview in days.
(DOC)

S1 Questionnaire German. The questionnaire was designed using the same layout as the normal weekly menu of the cafeteria. Each column represents a working day when the canteen was open. The lines represent the 13 different food categories from which participants could choose a different serving every day. For analysis, the varying categories were grouped together: Giving 8 food item categories: main courses, side dishes, boiled potatoes (the non-varying third side dish), vegetable side dishes, desserts, fruit-salad (the non-varying third dessert), salad-bar (available every day) and bakery (available every day).
(PDF)

S1 Questionnaire English. The English questionnaire is a translation of the German original. It was not used in the study but produced exclulsively to facilitate reading of this report."
(PDF)

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3. Diskussion

3.1. Public Health-Bedeutung von Infektionen, die durch Lebensmittel übertragen werden

Das Vorkommen von lebensmittelbedingten Krankheiten wird weltweit, darunter auch in Deutschland durch Faktoren wie den globalisierten Handel mit Nahrungsmitteln und lebenden Tieren, moderne intensivierte Nahrungsmittelproduktionssysteme, internationale Reisen und die kontinuierliche Anpassung bekannter und aufkommender Krankheitserreger und Antibiotikaresistenz beeinflusst. Diese Krankheiten sind weit verbreitet und sind mit individuellem Leiden und hoher Zahl von Folgeerscheinungen verbunden. Darüber hinaus stellen sie ein ernsthaftes Problem für die öffentliche Gesundheit dar und jährlich werden hohe Summen für die Sicherstellung der öffentlichen Lebensmittelsicherheit ausgegeben. Darüber hinaus haben diese Erkrankungen erhebliche wirtschaftliche Auswirkungen auf die Kosten der Krankenversorgung, den Produktivitätsverlust sowie in der Agrar- und Lebensmittelindustrie.

Die Erreger in den Lebensmitteln stammen aus unterschiedlichen Quellen. Die Konzentration der Krankheitserreger, die im verzehrten Lebensmittel enthalten sind und somit das menschliche Erkrankungsrisiko bestimmen, hängen von mehreren Faktoren in der gesamten Produktionskette ab. Diese Kette reicht von den landwirtschaftlichen Betrieben (z.B.: *Salmonella*) über die Lebensmittelverarbeitung (z.B.: *Listeria*), über den Einzelhandel (z.B.: Hepatitis A-Virus), bis hin zur Lebensmittelhandhabung (z.B.: *Clostridium botulinum*). Um die mikrobiologische Qualität von Lebensmitteln für den Konsumenten sicherzustellen, sowie Strategien zur Verringerung der ausgehenden Gefahren zu entwickeln, muss Überwachung der Erreger auf alle Punkte in der Herstellungskette von der Tierhaltung bis zum Verzehr beruhen. Die Hauptaktivitäten in diesen Präventionsprogrammen sind zum einen die aktive Überwachung mit dem Ziel, den Erreger in verschiedenen Stadien der Kette vom Lebensmittel-Erzeuger zum Verzehr nachzuweisen, und zum anderen die Identifikation und Entfernung des kontaminierten krankheitsverursachenden Nahrungsmittels und somit die Verhinderung weiterer Übertragungen. Letzteres wird vor allem durch die Untersuchung von Ausbrüchen erreicht. Aber auch die Prävention profitiert von den Erkenntnissen aus Ausbruchsuntersuchungen. Jedes Jahr werden in der behördlichen Lebensmittelsicherheit hohe Summen in Programme zur Überwachung und Kontrolle von Lebensmitteln und zoonotischen Tierseuchen investiert. Bevölkerungsrepräsentative Studien wie in Kapitel 2.1.1. und Kapitel 2.1.2. bilden dabei einen Teil der empirischen Basis zur Rechtfertigung dieser Ausgaben.

Die Schätzungen zur Häufigkeit von Erkrankungen durch den Konsum kontaminierter Lebensmittel stellen eine große Herausforderung dar. Zunächst einmal sind über 250 unterschiedliche Erreger als lebensmittelübertragbar bekannt. Darüber hinaus sind

für die meisten Erreger auch Mensch-zu-Mensch-Übertragungen in unterschiedlichen Anteilen beschrieben. Hepatitis A bespielweise kann in Deutschland durch kontaminierte Lebensmittel übertragen werden (85, 86), aber auch in Massenunterkünften (87) oder durch sexuellen Kontakt (88). Norovirus-Infektionen sind die häufigsten Ursachen für Gastroenteritis, wobei die Schätzungen über den Anteil, den lebensmittelübertragene Infektionen ausmachen, zwischen unterschiedlichen Norovirus-Genotypen variieren. Modellierungen basierend auf molekularbiologischen Informationen schätzen sie übergreifend auf 14% (22). Epidemiologische Studien aus Großbritannien ermitteln den Anteil auf 10,8% (21).

Ein weiteres Problem bei der Schätzung von lebensmittelübertragenen Infektionen entsteht durch die Tatsache, dass eine große Bandbreite von Krankheitsmanifestationen verursacht werden, für die jeweils bevölkerungsbezogen Daten erhoben werden müssen und die Anteile lebensmittelbedingten Ursprungs geschätzt werden müssen. Neben Gastroenteritis als klassisches Outcome sind Hepatitiden, Septikämien, Aborte und Fehlbildungen bei Neugeborenen, neurologische Symptome, Augeninfektionen durch Parasiten und Nierensymptomatik zu nennen. Darüber hinaus zeigt die in den letzten Jahren regelmäßige Entdeckung, dass bedeutende Krankheitserreger lebensmittelübertragen sind (z.B.: Hepatitis E-Virus), das auch weitere bekannte und unbekannte Erreger eine signifikante Anzahl von Erkrankungen verursachen, die aber nicht explizit bekannt sind (3).

Eine große Herausforderung ist, dass das Überwachungssystem für Infektionskrankheiten in Deutschland Lebensmittel als Übertragungsweg nicht routinemäßig erfasst. Dies wäre nur mit sehr hohem Aufwand für die Gesundheitsämter zu ermöglichen. Surveillance ist wichtig für den Infektionsschutz vor-Ort und der Evaluierung von Maßnahmen. Es wird aber nur ein kleiner Anteil der Erkrankungsfälle im Meldesystem registriert. Daher kann nur mit Hilfe von Surveys wie in Kapitel 2.1.1. und Kapitel 2.1.2. repräsentative Angaben aller Fälle, auch der nicht-laboruntersuchten in der deutschen Bevölkerung erhoben werden. Die Studien zum Vorkommen von Gastroenteritis und *Toxoplasma*-Infektionen und Erkrankungen erheben somit zum ersten Mal wichtige Basisdaten in diesem Bereich.

Eine Vielzahl von Ländern hat Studien zum Vorkommen von Gastroenteritis bei Erwachsenen durchgeführt. Dabei wurde die gleiche Methodik angewandt (12, 20). Die Inzidenz in Deutschland stimmt im Allgemeinen mit Beobachtungen in ähnlichen Studien in anderen Ländern überein (70). Ein abnehmender Trend für das Alter wird in allen ähnlichen Studien berichtet. Die Inzidenz von Gastroenteritis in Deutschland liegt im Allgemeinen in der gleichen Größenordnung wie in Kanada (89), Hongkong (90) und Australien (91). Sie scheint niedriger als in den USA (92), in Neuseeland (93), Kuba (94) und Norwegen (95) und jungen Altersgruppen in Dänemark (96), jedoch höher als in Großbritannien und Irland (97, 98), sowie unter älteren Altersgruppen in Italien (99).

Eine Initiative des CDC der Vereinigten Staaten hat die jährliche Anzahl von in den USA erworbenen, lebensmittelbedingten Krankheiten, Krankenhausaufenthalten und Todesfällen basierend auf 31 spezifischen Krankheitserregern und durch nicht-spezifizierte Erregern geschätzt (3, 100). In diesen Untersuchungen werden jährlich insgesamt 47,8 (95%-KI: 28,7-71,1) Millionen Infektionserkrankungen durch Lebensmittelkonsum in den USA errechnet. Wenn man diese Schätzungen auf die deutsche Bevölkerung überträgt (nicht altersstandardisiert), würden dies insgesamt jährlich 12,3 Millionen (95%-KI: 7,4-18,3) Erkrankungen in Deutschland ergeben. Die Schätzungen des CDC für Hospitalisierungen und Todesfälle, ergeben für Deutschland übertragen jährlich 32.850 (16.068-55.392) Hospitalisierungen und 780 (383-1.280) Todesfälle durch den Konsum von kontaminierten Lebensmitteln. Eine parallel durchgeführte Untersuchung in Canada kommt auf gleiche Größenordnungen (101). Für eine Überschätzung bei der Übertragung der Ergebnisse von den USA auf Deutschland spricht, dass die bevölkerungsrepräsentativen Querschnittsstudien zu Gastroenteritis in dieser Arbeit (0,95 Episoden/Person/Jahr) niedriger liegen als die in den USA mit ähnlicher Methodik erhobenen (1,4 Episoden/Person/Jahr). Darüber hinaus sind schwere Infektionen mit EHEC O157 in den USA weiter verbreitet, als in Deutschland (54, 102). Für eine Unterschätzung, also höhere Bedeutung von lebensmittelübertragenen Erkrankungen in Deutschland im Vergleich zu den USA spricht, dass einige häufig vorkommende Infektionen in den USA deutlich weniger verbreitet sind. Bespielweise liegt die *Toxoplasma*-Seroprävalenz in den USA bei 6,7% (77) und ist somit weniger als ein Siebtel der im Rahmen dieser Arbeit für Deutschland ermittelten Seroprävalenz. Trotzdem ist *Toxoplasma* in den USA als vierthäufigster Erreger für Hospitalisierungen und zweithäufigster für Todesfälle aufgezeigt, was im Gegenschluss für eine deutlich höhere Belastung in Deutschland spricht. Darüber hinaus sind Hepatitis E-Meldezahlen hoch in Deutschland (103, 104), autochthone Fälle in den USA vom CDC als nahezu unbekannt angegeben (3, 100). Es ist auffällig, dass die beiden letztgenannten Erkrankungen durch den Konsum von roh und nicht durchgegartem Schweinefleisch übertragen werden, was immer noch eine Besonderheit der deutschen Esskultur darstellt und mit hohen Infektionsrisiken verbunden ist.

Erhebungen zur Krankheitslast von lebensmittelübertragenen Erregern können dabei helfen die Sensitivität und Spezifität der Surveillance-Systeme zu verstehen. Beispielhaft konnte die Studie zur *Toxoplasma* im Rahmen dieser Arbeit aufzeigen, das die Surveillance im Rahmen des Infektionsschutzgesetzes insuffizient ist (73).

In Deutschland sind nur wenige Studien bekannt, die die Krankheitslast für lebensmittelübertragenen Erreger bestimmen (2, 71). Es besteht daher ein großer Nachholbedarf -insbesondere auch krankheitsübergreifend- bezüglich Burden of Disease-Studien zu lebensmittelübertragenen Erregern.

3.2. Veränderungen in der Epidemiologie lebensmittelübertragener Ausbrüche

Die meisten der in Kap. 3.1. beschriebenen lebensmittelbedingten Infektionserkrankungen sind sporadische Erkrankungsfälle oder kleine Ausbrüche in Haushaltsumgebung. Durch Ermittlungen der Gesundheitsämter, Landesgesundheitsbehörden oder des RKI können bei Vorliegen entsprechender epidemiologischen Informationen Einzelfälle zu Gruppen zusammengefügt werden, bei denen man von einer gemeinsamen Infektionsquelle ausgeht und die nachfolgend Ausbruch genannt werden.

Ähnlich wie für lebensmittelübertragene Krankheiten im allgemeinen beschrieben, muss man auch für das Auftreten von Ausbrüchen davon ausgehen, dass wirtschaftliche Änderungen und die Globalisierung einen signifikanten Einfluss haben. Die in dieser Arbeit beschriebenen Ausbrüche in Kapitel 2.2.1. bis 2.2.3. zeigen, dass die global vernetzte Nahrungsmittelversorgung eine Ursache darstellen kann. Nahrungsmittel für die Ernährung in Deutschland werden heute weltweit angebaut, verarbeitet und geliefert. Veränderungen der globalen Märkte und die Geschwindigkeit des Transports haben es profitabel gemacht, Nahrungsmittel zwischen den Kontinenten zu transportieren. Aufgrund der niedrigen Preise sind die Produzenten darauf angewiesen, dass eine große Anzahl von Wanderarbeitern manuelle Arbeiten auf Plantagen (z.B. Erdbeerfelder in China) ausführt. Diese können Ausscheider von Norovirus-Genotypen oder Hepatitis A-Virus sein, für die es in Mitteleuropa keine spezifische bevölkerungsbezogene Immunität gibt (105). Wenn in den Anbaugebieten keine angemessene Hygiene aufrechterhalten wird, können diese Erreger in die Nahrungsmittelversorgung in Deutschland gelangen. Der globale Lebensmittel-Vertrieb hat auch große Farmen und Verarbeitungsanlagen geschaffen, die große Chargen bestimmter Lebensmittel handeln, so wie sie vor einiger Zeit noch nicht möglich waren. Dadurch können aber auch große Ausbrüche verursacht werden.

Darüber hinaus hat sich in den letzten 20 Jahren in Mitteleuropa der Lebensmittelkonsum diversifiziert und eine Nachfrage nach Lebensmittel und Grundstoffen aus anderen Kontinenten geschaffen, dies nicht nur für Spezialitäten und Produkte mit langen Haltbarkeiten, sondern auch für Produkte des alltäglichen Bedarfs und für Salate und Früchte. Es entsteht somit aber auch eine Abhängigkeit von den Standards der Lebensmittelsicherheit in dortigen Ländern. In den USA, aber auch in Europa, registriert man seit zehn Jahren eine wachsende Belastung durch lebensmittelbedingte Ausbrüche aufgrund von kontaminierten Salaten und Früchten (106). Über die in dieser Arbeit berichteten Ausbrüche hinaus zeigen dies auch andere Geschehen in Europa, wie zum Beispiel in den letzten Jahren drei Salmonellen-Ausbrüche durch Wassermelonen aus Brasilien, Mungbohnen-Sprossen aus den Niederlanden, sowie durch Sesam aus Nigeria (107-109).

Das RKI veröffentlicht regelmäßig Daten zum Vorkommen von Ausbrüchen, die nach Infektionsschutzgesetz (IfSG) gemeldet wurden. Auf Ebene der Behörden der Lebensmittelsicherheit gibt es ein System der bundeseinheitlichen Erfassung von Krankheitsausbrüchen und der assoziierten Lebensmitteln. Die Daten beider Systeme werden auf Bundesebene regelmäßig abgeglichen und jährlich veröffentlicht (110).

Erkrankungsfälle bei Ausbrüchen werden in Deutschland nur erfasst, wenn es einen Labornachweis gibt oder die Fälle mit anderen laborbestätigten Fällen in Verbindung stehen. Nicht aber für Ausbrüche, die zunächst als reine Gastroenteritis erscheinen wie dem Norovirus-Ausbruch 2012. Daher werden ad-Hoc-Erfassungssysteme wie in der Studie in Kapitel 2.2.4. dargestellt in diesen Fällen sehr wichtig. Ähnlich wie für Einzelfälle von Erkrankungen wird auch bei Ausbrüchen in Deutschland Lebensmittel als Übertragungsweg nicht routinemäßig erfasst. Dies wäre nur mit sehr hohem Aufwand für die Gesundheitsämter zu ermöglichen. Für einige lokale Ausbrüche gibt es sehr gute bis gute epidemiologische Evidenz, während es für andere nur Anhaltspunkte gibt bzw. sämtliche Hinweise fehlen. Bei der Surveillance von Ausbrüchen innerhalb des IfSG wird je nach Grad der Sicherheit bezüglich der Lebensmittelassoziation unterschieden zwischen Ausbrüchen, bei denen aufgrund des Erregers eine lebensmittelbedingte Übertragung angenommen werden kann (potenziell lebensmittelbedingte Ausbrüche) (7). Ein Teil dieser Ausbrüche, bei denen in der Ausbruchsdokumentation Lebensmittel von den ermittelnden Behörden explizit genannt werden, werden als explizit lebensmittelbedingte Ausbrüche beschrieben. Im Jahr 2017 wurden 875 potenziell lebensmittelbedingte Ausbrüche (ohne Norovirus-Gastroenteritis-Ausbrüche) an das RKI übermittelt (7). Davon sind 316 Ausbrüchen explizit lebensmittelbedingt. Unter Betrachtung der externen Einflüsse könnte man annehmen, dass die Zahl der Ausbrüche in den letzten Jahren zunimmt. Die zeitliche Verteilung zeigt aber eine kontinuierliche Abnahme in den letzten Jahren (Abbildung 2), die vor allem auf eine Abnahme von Salmonellose-Ausbrüchen und auf Ausbrüchen assoziiert mit Eiern und Eiprodukten beruht. Eine mögliche Erklärung für den rückläufigen Trend, der sich auch auf sporadische Salmonellose-Fälle bezieht (111), ist die stufenweise Einführung der Impfpflicht für Legehennen und eine erfolgreiche, systematische Eradikation von Salmonellen in Elterntierbeständen für Legehennen. Gemüse und Gemüseprodukte, sowie Obst und Obstprodukte sind sehr selten Ursachen von lebensmittelbedingten Ausbrüchen. Interessant ist, dass nachfolgend zu den großen Ausbrüchen in 2011 und 2012 diese Vehikel nicht häufiger detektiert wurden. Dies obwohl man annehmen könnte, dass bei den mit der Aufklärung befassten Personen in den Gesundheits- und Veterinärämter bis zu diesen Ausbrüchen ein detection bias vorgelegen hat, der zu vorschnellen Assoziationen mit Produkten tierischer Herkunft führte und sich durch die prominenten Ereignisse assoziiert mit Produkten nicht-tierischer Herkunft vielleicht aufgelöst haben könnte. Die aktuellen Zahlen für 2017 zeigen diesbezüglich

aber eine Trendumkehr, also eine Zunahme. Eine Erklärung dafür gibt es bisher noch nicht.

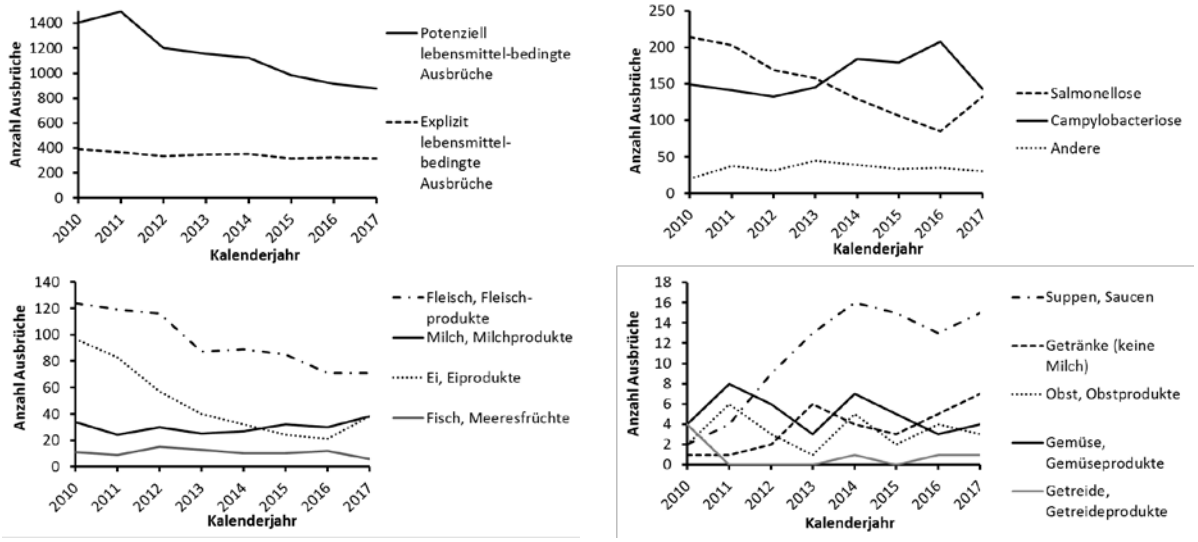

Abbildung 2: Zeitliche Verteilung zum Vorkommen von lebensmittelbedingten Ausbrüchen in Deutschland zwischen 2010 und 2017. Links oben: Nach Grad der Sicherheit bezüglich der Lebensmittelassoziation; Rechts oben: Explizit lebensmittelbedingte Ausbrüche nach Erreger; Links unten: Nach Lebensmittelvehikel (tierische Herkunft); Links unten: Nach Lebensmittelvehikel (nicht-tierische Herkunft). Quelle: Die Abbildung wurde erstellt von Hendrik Wilking basierend auf Daten des Robert Koch-Instituts.

3.3. Herausforderungen bei der Generierung von Evidenz bei der Erforschung von Ausbruchsursachen

Die Fehlklassifikation von Ausbruchsfällen bezüglich der Exposition (exposure misclassification) ist ein großes Problem bei der Untersuchung von Ausbrüchen. Es kann bei Nichtbeachtung zum fehlerhaften Auslassen des verursachenden Lebensmittelvehikels bei öffentlichen Warnungen führen. Lebensmittel, die aufgrund Erinnerungsfehler der Studienteilnehmer in der Studie nicht mit dem Vorkommen von Erkrankungen assoziiert werden, werden nachfolgend oft ignoriert, wenn ein anderes Lebensmittel signifikant mit einer Krankheit assoziiert erscheint. Der HUS/EHEC O104-Ausbruch in 2011 in Deutschland hat gezeigt, dass versteckte oder marginale und schlecht erinnerbare Inhaltsstoffe wie Sprossen, die manchmal nur zur Dekoration eines Gerichts verwendet werden, große Ausbrüche verursachen können. Neben dem HUS/EHEC O104-Ausbruch in 2011 in Deutschland betraf dies in den letzten Jahren auch andere große Ausbrüche. In den Vereinigten Staaten gab es im Jahr 2008 einen bundesstaatübergreifenden lebensmittelbedingten Ausbruch von *Salmonella Saintpaul* (112). Er betraf mehr als 1.400 Erkrankungsfälle. Obwohl die ersten Untersuchungen auf Tomaten als mögliches Vehikel hinwiesen, wurden

Jalapeño- und Serrano-Pepperoni später als Ausbruchsursachen gefunden (113). Die mit diesem Vorfall verbundenen Vorgänge veranlassten die Regierung, die Industrie und die Öffentlichkeit, die Wirksamkeit des US-amerikanischen Lebensmittelsicherheitssystems in Frage zu stellen (114).

Ausbrüche mit schwieriger Expositionserhebung stellen eine Herausforderung für die Epidemiologie dar, da herkömmliche Untersuchungsmethoden möglicherweise nicht ausreichen, um aussagekräftige Schlussfolgerungen zu ziehen. In dieser Situation sind eine aussagekräftige deskriptive Epidemiologie und eine gründlich durchgeführte Hypothesengenerierung noch wichtiger. Initiale Interviews mit Patienten können sehr aussagekräftig sein, um festzustellen, ob mit Fehlklassifikation bezüglich der Exposition gerechnet werden muss. Wenngleich in einem Fragebogen ein Gleichgewicht zwischen der Qualität und der Quantität der Fragen gefunden werden muss, müssen, wenn exposure misclassification vermutet wird, zusätzliche Aspekte in die Befragung eingeschlossen werden.

Ein wichtiger Schritt bei jeder Ausbruchuntersuchung ist die Phase der Hypothesengenerierung, die häufig mittels ausführlichen Befragungen (Exploratives Interview, Trawling interviews) mit den ersten Fallpatienten und anderen involvierten Personen durchgeführt wird. In dieser Phase kann mithilfe der Daten aus der Studie zum Erinnerungsfehler (Kapitel 2.2.4.) eingeschätzt werden, ob mangelhaftes Erinnerungsvermögen potenziell ein Problem darstellt und zu exposure misclassification führt. Dies gibt dem Ausbruchs-Untersuchungsteam die Möglichkeit zu entscheiden, welche Aspekte der Exposition in einer analytisch-epidemiologischen Studie untersucht werden sollten.

Die Verwendung der Informationen aus dem Abrechnungssystem der Kantine in der Fall-Kontroll-Studie zu EHEC O104 in Frankfurt (Kapitel 2.2.1.) ermöglichten eine schnelle und genaue Untersuchung, während exposure misclassification aufgrund von schlecht erinnerten Lebensmittelkäufen vermieden wurde (78). Dies zeigt die Nützlichkeit, wenden Datenquellen verwendet werden, die weitestgehend unabhängig vom Erinnerungsvermögen der Studienteilnehmern sind. In früheren Studien wurden ähnliche Werkzeuge erfolgreich zum Nachweis von Ausbruchsvehikeln eingesetzt. Kundentransaktionsaufzeichnungen aus Datenbanken von Supermärkten zur Übermittlung an Kreditkartenfirmen wurden während einer Untersuchung über EHEC in Rindswürsten in Dänemark genutzt (115). Daten aus Bonusprogrammen, ähnlich der PaybackCard oder DeutschlandCard, wurden innerhalb eines *Salmonella Montevideo*-Ausbruchs in den USA genutzt (116). Ähnlich geschah dies innerhalb eines Cyclosporiasis-Ausbruchs in Kanada (117). In Island wurden bei einem EHEC-Ausbruch die Daten direkt aus dem Supermarktsystem genutzt (118). Diese Informationen aus den Abrechnungsvorgängen können eine Fehlklassifizierung in der Expositionsermittlung nicht ganz verhindern, z.B. wenn Mitarbeiter Lebensmittel für andere kaufen oder Lebensmittel kaufen, die nicht gegessen wurden. Dies könnte die drei Fallpatienten

erklären, die in der Studie in Frankfurt keinen Salat gekauft hatten. Als weitere Limitation muss genannt werden, das Beilagen und Dekorationen von einigen beiseitegelegt werden. Ein Salat kann verschiedene Bestandteile enthalten, die nicht alle gerne gegessen werden, oder aus denen eine Person nur ein paar auswählt. Eine Beachtung dieser Umstände ist daher unerlässlich.

In einigen Ausbrüchen reichen einfach aufgebaute Studien im Fall-Kontroll-Design oder Kohorten-Design basierend auf persönlichen oder telefonischen Befragungen der Patientinnen und Patienten, um ad Hoc epidemiologische Evidenz zu generieren. In anderen komplexeren Situationen ist dies aber nicht möglich und dann müssen innovativ Wege des Studiendesigns oder der Datengewinnung gegangen werden. Für das innovative Studiendesign der Studie in Kapitel 2.2.2. hat sich das Ausbruchsteam entscheiden, nachdem Krankheitscluster und Infektionsorte bekannt wurden. Nach der Entscheidung konnte die Studie schnell innerhalb von 54 Stunden bis zur Publikation der Ergebnisse umgesetzt werden.

Da es häufig schwierig ist, aussagekräftige Ergebnisse zu erhalten, muss man annehmen, dass Studien zu Krankheitsausbrüchen, die durch Exposure misclassification erschwert werden, weniger wahrscheinlich veröffentlicht werden (publication bias). Dies führt daher seltener zu Empfehlungen für ein verbessertes Vorgehen. Durch die Studien zu diesem Thema in Kapitel 2.2.3. stehen nun Werkzeuge und Argumentationshilfen zur Verfügung, um dieses Problem zu berücksichtigen bzw. zu diskutieren.

3.4. Informationsintegrationen – Ein Ausblick in die Zukunft der Ausbruchsuntersuchungen

Die Untersuchung von lebensmittelbedingten Ausbrüchen ist mühsam und bleibt häufig leider auch erfolglos. Eine stetige Verbesserung der Untersuchungsmethoden ist notwendig, um frühzeitig Hypothesen zum Ausbruchsvehikel generieren zu können und nachfolgend Infektionsquellen zu identifizieren und abzustellen.

In Zukunft sind drei Punkte sehr wichtig und in Abbildung 3 ist ein Konzept für die Informationsintegration, d. h. ein Zusammenführen von Informationen aus verschiedenen Datenquellen in eine gemeinsame einheitliche Datenstruktur dargestellt. Um dieses zu erreichen sind in den letzten Jahren Vorarbeiten geleistet worden, die auch Teile dieser Arbeit darstellen. In der Abbildung sind die drei Punkte als (i), (ii), und (iii) dargestellt.

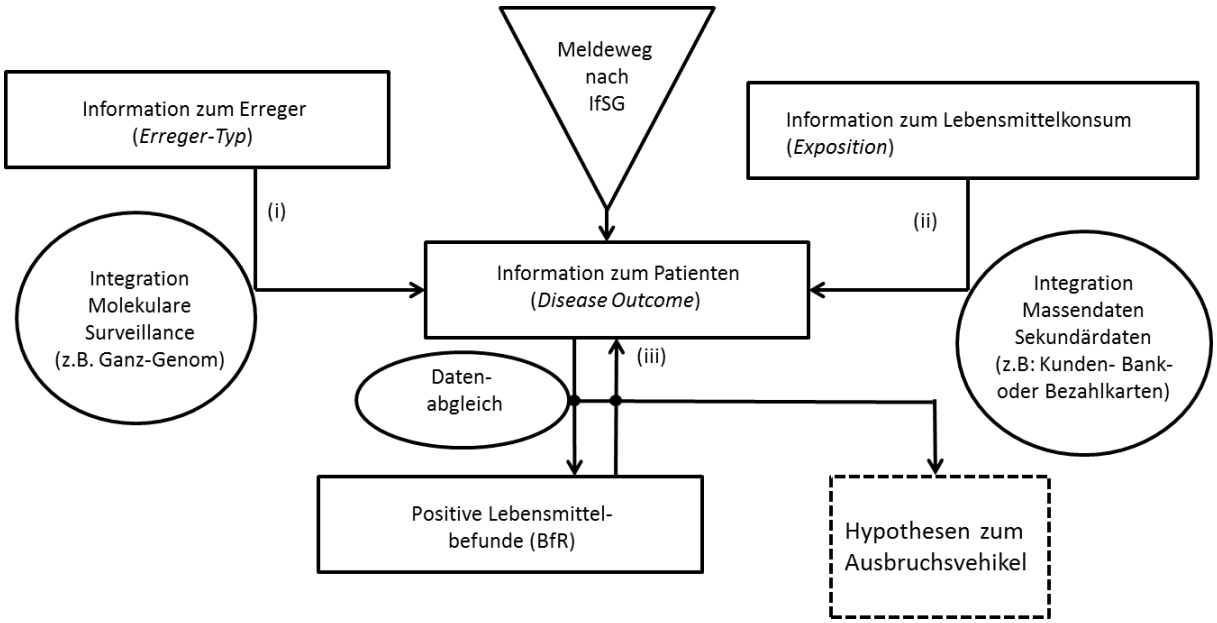

Abbildung 3: Konzept für die Integration von Daten aus der Krankheitsüberwachung, mit dem Ziel der Generierung von Hypothesen zum Ausbruchsvehikel und der nachfolgenden Identifikation von infektionsquellen bei lebensmittelübertragenen Ausbrüchen. (i) (Ruppitsch, et al. 2015, Vygen-Bonnet, et al. 2017); (ii) (Wilking, et al. 2012, Gertler, et al. 2015); (iii) (Kleta, et al. 2017). Quelle: Die Abbildung wurde erstellt von Hendrik Wilking.

Der erste zentrale Punkt ist der zukünftige Aufbau einer bundesweiten und mit internationalen Surveillance-Systemen kompatiblen systematischen Gewinnung, Zusammenführung und Analyse von sequenzbasierten Daten zu Erregertypen (integrierte molekulare Surveillance). Dies insbesondere für lebensmittelübertragene Infektionskrankheiten wie Listeriose, HUS/EHEC, Salmonellose, Hepatitis A, Hepatitis E, aber auch im gleichen Atemzug für einige nicht-lebensmittelübertragene Infektionskrankheiten wie Meningokokken, Tuberkulose, Influenza, HIV, *Clostridium difficile*-Enteritis oder Carbapenemase-bildende *Entererobacteriaceae*. So ein System sollte auf der Analyse von Ganzgenom-Daten (Whole Genome Sequencing, WGS) von Viren oder bakteriellen Isolaten basieren.

Beispielhaft für die Nutzung von WGS in Ausbruchssituationen in Deutschland war ein Listeriose-Ausbruch in 2012-2017 in Zusammenhang mit Bauchspeck (119) und einem HUS/EHEC O157-Ausbruch in 2017 (120). In den USA wurden Plattformen für den Austausch innerhalb des GenomeTrakr-Konsortiums geschaffen. In Europa innerhalb des COMPARE-Konsortiums (121, 122) und vom ECDC. Die Integration von Daten für den Infektionsschutz ist momentan die größte Herausforderung in der Überwachung und Untersuchung von lebensmittelbedingten Infektionskrankheiten.

Als zweiter Punkt ist die systematische Integration von Massendaten (Big data) zu Lebensmittelexpositionen von Patienten in Ausbruchssituationen zu nennen. Hier

bieten das Vorgehen beim HUS/EHEC O104-Ausbruch in 2011 dargestellt in Kap. 2.2.4. (78) und die Simulationsstudie dargestellt in Kap. 2.2.4. (84) erste methodische Ansätze. In Massendaten von Einkaufsdaten können Datensätze Patienten zugeordnet werden und im Rahmen von Ausbruchsuntersuchungen analysiert werden. Beim Auftreten von lebensmittelübertragenen Ausbrüchen in Krankenhäusern muss die Patientenverpflegung elektronisch ausgelesen werden können.

International werden gehäuft Untersuchungen dazu beschrieben und viele dieser Ausbrüche wären wahrscheinlich ohne Massendaten nicht gelöst worden. In Deutschland gibt es aber eine Reihe von methodologischen, rechtlichen und praktischen Hindernissen, die über die bisherigen Studien hinaus gelöst werden müssen. Big-Data-Informationen als Expositionsermittlung sind potenziell leistungsfähig und könnten mit einem zukünftig verbesserten Zugriff möglicherweise ein Routinewerkzeug für Ausbruchsuntersuchungen werden.

Als dritter bedeutender Punkt ist die routinemäßige nicht-anlassbezogene Integration von epidemiologischen Daten aus der Krankheitsüberwachung (Surveillance) von Patienten und der Überwachung von Lebensmitteln zu nennen. Durch systematische Auswertungen nach Erreger-Typ, aber auch Zeit und Geographie können sehr wertvolle Hinweise gefunden werden. Beispielhaft konnte bei einem Listeriose-Ausbruch durch systematisches Screening von Listeria-Isolaten am Bundesinstitut für Risikobewertung (BfR) und dem Vergleich zu Isolaten von Patienten in Lebensmitteln der gleiche *Listeria*-Ausbruchstyp gefunden werden, was schlussendlich zur Detektion des verursachenden Betriebs und zum Stopp des Ausbruchs führte (123).

In Zukunft braucht es neue Methoden, Werkzeuge und Datenquellen, mit denen die Überwachung von lebensmittelbedingten Krankheitserregern und Gefahren so verbessert werden kann, dass Überwachungsressourcen optimal zugeteilt und lebensmittelbedingte Krankheiten besser verhindert werden können. Das übergeordnete Ziel besteht darin, Wege zu entwickeln und aufzuzeigen, wie neue und bestehende Datenquellen besser genutzt werden können, damit Behörden und Industrie eine verbesserte Entscheidungshilfe haben.

4. Zusammenfassung

Der oral-alimentäre Übertragungsmechanismus ist für Infektionen des Menschen äußerst effektiv und lebensmittelbedingte Infektionen durch kontaminierte Nahrungsmittel sind häufig und die damit verbundene Krankheitslast für die Bevölkerung hoch. Die global vernetzte Nahrungsmittelversorgung, der grenzüberschreitende Handel mit lebenden Tieren und der anzunehmende Einfluss des zunehmenden Kostendrucks auf die Qualität der Nahrungsmittelproduktion beeinflussen als externe Faktoren das Erkrankungsrisiko. Die Diversität und kontinuierliche Anpassung bekannter und neu aufkommender Infektionserreger trägt zur Problemlage bei, was der bedeutende Ausbruch durch den neuartigen EHEC O104 im Jahr 2011 gezeigt hat.

Die Experten auf unterschiedlichen Ebenen in Public Health, Bevölkerungsmedizin und Lebensmittelsicherheit müssen sowohl neue Präventionsstrategien entwickeln, als auch routinemäßig kleinere Ausbrüche untersuchen und auf den nächsten großen lebensmittelbedingten Ausbruch vorbereitet sein. Entsprechend dieser Entwicklungen beschäftigt sich diese Arbeit mit epidemiologischen Aspekten dieser lebensmittelübertragener Erreger und zeigt neue Lösungsansätze für Vorgehensweisen in Ausbruchssituationen.

Die bundesweite Querschnittsstudie zum Vorkommen von akuter Gastroenteritis liefert Basisdaten für die Einschätzung der Krankheitslast in der Bevölkerung in Deutschland. Die *T. gondii*-Seroprävalenz-Studie zeigt auf, dass dreiviertel der Bevölkerung Deutschlands im Laufe ihres Lebens eine Infektion durchmachen und dass mit einer hohen Zahl an klinisch manifesten Erkrankungen gerechnet werden muss.

In einer Krisensituation konnte die Fall-Kontroll-Studie zu HUS/EHEC O104 in Frankfurt (Main) die Suche nach dem Ausbruchsvehikel stark eingrenzen und die Kohortenstudie in Lübeck konnte innerhalb dieser Krise Sprossen als Ursache identifizieren. Diese beiden Arbeiten zeigen dabei wertvolle Erfahrungen für Epidemiologinnen und Epidemiologen auf, die mit schwierigen Situationen der Datenerhebung konfrontiert sind. Es kann sehr zielführend sein, die einzelnen Komponenten einer Exposition zu untersuchen, wie sie von denjenigen berichtet werden, die es produzieren oder verkaufen, und nicht die vom Erkrankten berichteten Lebensmittel.

Bei einer anderen Krisensituation konnte durch die Implementierung einer Surveillance zu Norovirus Gastroenteritis das Ausmaß eines Geschehens aufgezeigt werden, sowie deren Verlauf beschrieben und das Infektionsvehikel detektiert werden. Die Studie zur Validität von Datenerhebungen zur Lebensmittel-Exposition konnte den Anteil von Fehlklassifikationen beschreiben und die Notwendigkeit von schnellen Untersuchungen belegen.

Die hier vorgestellten Arbeiten bilden die Grundlage für Präventionsempfehlungen und waren die empirische Grundlage für die Reaktion des Bundes auf bedeutende Public Health-Krisen.

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Erklärung

§ 4 Abs. 3 (1) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

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