UNIVERSITAT DE BARCELONA

Epidemiología de los brotes de gastroenteritis de etiología vírica en instituciones cerradas y semicerradas

Ignacio Parrón Bernabé

EPIDEMIOLOGÍA DE LOS BROTES DE GASTROENTERITIS DE ETIOLOGÍA VÍRICA EN INSTITUCIONES CERRADAS Y SEMICERRADAS

Memoria de tesis doctoral presentada por Ignacio Parrón Bernabé para optar al grado de doctor por la Universidad de Barcelona

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Si he logrado ver más lejos ha sido
porque he subido a hombros de gigantes.
(If I have seen further, it is by standing
on the shoulders of giants)
Sir Isaac Newton

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Escola de Doctorat

Informe director/ tutor sobre l'autorització del dipòsit de la tesi

Dr./a. Àngela Domínguez García, com a director/tutor de la tesi doctoral titulada "Epidemiología de los brotes de gastroenteritis de etiologia vírica en instituciones cerradas y semicerradas" i, d'acord amb el que s'estableix a l'article 35 Normativa reguladora del Doctorat a la Universitat de Barcelona, emeto el següent:

INFORME

(Informe detallat i motivat sobre el contingut de la tesi i sobre l'autorització de dipòsit de la tesi que s'ha demanat)

La tesi doctoral del doctorand Ignacio Parrón Bernabé sobre "Epidemiología de los brotes de gastroenteritis de etiologia vírica en instituciones cerradas y semicerradas" s'ha desenvolupat de forma adequada per assolir tots els objectius proposats inicialment.

Durant el procés de desenvolupant de la tesi el doctorant ha enfortit els seus coneixements científics tant pel que fa a la temàtica de la tesi com al mètode científic.

El doctorant ha planificat la realització dels diferents estudis que han donat lloc als articles que formen part de la tesi, ha analitzat les dades, ha fer una cerca bibliogràfica adequada sobre la temàtica particular de cada estudi i ha comparat adequadament els resultats propis amb els resultats d'altres estudis similars, analitzant les diferències entre els resultats propis i els altres i ha considerat possibles explicacions de les diferències trobades. En cada un dels estudis ha arribat a unes conclusions sobre els objectius plantejats, tot identificant línies de recerca sobre les que s'ha de continuar investigant per obtenir unes conclusions més sòlides.

Finalment el doctorant prenent en consideració els resultats de tots els estudis realitzats el doctorant ha pogut formular unes conclusions de gran interès en salut pública per a la recerca en el camp de l'epidemiologia i el control dels brots de gastroenteritis vírica.

Tenint en compte tot el que he senyalat, considero que el doctorant ha adquirit tots els coneixements necessaris per optar al grau de doctor i autoritzo el dipòsit de la tesi previ a la seva defensa que estableix la normativa reguladora del doctorat a la Universitat de Barcelona.

Barcelona, 16 de setembre de 2022.

(Signatura)

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El camino que culmina aquí se inició hace más de cincuenta años, cuando yo tenía siete u ocho años y mi padre comenzó a trabajar de conserje de la entonces llamada Jefatura Provincial de Sanidad. Nos trasladamos a aquel edificio en el que hoy es el Passeig de Lluís Companys y mi infancia creció rodeada por telegramas que notificaban meningitis, brotes de cólera y botellas de aceite de colza, además de un par de laboratorios de microbiología y una consulta de "enfermedades venéreas". Cualquiera podría pensar que estaba predestinado a trabajar en Salud Pública. Cualquiera menos yo. Después de dudar entre químicas o biología acabé en medicina y no fue hasta finalizada la carrera que, tras asistir a algunas de las jornadas organizadas por el Institut d'Estudis de la Salut, me di cuenta de que no tenía que ir muy lejos para encontrar lo que me gustaba, que estaba ahí, en la que siempre había considerado mi casa. Así que las jornadas me llevaron al Diplomado de Medicina Preventiva del Clínico y el diplomado me llevó a una oposición para optar a una plaza del Cos Superior de Salut Pública.

Y a finales de 1992 allí estaba yo, entrando en la Direcció general de Salut Pública y encontrándome con un elenco de profesiones que me han acompañado y servido de ejemplo a lo largo de mi carrera profesional. De entre ellos quiero especialmente mostrar mi agradecimiento al Dr. José Alcaide y al Dr. Lluís Salleras, de la mano de ellos di mis primeros pasos en este mundo de la epidemiología. También a Pep Álvarez, a quien más considero un amigo que un compañero.

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Índice

ABREVIATURAS Y ACRÓNIMOS

CDC: Centers for Disease Control and Prevention

DNA: Ácido desoxirribonucleico, por el nombre en inglés

ECDC: European Centre for Disease Prevention and Control

GI: Genogrupo I

GII: Genogrupo II

GII.3: Genotipo 3

GII.4: Genotipo 4

GEA: Gastroenteritis aguda

HBGA: Grupo de Antígenos de Histocompatibilidad sanguíneos, por el nombre en inglés

IC95%: Intervalo de confianza del 95%

NTPasa: Nucleósido trifosfatasa

OCDE: Organización para la Cooperación y el Desarrollo Económicos

OMS: Organización Mundial de la Salud.

OR: Odds ratio

ORFs: Marcos de lectura abiertos

Pro: Proteasa

RNA: Ácido ribonucleico, por el nombre en inglés

RpRd: RNA polimerasa dependiente de RNA

RR: Riesgo relativo

RTqPCR: Reacción en cadena de la polimerasa con transcriptasa inversa, por el nombre en inglés

TA: Tasa de ataque

VP1: Proteína viral principal

VP2: Proteína viral menor

VPg: Proteína vinculada al genoma del virus

RT: Razón de tasas

ENUMERACIÓN DE LOS ARTICULOS

Tesis en formato de compendio de artículos. La tesis consta de un objectivo principal y cuatro objetivos secundarios y se desarrolla en los 6 artículos que se enumeran a continuación

Artículo 1

Parrón I, Álvarez J, Jané M, Cornejo Sánchez T, Razquin E, Guix S, Camps G, Pérez C, Domínguez À; Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. A foodborne norovirus outbreak in a nursing home and spread to staff and their household contacts. Epidemiol Infect. 2019;147:e225. doi: 10.1017/S0950268819001146.

Factor de Impacto y cuartil de la revista en 2019: Cuartil Q2; Factor de Impacto: 2,152

Artículo 2

Parrón I, Plasencia E, Cornejo-Sánchez T, Jané M, Pérez C, Izquierdo C, Guix S, Domínguez À, on Behalf of The Working Group for the Study of Acute Gastroenteritis Outbreaks in Catalonia. Human astrovirus outbreak in a daycare center and propagation among household contacts. Viruses. 2021;13(6):1100. doi: 10.3390/v13061100.

Factor de Impacto y cuartil de la revista en 2021: Cuartil: Q2; Factor de Impacto: 5.818

Artículo 3

Parrón I, Barrabeig I, Alseda M, Rius C, Cornejo-Sánchez T, Jané M, Pérez C, Guix S, Domínguez À; Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. Norovirus outbreaks in long-term care facilities in Catalonia from 2017 to 2018. Sci Rep. 2021;11:23218. doi: 10.1038/s41598-021-02348-2.

Factor de Impacto y cuartil de la revista en 2021: Cuartil: Q2; Factor de Impacto: 4,996

Artículo 4

Parrón I, Barrabeig I, Alseda M, Cornejo-Sánchez T, Guix S, Jané M, Izquierdo C, Rius C, Domínguez À, The Working Group for the Study of acute gastroenteritis outbreaks in Catalonia. Involvement of workers in closed and semiclosed institutions in outbreaks of acute gastroenteritis due to norovirus. Viruses. 2020;12:1392. doi: 10.3390/v12121392.

Factor de Impacto y cuartil de la revista en 2020: Cuartil: Q2; Factor de Impacto: 5,048

Articulo 5

Parrón I, Barrabeig I, Soldevila N, Bartolomé R, Guix S, Rius C, Cornejo-Sánchez T, Izquierdo C, Domínguez À; Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. Outbreaks of gastroenteritis due to norovirus in schools and summer camps in Catalonia, 2017-2019. Microbiol Spectr. 2022;10:e0011922. doi: 10.1128/spectrum.00119-22.

Factor de Impacto y cuartil de la revista en 2022: Cuartil: Q1; Factor de Impacto: 9,043

Articulo 6 (enviado a International Journal of Infectious Diseases)

Parrón I, Carol M, Bes N, Izquierdo C, Godoy P, Barrabeig I, . Sala MR, Minguell S, Ferras J, Rius C, Martínez A, Domínguez A, Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. Impact of the COVID-19 pandemic on the incidence of acute gastroenteritis outbreaks in Catalonia (Spain)

1.- Introducción

La gastroenteritis aguda (GEA) es un cuadro clínico de menos de dos semanas de evolución caracterizado por diarrea que puede ir acompañado de náuseas, vómitos, dolor abdominal, sensación distérmica y/o fiebre (1). El síntoma más característico es la diarrea, aunque pueden producirse cuadros clínicos en los que ésta sea poco importante.

La Organización Mundial de la Salud (OMS) define la diarrea como:“la expulsión de tres o más deposiciones líquidas con o sin sangre en 24 h que adoptan la forma del recipiente que las contiene” y el episodio diarreico como “el que cumple el criterio anterior y termina cuando el último día de diarrea es seguido por al menos 48 h de deposiciones normales” (2). Se puede considerar diarrea aguda la que dura menos de dos semanas, diarrea persistente si dura de dos a cuatro semanas y diarrea crónica la que dura más de cuatro semanas (3).

Según las características clínicas, la enfermedad diarreica puede dividirse en (4):

- Diarrea acuosa aguda, que se presenta en aquellos cuadros clínicos producidos por toxinas bacterianas preformadas, bacterias productoras de endotoxinas y patógenos con adherencia a la mucosa intestinal. En los cuadros clínicos producidos por toxinas preformadas y endotoxinas la diarrea se presenta en cuestión de horas y suele ir acompañada de vómitos; la fiebre es escasa o ausente. En los patógenos con adherencia a la mucosa suele aparecer fiebre elevada y los vómitos son menos intensos (3). La diarrea suele durar varios días y los enfermos tienen un alto riesgo de deshidratación. Ejemplos de este tipo de diarreas son las producidas por *Vibrio cholerae* o por rotavirus.

- Disentería aguda, que se caracteriza por diarreas sanguinolentas. Son producidas por agentes invasores como *Campylobacte*r, *Salmonella*, *Shigella*, *E. coli* enterohemorrágica y *Entamoeba histolytica*. Suelen producir cuadros clínicos con fiebre alta e intenso dolor abdominal. Pueden aparecer afectaciones extradigestivas como artritis, uretritis, conjuntivitis o síndrome hemolítico-urémico (3). Además de la deshidratación, otras complicaciones que se pueden presentar son daño de la pared intestinal, sepsis y desnutrición.

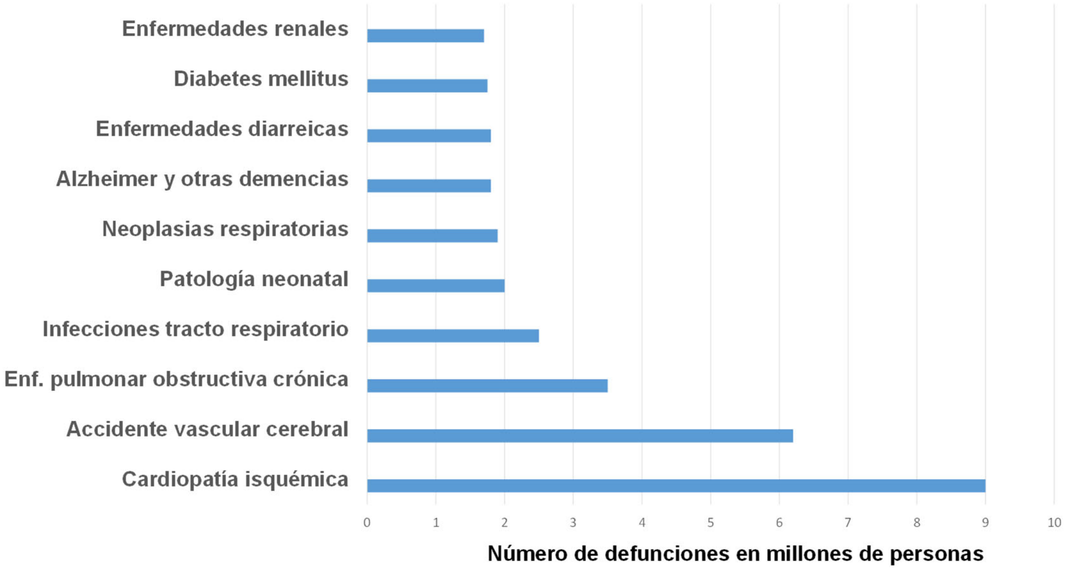
- Diarrea persistente, cuya duración es de 14 o más días. Suele estar provocada por parásitos o por causas no infecciosas como la enfermedad inflamatoria intestinal. Puede complicarse con infecciones extraintestinales, deshidratación y desnutrición.

La mayoría de episodios de GEA son autolimitados y no suelen complicarse, pero la pérdida de líquidos y electrolitos puede provocar cuadros clínicos graves de deshidratación en personas especialmente vulnerables (niños y ancianos).

2.- Epidemiología de las gastroenteritis agudas a nivel mundial

Las enfermedades diarreicas representan la octava causa de muerte a nivel mundial y la segunda causa por etiología infecciosa, después de las infecciones de las vías respiratorias (5,6,7) (Figura 1).

Figura 1. Las 10 principales causas de defunción a nivel mundial, año 2019.



Fuente: Organización Mundial de la Salud. Disponible en:

https://www.who.int/es/news-room/fact-sheets/detail/the-top-10-causes-of-death

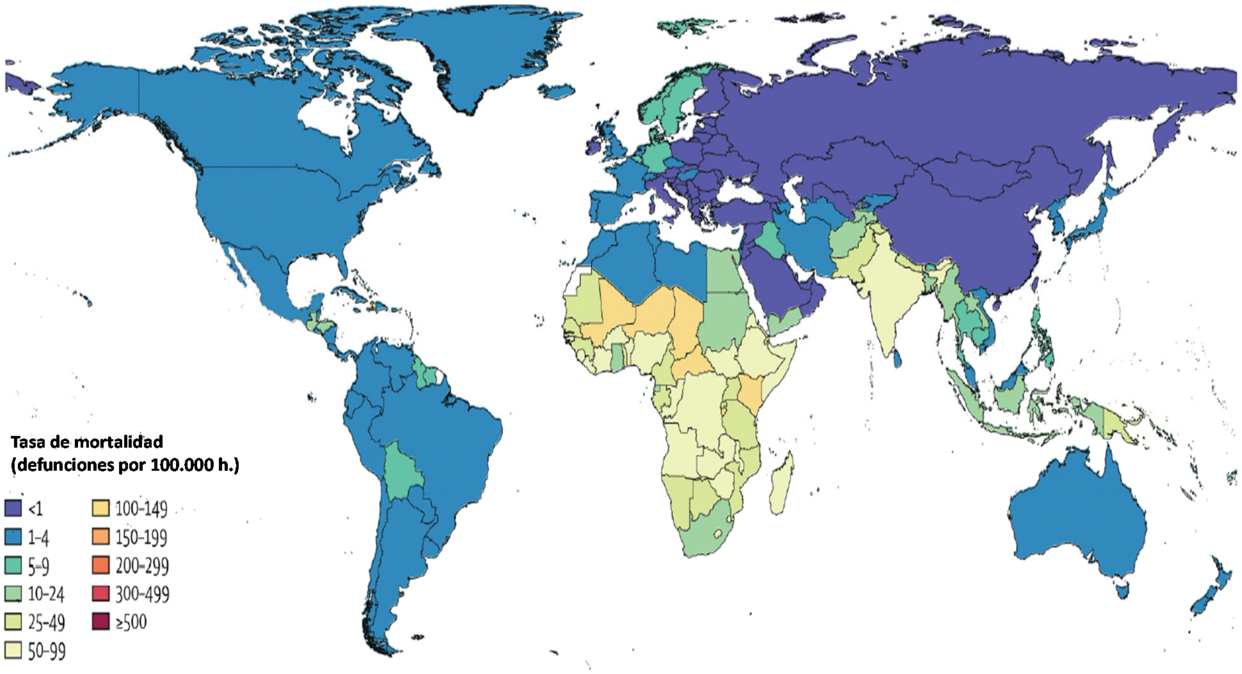
Consultado el 2 de agosto de 2022 (8).

Las GEA están altamente relacionadas con el nivel socioeconómico del país. En 2019 las enfermedades diarreicas representaron la 5ª causa de muerte en países con bajos ingresos, y la 6ª causa en países con ingresos medianos-bajos. En países con ingresos medianos-altos y países con ingresos altos las enfermedades diarreicas no se encuentran dentro de las 10 primeras causas de muerte (8).

En general, las tasas de mortalidad son más altas en el África subsahariana y en el sur de Asia, donde oscilan entre 50 y 150 defunciones por 100.000 habitantes al año. En algunos países como la República Centroafricana y el Chad se pueden observar tasas de hasta 150 defunciones por 100.000 habitantes al año. En 2019 la tasa de mortalidad por enfermedad diarreica para toda la Región Africana de la OMS fue de 67,12 defunciones por 100.000 habitantes (9).

La mortalidad anual en el resto del mundo es inferior a 5 casos por 100.000 habitantes. En la Región Europea de la OMS fue de 1,15 casos por 100.000 habitantes en 2019 (Figura 2). En España se producen anualmente unas 1000 muertes por enfermedades infecciosas intestinales; en 2019 se atribuyeron a esta causa 1034 muertes, lo que corresponde a una tasa de 2,20 defunciones por 100.000 habitantes (10).

Figura 2. Distribución mundial de la tasa de mortalidad por enfermedades diarreicas por 100.000 habitantes al año, 2016.



Fuente: Troeger C et al. The Lancet Infectious Diseases, 2018 (11).

Durante los últimos años se han producido importantes cambios en la morbi-mortalidad mundial por GEA, pasando de 2,6 millones de muertes en 2000 a 1,5 millones en 2019 (8).

La OMS el 11 de marzo de 2020 declaró la enfermedad causada por el virus SARS-CoV-2 como pandemia de COVID-19 y recomendó la aplicación de medidas para prevenir la transmisión por gotas y contacto, incluida la higiene de manos (12); posteriormente se observó también la transmisión mediante aerosoles (13-16).

Era previsible que las medidas adoptadas frente a COVID-19 también repercutieran en la transmisión de otras enfermedades con mecanismos de transmisión parecidos, observándose una importante reducción en la incidencia de gripe aquella misma temporada (de febrero a septiembre de 2020) en los países del cono sur (17).

La disminución de notificación de enfermedades transmisibles no se observó sólo en enfermedades que compartían mecanismos de transmisión similares al SARS-CoV-2. En Taiwan, entre 2019 y 2020, Lai et al. (18) describieron una reducción en la notificación de enfermedades transmitidas por vectores. Algunos autores indicaron que la disminución en la notificación de casos podía explicarse por una menor incidencia, pero también por una menor detección y comunicación de casos a los servicios de vigilancia epidemiológica (19).

Más de 90% de los casos de GEA se deben a agentes infecciosos. El restante 10% se debe a fármacos, ingestión de sustancias tóxicas, problemas alimentarios, isquemia y otros trastornos (3,20) (tabla 1).

A nivel mundial los patógenos que más contribuyen a la producción de GEA durante el primer año de vida son norovirus GII, rotavirus, *Campylobacter* spp., astrovirus y *Cryptosporidium* spp. y durante el segundo año de vida lo son *Campylobacter* spp., norovirus GII, rotavirus, astrovirus y *Shigella* spp. (21).

Tabla 1. Causas de GEA a nivel mundial.

Causas	Menores de 5 años	Con 5 o más años
Infecciones entéricas	Rotavirus Norovirus Adenovirus *Campylobacter* *Salmonella*	*Campylobacter* *Salmonella* Rotavirus Adenovirus
Infecciones extraintestinales	Infecciones respiratorias	Gripe Infecciones virales
Alergias alimentarias	Alergia a proteínas leche de vaca Alergia a proteínas de soja	
Transtornos de absorción	Déficit de lactasa Déficit de sacarosa-isomaltasa Enfermedad celíaca Fibrosis quística	Intolerancia a la lactosa de tipo adulto
Patologías gastrointestinales		Colon irritable Enf.inflamatoria intestinal
Endocrinopatías	Hipertiroidismo Diabetes mellitus Síndrome de Addison	
Neoplasias	Feocromocitoma Síndrome carcinoide	
Causas quirúrgicas	Invaginación	
Fármacos	Antibióticos Laxantes	
Intoxicaciones	Metales pesados (cobre, zinc)	

Fuente: Benéitez Maestre AM, de Miguel Durán F. Pediatría Integral, 2015 (20)

La etiología de las GEA varía según las condiciones socioeconómicas del país. En países desarrollados predominan las gastroenteritis víricas, mientras que en países en vías de desarrollo, si bien los virus siguen siendo la causa más común de gastroenteritis, es frecuente identificar también etiologías bacterianas (22).

El mismo fenómeno se observa en las edades infantiles. Los virus entéricos predominan en niños tanto en países desarrollados como en países en vías de desarrollo, pero en estos últimos se observa afectación por protozoos (principalmente *Giardia intestinalis* y *Entamoeba* spp.) con mayor frecuencia (23).

Los virus son la principal causa de GEA tanto en países con altos ingresos como en países con bajos ingresos. En menores de 5 años rotavirus es la principal causa de diarrea grave a nivel mundial y norovirus, sapovirus, astrovirus y adenovirus también se asocian frecuentemente a cuadros clínicos diarreicos. Las gastroenteritis víricas generalmente producen diarreas no sanguinolentas y vómitos. Rotavirus puede producir clínicos más graves y de una mayor letalidad respecto a otras etiologías víricas (24,25).

Se estima que la prevalencia de norovirus en pacientes con GEA en países en vías de desarrollo es del 17%. En países con bajos ingresos, en los que se producen más GEA que en los países desarrollados, norovirus está menos involucrado en la aparición de estos cuadros clínicos que en los países con mayores ingresos. Una posible explicación es que las personas de países con bajos ingresos no acuden a servicios médicos cuando presentan cuadros clínicos leves o moderados de gastroenteritis (como son los producidos por norovirus), lo que puede ocasionar una subestimación de la prevalencia de norovirus (26).

3.- Virus causantes de gastroenteritis aguda

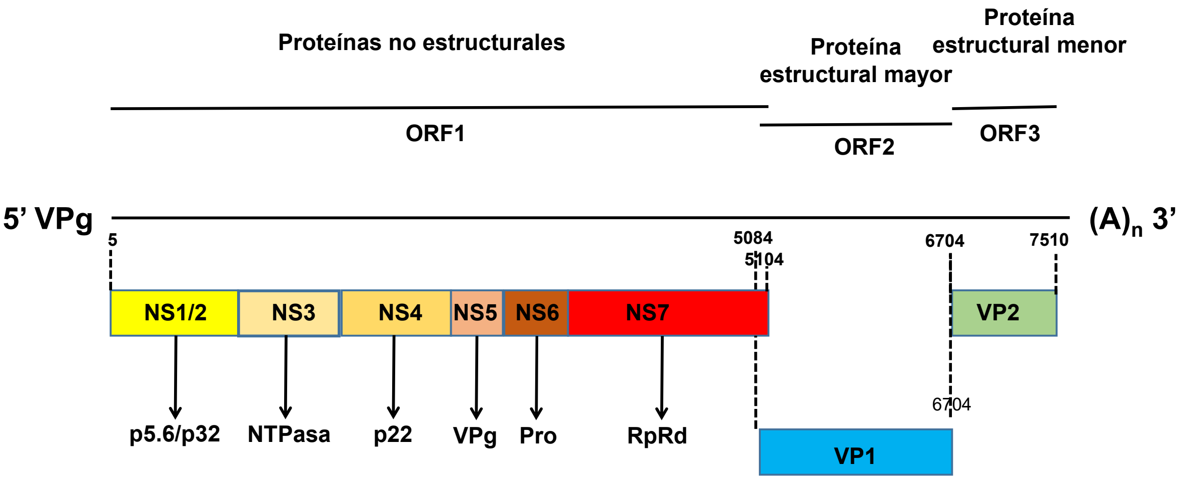
3.1.- Familia *Caliciviridae*

Tanto norovirus como sapovirus pertenecen a la familia *Caliciviridae.*

Los virus de la familia *Caliciviridae* son virus con un genoma monocatenario positivo de ácido ribonucleico (RNA) de 7,4 a 8,3 kb, no envueltos y de simetría icosaédrica (27) (Figura 3).

Su genoma posee tres marcos abiertos de lectura (ORF), una zona de RNA genómico que forma la ORF1 y que codifica 6 proteínas no estructurales: nucleósido trifosfatasa (NTPasa), VPg, proteasa (Pro), RNA polimerasa RNA dependiente (RpRd), y las proteínas NS1/2 (p5.6/p32) y NS4 (p22) y una zona de RNA subgenómico con dos ORF que codifican proteínas de la cápside, la ORF2 que codifica la proteína viral principal (VP1) y la ORF3 que codifica la proteína viral menor (VP2). El extremo 5' carece de estructura Cap característica de los RNA mensajeros eucariotas y, en su lugar, está unido covalentemente a la proteína viral VPg que es considerada una cap proteína (28).

Figura 3. Genoma de los calicivirus humanos.



Fuente: Vinjé J et al. Journal of General Virology, 2019 (27)

La familia está formada por 11 géneros, 5 de ellos admitidos inicialmente (*Norovirus*, *Nebovirus*, *Sapovirus*, *Lagovirus*, *Vesivirus*) y otros 6 admitidos más recientemente (*Nacovirus, Bavovirus*, *Recovirus*, *Salovirus*, *Minovirus* y *Valovirus*) (28,29).

Afectan a mamíferos, aves y peces. Siete géneros (*Logovirus*, *Norovirus*, *Nebovirus*, *Recovirus*, *Sapovirus*, *Valovirus* y *Vesivirus*) son patógenos primarios en mamíferos; dos (*Bacovirus* y *Nacovirus*) afectan a aves y otros dos (*Minovirus* y *Salovirus*) afectan a peces (27).

3.2.- Norovirus

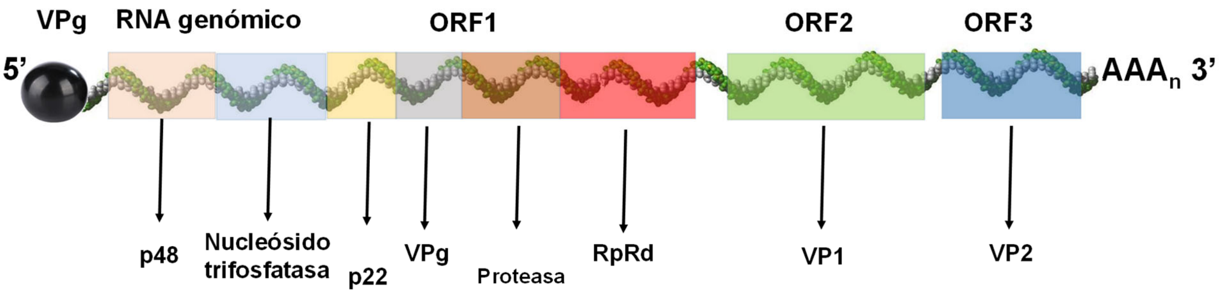
Norovirus fue identificado por primera vez al observarse partículas de 25 nm mediante microscopía electrónica en el filtrado de heces de una persona afectada de gastroenteritis no bacteriana en un brote ocurrido en Norwalk (Ohio, Estados Unidos), induciéndose el cuadro clínico a voluntarios a los que les administró el filtrado (30). La enfermedad ya había sido descrita en 1929 por Zahorsky, que indicó que se trataba de un cuadro clínico que se presentaba preferentemente en los meses fríos del año y en el que la sintomatología más predominante eran los vómitos, por lo que se refirió a ella como “enfermedad del vómito invernal” (31).

En 1969 Adler et al. describieron la enfermedad como un cuadro clínico en que predominan las náuseas y los vómitos (98% y 92% de los casos, respectivamente), dolor abdominal en el 58% de los enfermos y diarrea y fiebre en aproximadamente el 35% de los enfermos. Estimaron un periodo de incubación de 48 horas, observando que la enfermedad se autolimitaba y que los pacientes se recuperaban en aproximadamente 24 horas (32).

En 1982 Kaplan et al. describieron los criterios para sospechar que un brote estaba producido por norovirus: muestra de heces negativa para bacterias, periodo de incubación entre 15 y 48 horas, vómitos en la mitad o más de los casos y duración de la clínica entre 12 y 60 horas (33).

Norovirus es un virus no envuelto cuyo genoma está formado por una única cadena positiva de RNA con un tamaño de unos 7,9 kb. En el extremo 5’ está unido por un enlace covalente con la proteína viral genómica (VPg) y en el extremo 3' posee una cadena de poliA. Dentro de la cadena de RNA existen códigos de finalización que determinan tres marcos abiertos de lectura (ORFs). El mayor de los tres ORF es el ORF1 que codifica una única poliproteína que después se divide en un proceso de proteólisis para dar lugar a 6 proteínas no estructurales, incluida la RNA polimerasa RNA dependiente. ORF2 codifica la proteína VP1, que contiene el dominio P cuya variabilidad se utilizará para clasificar taxonómicamente al virus y ORF3 codifica la proteína VP2. Ambas proteínas son proteínas estructurales de la cápside vírica que posee 90 dímeros de VP1 que le dan una estructura icosaédrica con unas formaciones con aspecto de copa (*calyx* en latín) típicas de los calicivirus (34,35) (Figura 4). El dominio P tiene gran variabilidad e interacciona con los grupos de antígenos de histocompatibilidad sanguíneos (HBGA) (36).

Figura 4. Estructura genómica de norovirus.



Fuente: Robilotti E et al. Clinical Microbiology Review, 2015 (34)

Norovirus se puede clasificar en diferentes genogrupos. La secuencia de aminoácidos de la proteína estructural VP1 se ha utilizado para los análisis filogenéticos (37,38).

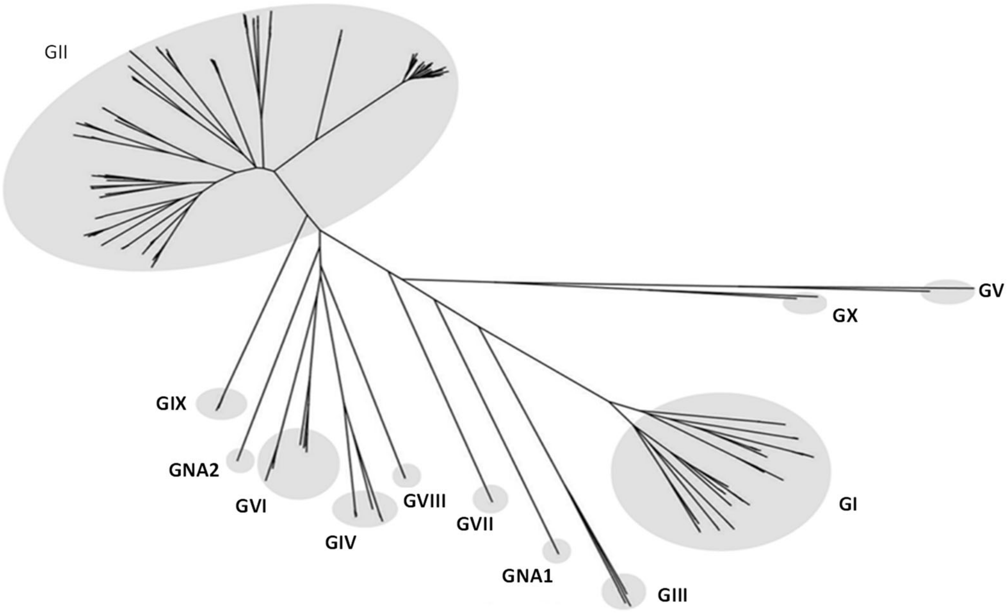
Clásicamente los norovirus se han clasificado en 6 genogrupos (GI a GVI) (39,40). En 2007 un norovirus canino fue secuenciado en perros en Hong Kong (40,41) y posteriormente identificado en 2014 en aguas residuales en Uruguay

(42), confirmándose como un nuevo genogrupo (GVII). En 2004 y 2011 se identificó un nuevo norovirus en heces de pacientes con gastroenteritis en Chiba y en Yuzawa (Japón) (43) y la secuenciación de la VP1 confirmó que se trataba de un nuevo genogrupo (GVIII). En la última actualización del Comité Internacional de Taxonomía de Virus (36), la diversidad en la VP1 de un genotipo de GII (el GII.15) ha provocado que se haya reclasificado como un nuevo genogrupo (GIX) y el análisis filogenético de un importante número de muestras de frotis anales y faríngeos en China ha dado lugar a la consideración del genogrupo GX (44).

Actualmente, según la diversidad de la proteína VP1, norovirus se clasifica en 10 genogrupos (GI a GX) (Figura 5) (36). Se consideraba que solo GI, GII y GIV eran capaces de infectar a humanos, pero actualmente se sabe que GVIII y GX también pueden producir infecciones en humanos (45).

A su vez, cada genogrupo se divide en genotipos cuando la secuencia de aminoácidos de VP1 difiere en más de un 20% del resto de genotipos conocidos. Según este criterio existen 49 genotipos (9 GI, 27 GII, 3 GIII, 2 GIV, GV y GVI y 1 GVII, GVIII, GIX y GX) (36).

Figura 5. Clasificación filogenética de norovirus basada en la secuenciación de aminoácidos de VP1.

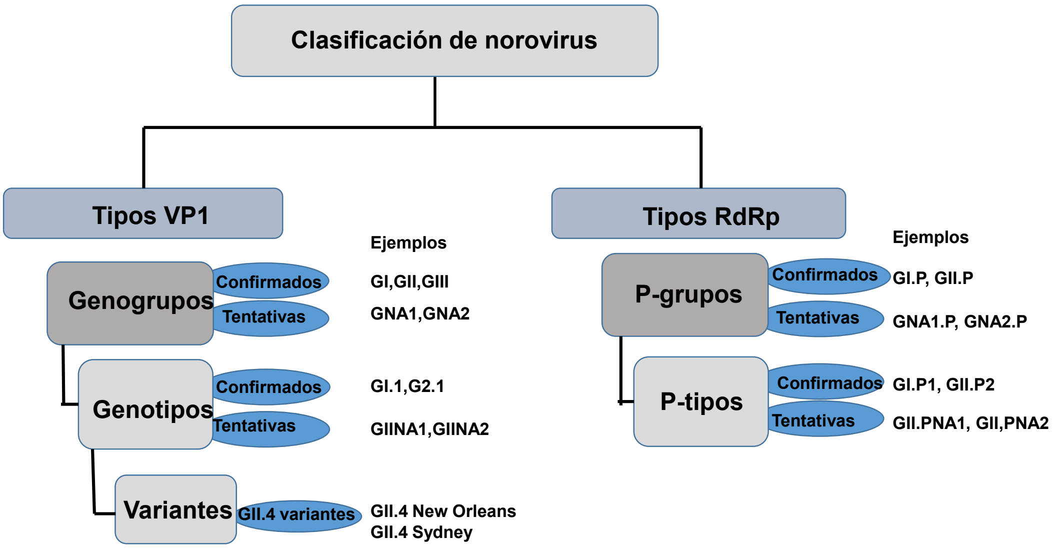


Fuente: Chhabra A et al. Journal of General Virology, 2019 (36)

Los criterios de clasificación no permiten distinguir entre las variantes de GII.4, por lo que el Comité Internacional de Taxonomía de Virus acordó que el genotipo GII.4 se subdividía en variantes según la diversidad de secuencias (46-49). Estas nuevas variantes de GII.4 son reconocidas cuando se convierten en linajes epidémicos en dos o más ubicaciones geográficas distintas, siendo la variante GII.4 Sydney la de más reciente aparición (36).

Según la diversidad de nucleótidos en la región RpRd de ORF1, norovirus puede dividirse en 8 grupos P (GI.P, GII.P, GIII.P, GIV.P, GV.P GVI.P, GVII.P y GX.P) y estos grupos en 60 tipos P diferentes (14 GI.P, 37 GPII.P, 2 GIII.P, 1 GIV.P, 2 GV.P y GVI.P, 1 GVII.P y GX.P); así mismo existen 14 tentativas de tipus P (4 GI.P, 9 GII.P y 1 GIV.P) (36) (Figura 6).

Figura 6. Clasificación de norovirus según secuenciación de VP1 o de la región RpRd.



Fuente: Chhabra A et al. Journal of General Virology, 2019 (36)

Las recombinaciones suelen producirse entre la ORF1 y la ORF2, por lo que pueden afectar tanto a la secuenciación de la polimerasa tipo P como a la proteína VP1. Estas recombinaciones son las que generan diversidad en norovirus (50), por lo que es importante determinar tanto la secuenciación de la polimerasa como la de la cápside para describir las características genotípicas de las cepas clínicas (51,52).

Dado el elevado número de recombinaciones descritas en los últimos años, se hace necesaria una clasificación dual de norovirus que informe tanto de la secuenciación de VP1 como de la región RpRd, por lo que se ha propuesto una clasificación en la que primero se indica el genotipo de VP1 seguido del tipo P (Tabla 2).

Tabla 2. Ejemplos de la nueva propuesta de denominación dual de norovirus.

Denominación habitual	**Nueva denominación**
GI.P6-GI.6	GI.6[P6]
GIPd-GI.3	GI.3[P13]
GIIP1-GII.1	GII.1[P1]
GII.P12-GII.3	GII.3[P12]
GII.Pe-GII.4 Sydney	GII.4 Sydney[P31]
GII.P16-GII.4 Sydney	GII.4 Sydney[P16]
GII.P4 New Orleans-GII.4 Sydney	GII.4 Sydney [P4 New Orleans]
GII.Pe-GII.17	GII.17[P31]
GII.P15-GII.15	GIX.1 [GII.P15]
GIV.P1-GIV.2	GIV.2[GVI.P1]

Fuente: Chhabra A et al. Journal of General Virology, 2019 (36)

Los virus de los que en las bases de datos se dispone de una sola secuenciación se clasifican en tentativas de genogrupos (GNA1 y GNA2) o tentativas de genotipos (GII.NA1, GII.NA2 y GIV.NA1), en espera de tener secuenciaciones adicionales para su asignación definitiva.

De los 10 genogrupos descritos, GI, GII, GIV, GVIII y GIX producen enfermedad en humanos (53).

El periodo de incubación es de 24 a 48 h y produce un cuadro clínico que suele autolimitarse en 2 o 3 días. Una de las características que permite diferenciar las gastroenteritis por norovirus de las producidas por otros virus es la gran proporción (50% o más) de personas que presentan vómitos. El principal modo de transmisión es el contacto directo persona a persona, por vía feco-oral. También puede transmitirse indirectamente por consumo de alimentos o agua contaminada (54).

La transmisión de norovirus se ve favorecida por su baja dosis infectiva (una media de 18 partículas víricas) (55), su alta resistencia al cloro (56), al calor y a la refrigeración (57), a los disolventes orgánicos y al pH ácido (58,59).

Norovirus es la causa del 60% de los casos esporádicos de GEA en los países desarrollados (34) y se estima que a nivel mundial es responsable del 90% de todos los brotes de gastroenteritis víricas (55).

Hardstaff et al. en una revisión sistemática de artículos sobre brotes de norovirus con transmisión alimentaria publicados entre 2003 y 2017 encontraron que, aunque este tipo de brotes se presentaba en cualquier parte del mundo, la mayor proporción correspondía a Europa, donde el 57% de los brotes fueron atribuibles a norovirus (60).

Norovirus es una de las principales causas de brotes transmitidos por alimentos. En Estados Unidos aproximadamente el 50% de los brotes detectados que han sido de transmisión alimentaria fueron causados por norovirus, ya fuera a partir de un manipulador de alimentos infectado o directamente por el consumo de alimentos contaminados en origen. El rango de personas expuestas en los brotes fue muy amplio (de 2 a 1580 personas) y el rango de enfermos fue de 2 a 305 (media de 23 personas por brote) (61) .

En 2020 en el Reino Unido norovirus fue responsable de unos 380.000 casos de intoxicación alimentaria (62).

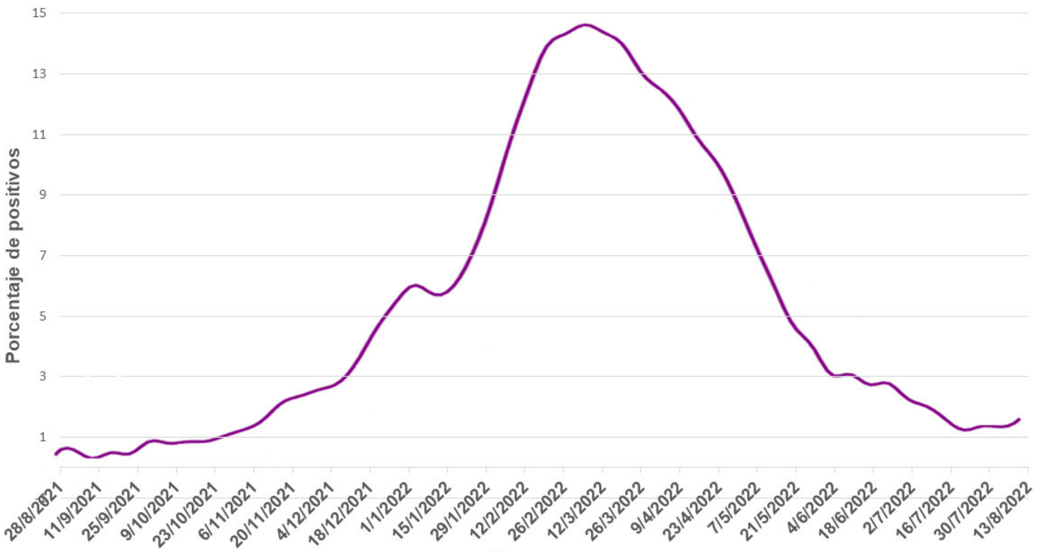
Alimentos frecuentemente implicados son los vegetales de hojas verdes (como por ejemplo la lechuga), la fruta fresca y el marisco (61). En la revisión realizada por Hardstaff et al. el alimento más frecuentemente implicado fue el marisco (el 61% de los brotes), destacando especialmente los brotes producidos por consumo de ostras (89% de los brotes vehiculados por marisco) (60).

La transmisión hídrica de norovirus ha sido ampliamente documentada. Graun et al. describieron 422 brotes de norovirus debidos al consumo de agua contaminada entre 1971 y 2006 en Estados Unidos (63). En 2018 Kauppinen et al. describieron dos brotes transmitidos por aguas contaminadas en Finlandia en los que observaron una larga persistencia de norovirus y adenovirus en muestras ambientales (64). También están ampliamente descritos brotes por norovirus relacionados con aguas recreativas. En Holanda en 2012 en un brote que afectó a 100 personas relacionado con el uso recreativo de un lago se identificó norovirus GI como agente causal (65) y en un estudio del agua de 4 playas del estuario del Amazona en Brasil se identificó norovirus en el 37% de las muestras (66).

Frecuentemente la contaminación de aguas de consumo es debida a una conexión entre el sistema de aguas sépticas y el de agua de consumo o el de aguas recreativas (67).

Los brotes por norovirus ocurren más frecuentemente durante los meses fríos del año, final del otoño, invierno y principio de la primavera (Figura 7) (68). Steele et al. en un estudio de brotes por norovirus en 2009 en Estados Unidos observaron que el 68% de estos brotes se producían durante los meses de otoño e invierno (69) y en un metaanálisis realizado por Ahmed et al. sobre 293 estudios de brotes por norovirus se observó que el 71% ocurrieron en los meses fríos del año (octubre a marzo en el hemisferio norte y abril a septiembre en el hemisferio sur) (70).

Figura 7. Porcentaje de muestras positivas del total de muestras analizadas para norovirus. Estados Unidos, agosto de 2021 a agosto de 2022.



Fuente: CDC. The National Respiratory and Enteric Virus Surveillance System (NREVSS). Disponible en: https://www.cdc.gov/surveillance/nrevss/norovirus/natl-trend.html. Consultado el 27 de agosto de 2022 (68)

2.3.- Sapovirus

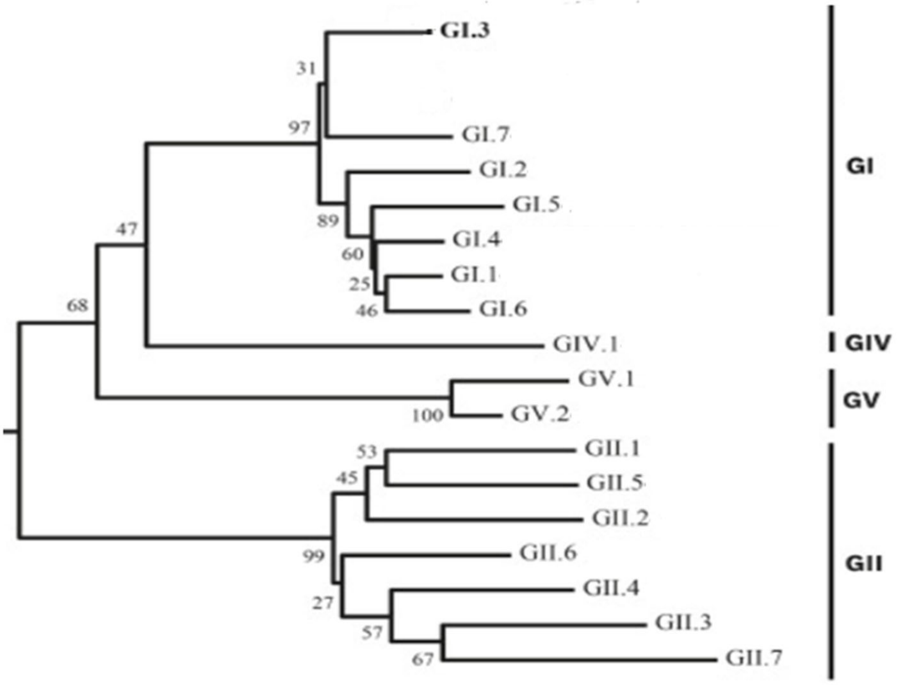
Fue identificado por primera vez en 1976 en muestras de heces provenientes de pacientes con gastroenteritis en el Reino Unido mediante microscopía electrónica, observándose partículas de 30 a 38 nm de diámetro de geometría icosaédrica con la típica morfología en su superficie de formaciones con depresión en forma de copa propia de los calicivirus. Se le denominó sapovirus después de que fuera descrito un brote por este agente en Sapporo, Japón, en el año 1982. A partir de entonces se le denominó “Sapporo-like viruses”. En el año 2002 el Comité Internacional de Taxonomía de Virus denominó a la especie *Sapporo virus* formando parte del género *Sapovirus* y de la familia *Caliciviridae* (71).

Junto con norovirus, es el otro miembro de la familia *Caliciviridae* que puede causar enfermedad en los humanos provocando cuadros clínicos de GEA en forma de casos esporádicos y de brotes.

Los sapovirus presentan gran diversidad genómica y son clasificados según la secuenciación de aminoácidos de la proteína VP1 en 15 genogrupos que afectan, además de al hombre, a cerdos, monos, perros, leones marinos y murciélagos (29). Recientemente se han propuesto nuevos genogrupos, lo que aumentaría el número de genogrupos hasta un total de 19 (72).

Cuatro de los 5 genogrupos (GI, GII, GIV y GV) con 17 genotipos (GI.1 a GI.7, GII.1 a GII.7, GIV.1, GV.1 y GV.2) son patógenos humanos (Figura 8) (71).

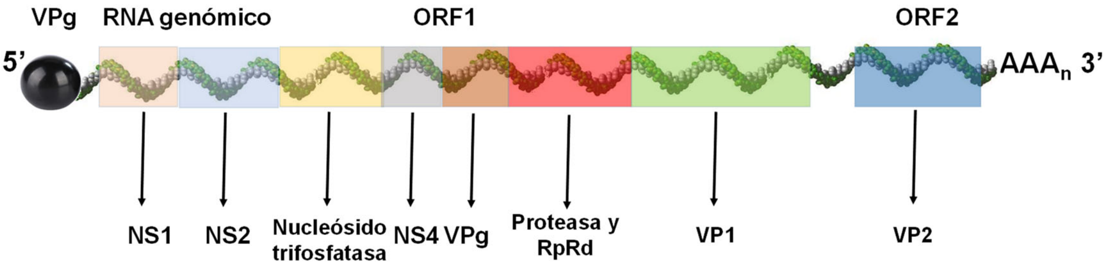
Figura 8. Árbol poligenético de los sapovirus patógenos humanos.



Fuente (modificado): Cho SR et al. Journal of Korean Medical Science, 2020 (73)

Posee un RNA monocatenario positivo de 7,1 a 7,7 kb. de tamaño; el extremo 5' está unido covalentemente a la proteína viral genómica (VPg) y el extremo 3' posee una cola de poli-A. A diferencia de norovirus, posee solo dos ORFs: ORF1 codifica 6 proteínas no estructurales, incluida la RNA polimerasa RNA dependiente, y la proteína mayor de la cápside VP1 y ORF2 codifica la proteína menor de la cápside VP2 (Figura 9). En algunos sapovirus humanos y de murciélago se ha detectado un tercer ORF, aunque su función es desconocida. Esta organización del genoma en dos ORF también se observa en otros calicivirus como lagovirus y nebovirus, mientras que otros como vesivirus y recovirus tienen una organización similar a norovirus con tres ORFs (71).

Figura 9. Estructura genómica de sapovirus.



Fuente: Oka T et al. Clinical Microbiology Review, 2015 (71)

ORF1 codifica una poliproteína que posteriormente es dividida por la proteasa (NS6) en las 6 proteínas no estructurales y la VP1. La región de unión entre la zona de transición entre la proteína NS7 (la RNA polimerasa RNA dependiente) y la proteína mayor de la cápside VP1 se ha utilizado como zona diana para la detección de astrovirus mediante PCR dada la elevada sensibilidad de las pruebas dirigidas a dicha zona.

Causan cuadros clínicos de GEA en humanos en todo el mundo, tanto en forma de casos esporádicos como de brotes, normalmente en recién nacidos y niños pequeños (74-78). Representan entre el 2 y el 12% de las diarreas agudas en niños menores de 2 años (79).

En los cuadros clínicos que ocasionan predominan los vómitos y el dolor abdominal, aunque también se pueden presentar diarreas y, en menor medida, fiebre.

Tras la introducción de la vacuna contra rotavirus, algunos autores consideran a sapovirus la segunda causa de gastroenteritis aguda en niños después de norovirus (80).

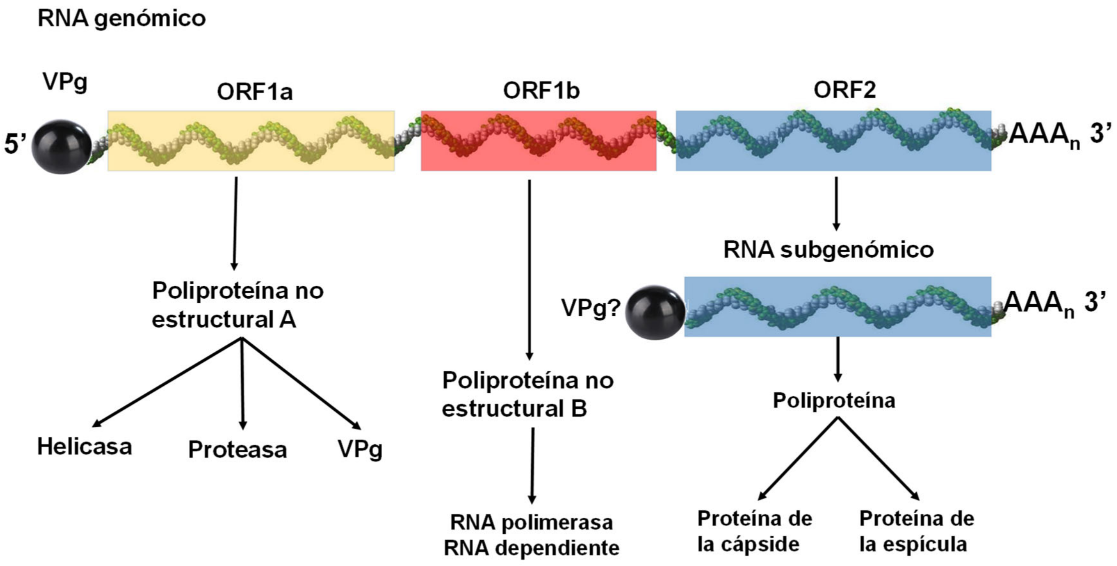
Tiene un periodo de incubación de 1 a 3 días, con una media de 1,7 días (81). Normalmente la clínica se autolimita en una semana y son raras las complicaciones. Se han reportado brotes en residencias geriátricas (82).

3.4.- Astrovirus

Astrovirus fue descrito por primera vez en 1975 por Appleton y Higgins (83) al observar mediante microscopía electrónica unas partículas de 28 a 30 nm en las heces de niños con cuadros clínicos de diarreas en una sala de maternidad en Inglaterra. Al mismo tiempo, Madeley y Cosgrove (84) describieron pequeños virus redondos con aspecto de estrella en recién nacidos hospitalizados por gastroenteritis a las que denominaron astrovirus (de la palabra griega "*astron*" que significa estrella).

En 1995 el Comité Internacional de Taxonomía de Virus definió la familia *Astroviridae* como virus icosaédricos de 28 a 41 nm de diámetro no envueltos en los que el 10% de los virones presentan un aspecto característico de estrella de cinco o seis puntas; el genoma está formado por una cadena positiva de RNA de 6,4 a 7,7 kb con un poly(A) en su extremo 3' y su extremo 5' unido por un enlace covalente a la proteína viral genómica VPg. Posee tres ORF, ORF1a y ORF1b en el extremo 5' que codifican las proteínas no estructurales (nsPs) que participan en la transcripción y replicación del virus y un tercer ORF en el extremo 3' (ORF2) que codifica las proteínas estructurales expresadas por un RNA subgenómico (85) (Figura 10).

Figura 10. Organización del genoma y de la proteína de astrovirus.



Fuente (modificado): Bosch A et al. Clinical Microbiology Review, 2014 (85)

Inicialmente, la família *Astroviridae* constava de un solo género, *Astrovirus*, basado en la morfología del virión. Más tarde se establecieron dos géneros basados en sus huéspedes originales: *Mamastrovirus* (MAstV) y *Avastrovirus* (AAstV), que infectan especies de mamíferos y de aves, respectivamente. Son virus no envueltos patógenos para varias especies de mamíferos (género *Mamastrovirus*) y de aves (género *Avastrovirus*). El género *Mamastrovirus* comprende 19 especies (con 33 genotipos) y el de *Avastrovirus* 3 especies (con 11 genotipos). Cuatro de los genotipos del género *Mamastrovirus* son patógenos humanos: HAstV clásico (*Mamastrovirus* 1), HAstV-MLB (*Mamastrovirus* 6), *Mamastrovirus* 8 y *Mamastrovirus* 9.

Del genotipo HAstV clásico se conocen ocho serotipos patógenos humanos (HAstV1 a HAstV8) que pueden producir gastroenteritis no bacterianas. HAstV1 es el serotipo más aislado a nivel mundial (86-88), aunque ocasionalmente otros serotipos se han mostrado más prevalentes en alguna zona geográficas (89). La infección con un serotipo no parece conferir inmunidad para otros serotipos (90).

A partir de 2008 se han encontrado nuevas variedades de astrovirus (MLB1/MLB2, VA1/VA2/VA3, y HMO A/B/C) en muestras clínicas de pacientes con y sin diarrea (91-93)

Clínicamente la infección por astrovirus produce cefalea, náuseas, vómitos, diarrea y dolor abdominal, aunque los vómitos son menos frecuentes que en las gastroenteritis por rotavirus o calicivirus (94,95). Su periodo de incubación, de 4,5 días de media, es largo en comparación a otros virus productores de GEA (81). La transmisión es por contacto directo con las personas afectadas, sus vómitos o sus heces (62), por consumo de alimentos o agua contaminados y por fómites (85,96).

Los astrovirus humanos causan el 10% de los cuadros clínicos de gastroenteritis en niños menores de 3 años de edad (87,90,97) y son la tercera o cuarta causa de gastroenteritis vírica en niños de esta edad (89,98-100).

Estudios de seroprevalencia muestran que la mayoría de niños ya se han infectado por astrovirus a la edad de 6 años (101) y que el 75% de los niños entre 5 y 10 años presentan anticuerpos frente al virus (102). Más del 50% de los niños con diarreas en los que se ha detectado astrovirus tenían menos de 2 años de edad (6,14). Las infecciones asintomáticas son frecuentes (103), especialmente en guarderías, y pueden representar más del 50% de todas las infecciones (104).

Astrovirus puede causar brotes de GEA, especialmente en instituciones sanitarias (105,106) y guarderías (104,107,108), aunque también se han descrito brotes en adultos en instituciones semicerradas (109).

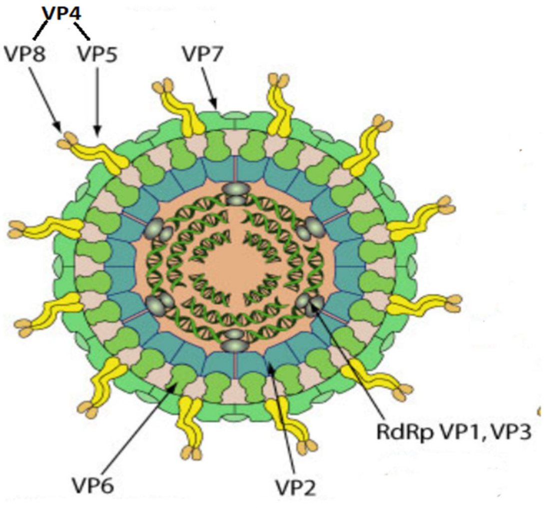
3.5.- Rotavirus

En 1943 Light et al. (110) estudiaron seis brotes de diarreas que afectaron a recién nacidos en tres hospitales del área de Washington (Estados Unidos) y observaron que el ultrafiltrado de las heces de los enfermos producía diarrea cuando se administraba a becerros, concluyendo que los brotes de gastroenteritis en recién nacidos podían deberse a un nuevo agente de naturaleza vírica. En 1976, Mebus et al. (111) informaron de la existencia de partículas víricas detectadas por inmunoelectromicroscopía en muestras liofilizadas procedentes del estudio de Light et al. En 1979 el Comité Internacional de Taxonomía de Virus (112) denominó a este virus como Rotavirus dado su parecido a una rueda cuando era observado al microscopio electrónico.

Rotavirus pertenece a la familia *Rotaviridae;* es un virus no envuelto con una cápside de geometría icosaédrica. Posee 11 segmentos de RNA bicatenario y cada segmento constituye un único marco abierto (ORF) de lectura, a excepción del segmento 11 en rotavirus A en el que hay dos ORF. Estos segmentos de RNA codifican 6 proteínas estructurales (VP1, VP2, VP3, VP4, VP6, y VP7) y 6 proteínas no estructurales (NSP1-NSP6). Las proteínas no estructurales están implicadas en la replicación del virus, mientras que las estructurales se relacionan con el mecanismo de entrada a la célula y la especificidad para el huésped. El material genético está contenido en una estructura de triple capa formada por un núcleo, una cápside interna y una cápside externa (113).

El núcleo está formado por el RNA vírico, la proteína VP2 y pequeñas cantidades de VP1 y de VP3. A su alrededor se encuentra la proteína VP6 formando la cápside interna y por fuera de ésta se encuentra la cápside externa formada por la glicoproteína VP7 y por 60 espículas constituidas por la proteína VP4 (la proteína que interacciona con la célula para unirse a ella). La proteína VP4 puede dividirse por la tripsina en dos polipéptidos de menor peso molecular, la VP5 y la VP8 (Figura 11) (113).

Figura 11. Esquema de la partícula viral de rotavirus con sus tres capas.



Fuente: Swiss Institute of Bioinformatics. ViralZone. Rotavirus. Disponible en: https://viralzone.expasy.org/107?outline=all_by_species. Consultado 28 agosto 2022 (114).

La secuenciación de la proteína VP6 permite clasificar a rotavirus en 10 grupos (A-J). Los grupos A, B y C son patógenos humanos, siendo el grupo A el causante de casi todos los brotes (115).

Los genes que codifican las proteínas VP4 y VP7 se utilizan para realizar una clasificación binaria de rotavirus que permite distinguir genotipos P (por ser VP4 sensible a proteasa) y genotipos G (por ser VP7 una glicoproteína) (116). Se han identificado 32 genotipos G (12 de ellos causan enfermedad en humano) y 47 genotipos P (de los que 15 son patógenos humanos) (115).

Su RNA tiene una estructura Cap en su extremo 5', pero en lugar de tener una poli-A en 3' posee una secuencia GACC en este extremo, esta secuencia en 3' está presente en los 11 segmentos del genoma (117). La extructura Cap en el extremo 5' del RNA mensajero de las células eucariotas y en el RNA vírico confiere resistencia a la degradación del RNA por acción de las exonucleasas

A nivel mundial los predominantes son G1 a G4 y G9. Cinco genotipos de rotavirus (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) provocan el 90% de las GEA causadas por este virus. G1P[8] representa más del 75% de las muestras positivas. P1B[4]G2 se asocia con cuadros clínicos más graves en niños pequeños. En la era posvacunal, G3P[8] ha sido el genotipo predominante hasta 2011 y posteriormente ha sido reemplazado por G12P[8] (118).

Tiene un tropismo muy selectivo, infectando exclusivamente las puntas de las vellosidades del intestino delgado. *In vitro* también es capaz de infectar a las células derivadas del epitelio renal además de las del epitelio intestinal. Esto es debido a la existencia de receptores específicos para la entrada del virus en el interior de las células huésped (119).

Rotavirus es una de las causas más comunes de GEA grave en niños menores de 5 años. Junto con norovirus es la principal causa de casos esporádicos y brotes a nivel mundial. En la era prevacunal se estima que rotavirus A causaba 453.000 muertes en menores de 5 años (120). Aunque con menor frecuencia que norovirus, rotavirus puede causar brotes, principalmente en guarderías, centros hospitalarios y residencias geriátricas (121). La frecuencia de aparición de brotes por rotavirus es baja. De los 263 brotes que fueron investigados entre 1998 y 2000 por los Centers for Disease Control and Prevention (CDC) en Estados Unidos sólo en 3 (1%) se identificó rotavirus A mientras que en 231 (87,9%) se identificó norovirus (122). De los 638 brotes notificados en Cataluña entre 2015 a 2019 el 44,8% (286 brotes) fueron debidos a norovirus y sólo en el 1,1% (7 brotes) se identificó rotavirus como agente causal del brote (123)

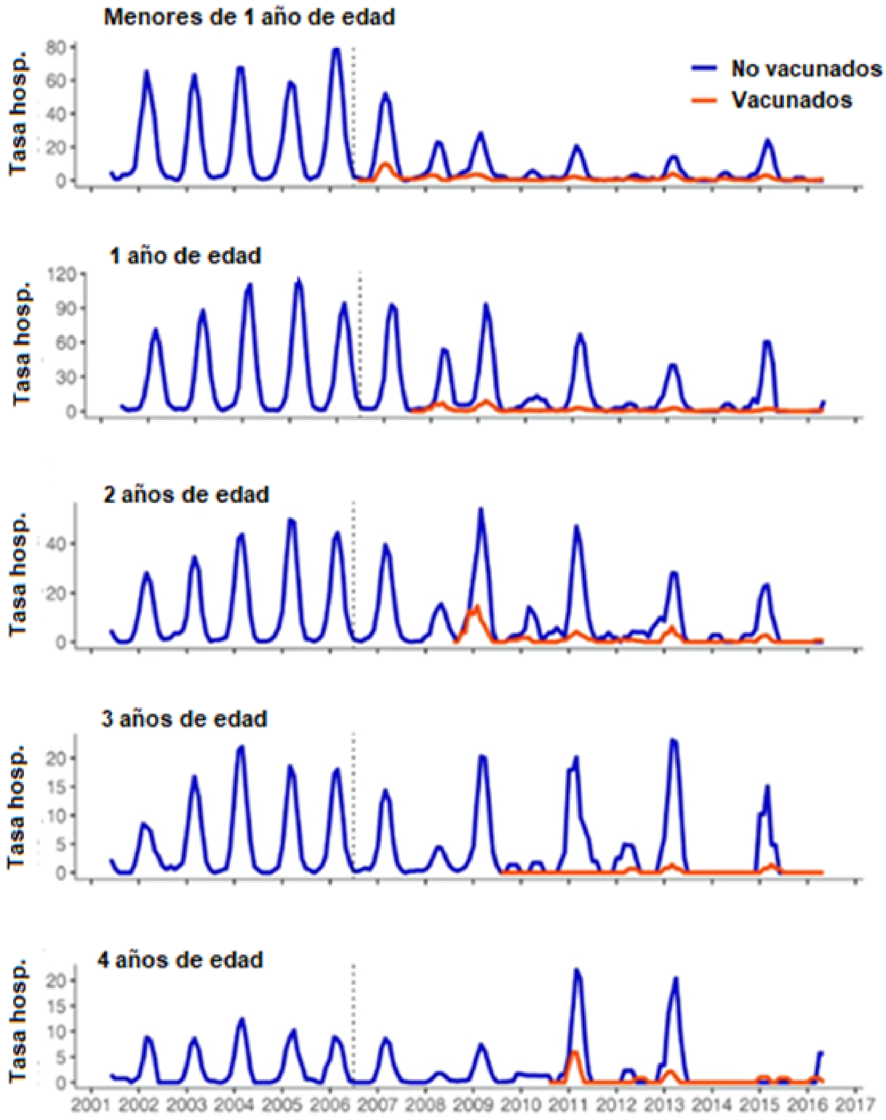
Rotavirus es altamente transmisible por vía feco-oral, tanto por contacto directo como mediante fómites. Aparece en altas concentraciones en las heces de las personas afectadas. El periodo de incubación es de 1 a 3 días y el cuadro clínico que produce es una diarrea acuosa de presentación aguda, sin sangre y que frecuentemente se acompaña de náuseas, vómitos y fiebre (124).

Antes de que se dispusiera de vacuna en Estados Unidos anualmente se producían 2,7 millones de infecciones por rotavirus, más de 400.000 consultas médicas y aproximadamente 70.000 hospitalizaciones, siendo responsable de hasta el 50% de todas las hospitalizaciones por gastroenteritis en menores de 5 años (125).

Se estima que en 2916 a nivel mundial en niños menores de 5 años rotavirus produjo 258 millones de GEA y 128.000 muertes de las que más de 104.000 ocurrieron en África subsahariana (126).

En la era prevacunal las GEA ocasionadas por este virus mostraban una estacionalidad con picos que se presentaban en invierno y principios de la primavera. En la era posvacunal los periodos epidémicos se han hecho más cortos y de menor magnitud y, en algunos países se ha observado un cambio a picos bianuales de incidencia en los menores de 5 años (Figura 12) (127).

Figura 12. Ingresos por gastroenteritis por rotavirus en niños de 0 a 4 años. Tasa por 10.000 persona-año. Estados Unidos, 2001 – 2016.



Fuente: Baker JM et al. BMC Infectious Diseases, 2019 (127)

En 2006 se dispuso de dos vacunas orales para rotavirus, una vacuna monovalente (Rotarix®, GlaxoSmithKline) y otra pentavalente (RotaTeq®, Merck and Co). Los primeros ensayos clínicos mostraron eficacias de ambas vacunas para prevenir la diarrea grave de 85% a 98% (128,129), aunque en países con bajos ingresos la eficacia fue menor (del 50% al 64%) (129,130). Dada la elevada incidencia de la enfermedad, estas eficacias representaban un gran impacto para la salud de la población en estos países. La vacuna monovalente ha presentado una efectividad para prevenir la enfermedad moderada o grave de entre el 57% y el 85% y la pentavalente de entre el 45% y el 90%, siendo esta efectividad mayor en los países con baja mortalidad infantil (131). Otras vacunas orales han sido licenciadas, como Rotavac® (Bharat Biotech, Hyderabad, India) (132) o Rotasiil® (Serum Institute of India, Pune, India) (133)

La introducción de la vacuna ha tenido un fuerte impacto en su incidencia. Aliabadi et al., en un estudio sobre el impacto de la vacuna en la hospitalización de niños menores de 5 años entre 2008 y 2016 en 69 países pertenecientes a la WHO-coordinated Global Rotavirus Surveillance Network, observaron disminuciones en la hospitalización que oscilaban entre el 26,4% (Región del Mediterráneo oriental de la OMS) y el 55,2% (Región europea de la OMS) (134).

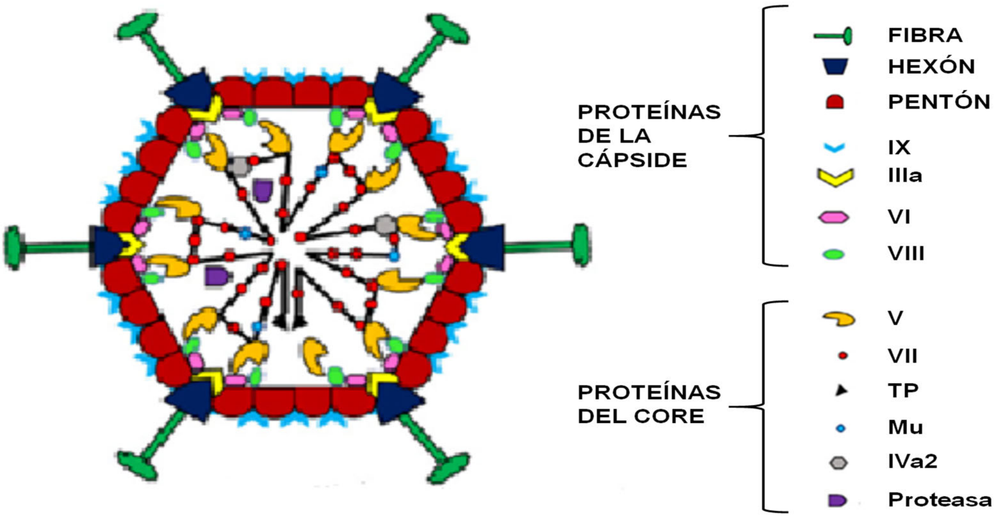
Aunque la introducción de las vacunas de manera extensiva también ha generado cierta preocupación sobre si podría producir cambios en los virus circulantes seleccionando genotipos no incluidos en las vacunas o la aparición de nuevas variantes (115), la OMS recomienda la inclusión de la vacuna para rotavirus en los programas nacionales de inmunización, especialmente en aquellos países con altos niveles de mortalidad como los del sur de Asia o los de África subsahariana (124).

3.6.- Adenovirus

Los adenovirus pertenecen a la familia *Adenoviridae* del género *Mastadenovirus*.

Son virus no envueltos de 65 a 90 nm con un genoma compuesto por una doble cadena lineal de ácido desoxirribonucleico (DNA) protegido por una nucleocápsida de simetría icosaédrica formada por 252 capsómeros (240 capsómeros hexagonales, hexones, que ocupan las caras y aristas, y 12 pentagonales, pentones, que se sitúan en los vértices). Una glicoproteína llamada fibra sobresale desde el centro de cada pentón (135,136) (Figura 13). Otras proteínas de la cápside son la IIIa, la VI, la VIII y la IX.

Figura 13. Estructura de adenovirus.



Fuente: Kulanayake S et al. Viruses, 2021 (137)

El núcleo está formado por una doble cadena lineal de DNA no segmentado de 26 a 48 kb, que codifica 12 proteínas, y las proteínas VII, V, Mu, IVa2, proteína terminal TP y una proteasa. La proteína terminal TP está unida a los extremos 5' y participa en el inicio de la replicación del DNA, facilitando la función de cebador de los primeros nucleótidos del DNA vírico durante la replicación del virus.

La proteína VII parece actuar como un mediador para el transporte del genoma viral al núcleo y actuaría sobre el DNA vírico con una función similar a las histonas, regulando el ciclo viral y desempeñando un importante papel en la prevención de la respuesta inmune antiviral. Se pensaba que la proteína V también estaba involucradas en la translocación del genoma viral al núcleo, pero se ha observado que en ausencia de proteína V el genoma viral se traslada al núcleo, aunque la replicación vírica da lugar a viriones termolábiles con morfología alterada. La proteína V parece ser que tiene una función de establecer puentes entre el DNA vírico, el core y la cápside dando estabilidad al virus. La proteína Mu se unen electrostáticamente al DNA y ayudan a condensar en genoma viral. La proteína IVa2 tiene diferentes funciones en la replicación vírica, participando en la inserción de DNA vírico en las cápsides para formar los viriones (137,138).

Existen más de 100 serotipos de adenovirus, 57 de ellos son patógenos humanos y se clasifican en 7 especies o subgéneros (A-G) (Tabla 3) (139). Afectan a diversos aparatos de nuestro organismo. Son frecuentes las infecciones del aparato respiratorio, las infecciones oculares, las infecciones genito-urinarias y las del tracto digestivo. Los serotipos 40 y 41, pertenecientes al subgénero F, producen gastroenteritis principalmente en niños en los que se pueden presentar vómitos, diarreas, dolor abdominal y fiebre (140). El cuadro clínico suele autolimitarse en individuos inmunocompetentes, aunque puede ser grave en personas inmunodeprimidas (141).

En abril de 2022 la OMS anunció que se habían notificado 169 casos de hepatitis agudas de causa desconocida en 12 países. La mayoría de estos casos presentaron un cuadro clínico de gastroenteritis que incluía diarrea, dolor abdominal y vómitos. Algunos de ellos evolucionaron a fallo hepático agudo. A fecha de 25 de agosto de 2022 han sido notificados 513 casos en 21 países; de ellos el 8% (22 casos) han requerido trasplante hepático y 3 han sido éxitus. De los 513 casos, en 404 se ha analizado la presencia de adenovirus identificándose en el 54% (218 casos). El tipado sólo se realizó en 31 casos, siendo el serotipo 41 el más frecuente (142).

Aunque con menor frecuencia, otros serotipos del subgénero A (31,12 y 18) y del subgénero C (1,2,5 y 6) también se han descrito como causantes de GEA (143).

Tabla 3. Clasificación de los adenovirus en especies y tipos.

Especie	**Tipos**
A	**12, 18, 31**
B	**3, 7, 11, 14, 16, 21, 34, 35, 50, 55**
C	**1, 2, 5, 6, 57**
D	**8-10, 13,15,17, 19,20, 22-30, 32,33,36-39, 42-49, 51, 53, 54, 56**
E	**4**
F	**40, 41**
G	**52**

Fuente: Avendaño C. Neumología Pediátrica, 2019 (139)

Adenovirus es uno de los patógenos responsables de gastroenteritis en niños tanto en los países desarrollados como en países en vía de desarrollo, especialmente en niños menores de 3 años. A nivel comunitario es responsable de entre el 4 y el 10% de las GEA. Un estudio realizado en diferentes países de Asia detectó la presencia de adenovirus en el 4,4% de los niños con diarrea, variando la prevalencia entre 2,8% en Vietnam y 8,7% en Corea (144). Lui et al. describieron que en el 10% de los niños hospitalizados que presentaron diarreas inmediatamente después o durante el ingreso se detectaba adenovirus en sus heces (145).

Después de un periodo de incubación de 3 a 10 días aparece una diarrea acuosa que se acompaña de náuseas, vómitos, fiebre y deshidratación leve y que se autolimita entre 5 y 12 días después. Produce una atrofia de las vellosidades del enterocito y una hiperplasia de las criptas, con la consecuente malabsorción y pérdida de líquidos. También induce una hipertrofia de las placas de Peyer, lo que puede dar lugar a la intususcepción intestinal y oclusión (143,146).

La respuesta inmune provocada por la infección genera la aparición de anticuerpos neutralizantes que previenen la reinfección por el mismo serotipo (143).

4.- Gastroenteritis víricas en instituciones cerradas y semicerradas

El Centro Europeo para el Control y Prevención de Enfermedades (ECDC) define una institución semicerrada como aquella en la que las personas comparten el espacio con otros usuarios del mismo centro durante una parte importante de su tiempo. En este tipo de instituciones se permite cierta movilidad a los usuarios para salir y entrar. Ejemplos: escuelas, guarderías, casas de colonias, residencias de estudiantes, centros de día para personas mayores y centros para personas con diversidad funcional (147).

A efectos de esta tesis hemos considerado institución cerrada aquel centro o institución en el que las personas permanecen la mayor parte de su tiempo, actuando de hecho la institución como el domicilio de la persona, compartiendo los espacios y servicios con otros usuarios. La movilidad para salir y/o entrar al centro está muy restringida. Ejemplos: residencias geriátricas, centros para personas con diversidad funcional, cuarteles militares o prisiones.

Las GEA pueden propagarse rápidamente en ambientes cerrados y semicerrados. Las tasas de ataque que se presentan se relacionan con la capacidad de las instituciones y con la duración de la exposición (148,149). Jenkins et al. (148) estudiaron brotes de GEA que se produjeron en cruceros en los Estados Unidos entre 2006 y 2019, observando que el 94,2% de los brotes analizados se presentaban en viajes cuya duración oscilaba entre 11 y 21 días, mientras que el 5,8% lo hacían en viajes con duración inferior a 7 días. El principal agente causal de estos brotes fue norovirus (149).

El 50% de los brotes de GEA se producen en residencias geriátricas, siendo norovirus la causa más frecuente, que puede suponer hasta el 80% (150).

Entre 1960 y 2017 la proporción de población de 65 o más años de edad en los países de la Organización para la Cooperación y Desarrollo Económico (OCDE) ha pasado del 9% al 17%. Este aumento se ha acompañado de un incremento proporcional de población que requiere cuidados de larga estancia y de población que vive en residencias geriátricas. Las personas residentes en estas instituciones suelen ser personas más frágiles que el resto de personas de su edad, presentan más comorbilidad y más factores de riesgo para desarrollar eventos que comprometan su salud. En 2017 en estos países un promedio de 10,8% de la población de 65 o más años de edad necesitaban cuidados de larga estancia, fundamentalmente servicios de residencias geriátricas; este promedio representa un incremento del 5% respecto al número de personas que requerían dichos servicios en 2007. Los usuarios de los servicios de cuidados de larga estancia son personas de edad avanzada; en España el 56% de los usuarios de estas instituciones son personas de 80 o más años de edad y solo el 26% tienen menos de 65 años de edad (151).

En centros geriátricos las tasas de ataque pueden ser elevadas y la edad y comorbilidad de los usuarios comporta un mayor riesgo de complicaciones (152). Lindsay et al. (150) en un metaanálisis sobre brotes por norovirus en países con ingresos medios y altos describen tasas de ataque de hasta el 45%, oscilando las tasas de hospitalización entre el 0,5 y el 6% y las de letalidad entre el 0,3 y el 1,6%.

Frecuentemente los trabajadores de estas instituciones desempeñan un importante papel en la transmisión. Wu et al. describen afectación del 10,5% de los trabajadores en los brotes por norovirus en Shanghai en el periodo 2015 a 2017(153). Para la transmisión tendría un papel relevante el contacto estrecho que se produce entre los usuarios y los cuidadores (154).

Tian et al. estudiaron los factores de riesgo que favorecen la aparición de brotes de GEA en residencias geriátricas, especialmente los brotes por norovirus, y destacaron que la aparición de brotes se ve favorecida por el tamaño del centro (a mayor tamaño más riesgo) y el porcentaje de residentes mayores de 75 años.

También describieron como factor de riesgo una mayor relación entre cuidadores y residentes y la facilidad de movimiento de los residentes dentro del centro. Atribuyeron estos hallazgos a que cuantos más cuidadores, más fácil es introducir el agente etiológico desde el exterior y a que la movilidad de los usuarios puede favorecer la transmisión de unas personas a otras (155).

También son frecuentes los brotes de GEA en casas de colonias. En Estados Unidos más de 14 millones de personas realizan estancias en centros de colonias cada año (156). El consumo de agua no potable relacionado con algunas de las actividades desarrolladas en estas instituciones, así como las limitaciones en la formación sanitaria de los trabajadores, facilita la aparición de brotes, siendo los más frecuentes los de etiología vírica. Kambhampati et al. (157) estudiaron los brotes de GEA notificados entre 2009 y 2016 en Estados Unidos en centros de colonias: de los 229 brotes detectados el 65% (110 brotes) fueron de etiología vírica (107 brotes por norovirus, 2 brotes por sapovirus y un brote de etiología mixta norovirus/*S. sonnei*).

Norovirus también es una causa frecuente de brotes en escuelas y guarderías. En Inglaterra el 18,4% de los brotes por norovirus ocurrieron en centros educativos en el periodo 2014-2019 (158). En 2016 y 2017 en Shanghai fueron notificados 215 brotes por norovirus, de los que el 87,9% se produjeron en escuelas y guarderías (159). En Estados Unidos el 10,2% de los brotes por norovirus entre 214 y 2019 fueron en escuelas y universidades, afectando a 81.695 personas y provocando 131 ingresos hospitalarios (160). En Cataluña en 2017 el 35% de los brotes que se produjeron en instituciones cerradas y semicerradas fueron en escuelas (161).

Aunque con menor frecuencia que norovirus, astrovirus y sapovirus también se han descrito como agentes de brotes de GEA en centros escolares. En 2017 un brote causado por astrovirus serotipo 4 produjo 98 casos de gastroenteritis y afectó el 63% de las clases de una escuela en Shenzhen (China), siendo la tasa de ataque del 3,2% (162). También en la región china de Guangxi en 2017 un brote afectó a adolescentes de una escuela de secundaria, siendo la tasa de ataque de 8,5% (108). Cho et al. (73) describieron un brote producido por sapovirus GI.3 en 218 en una escuela de primaria en Gyeonggi-do (Corea) en el que 17 de los 999 expuestos desarrollaron un cuadro clínico en el que el síntoma principal fueron los vómitos.

Enserink et al. (163) estudiaron los casos aislados de GEA que se presentaron durante 2010 a 2013 en cien guarderías de Holanda; el 75,4% de los episodios fueron de etiología vírica y el 24,6% de etiología bacteriana. El tamaño de los grupos, el hacinamiento, tener animales, tener zonas de juegos con arena o piscinas portátiles se asoció a un mayor riesgo de que los niños presentasen gastroenteritis, mientras que el lavado diario de los juguetes se relacionó con una menor incidencia.

Los establecimientos y bases militares constituyen otro tipo de instituciones cerradas en las que son frecuentes los brotes de gastroenteritis, pudiéndose producir afectación de gran parte de las personas expuestas. Las tasas de ataque de cuadros clínicos compatibles con gastroenteritis víricas entre militares pueden llegar al 29% por mes cuando se realizan maniobras de despliegue de tropas (164). El movimiento de tropas entre diferentes campos puede ocasionar que los brotes se extiendan más allá de la propia base militar. Norovirus es la principal causa de brotes de GEA en estos establecimientos militares. En El Salvador durante unas maniobras militares en 2011 se produjo un brote de GEA por norovirus que afectó al 27% de los soldados desplegados (165). En 2018 se produjo un brote por norovirus GI en un campo militar en Kuwait en el que había 14,000 soldados de los cuales unos 4.000 estaban en tránsito hacia otros destinos. Coincidiendo en el tiempo se produjo otro brote compatible con norovirus en un campo militar relacionado con el anterior, aunque no pudo establecerse una relación clara entre ambos brotes (166). En este tipo de instituciones es posible la aparición de brotes con afectación multifocal. Así, en 2017 se produjo un brote por norovirus que afectó al mismo tiempo a tres bases militares de Portugal, aunque en este caso la tasa de ataque (3,5%) fue menor que las descritas en otros brotes (167).

5.- Hipótesis

- Los brotes de gastroenteritis aguda de etiología vírica son una importante causa de morbilidad en las instituciones cerradas y semicerradas.

- Cuando se produce un brote de gastroenteritis aguda de etiología vírica en una institución cerrada o semicerrada la transmisión de la infección no queda limitada a dicha institución, pudiéndose producir casos secundarios entre los familiares de trabajadores y usuarios. La detección de estos casos secundarios no es fácil y frecuentemente no se contabilizan como vinculados al brote, por lo que la magnitud de dichos brotes puede estar infraestimada.

- La carga viral detectada en heces puede variar según la presencia o no de síntomas y del tipo de virus implicado, aspectos que puede influir en la extensión del brote.

- La notificación de brotes de gastroenteritis aguda ha disminuido durante la pandemia de COVID-19, por lo que parece razonable comparar las características de dichos brotes durante la pandemia respecto al periodo prepandémico.

6.- Objetivos

El objetivo principal de trabajo es investigar los brotes de GEA de etiología vírica en instituciones cerradas y semicerradas y sus determinantes, tanto a nivel de la afectación de las personas expuestas en la institución en la que ha aparecido el brote como entre sus contactos domiciliarios.

Para ello se plantean los siguientes objetivos específicos:

1 Estimar las tasas de ataque y los modos de transmisión en los brotes de gastroenteritis aguda en centros geriátricos, centros educativos y casas de colonias, así como en los trabajadores de dichas instituciones

2. Investigar la afectación entre los trabajadores y entre los contactos domiciliarios de las personas expuestas en las instituciones en que se han producido brotes de gastroenteritis aguda.

3. Analizar la carga viral en los infectados sintomáticos y asintomáticos detectados en los brotes de gastroenteritis aguda.

4. Analizar la repercusión de la pandemia de COVID-19 en la incidencia y características de los brotes de gastroenteritis aguda declarados a los servicios de vigilancia epidemiológica.

7.- Material, métodos y resultados.

7.1.- Artículo 1: A foodborne norovirus outbreak in a nursing home and spread to staff and their household contacts.

Parrón I, Álvarez J, Jané M, Cornejo Sánchez T, Razquin E, Guix S, Camps G, Pérez C, Domínguez À; Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. A foodborne norovirus outbreak in a nursing home and spread to staff and their household contacts. Epidemiol Infect. 2019;147:e225.

Título

Brote de norovirus en un centro geriátrico y su propagación a trabajadores y a contactos domiciliarios

Resumen

Introducción:

Norovirus es la primera causa de brotes de gastroenteritis aguda en residencias geriátricas. La extensión de estos brotes no se limita únicamente a los residentes y trabajadores de los centros, sino que con frecuencia afecta también a los familiares de residentes y trabajadores.

El objetivo del estudio fue describir un brote de toxiinfección alimentaria por norovirus en una residencia geriátrica y analizar los factores relacionados con los trabajadores que pueden influir en la propagación del brote dentro y fuera de la residencia.

Material y método:

En una primera fase se realizó un estudio descriptivo dirigido a orientar sobre el origen y mecanismo de transmisión del brote, seguido de un estudio caso-control para determinar el alimento responsable. Se determinó la etiología del brote mediante detección de norovirus por RT-PCR en tiempo real. En una segunda fase se determinó la extensión del brote dentro y fuera de la residencia y, mediante análisis multivariante, se estudió que factores relacionados con los trabajadores habían influido en la propagación de persona a persona.

Resultados:

El brote se originó por el consumo de un plato a base de pavo durante la cena del día 15 de marzo de 2018 (odds ratio 4,22). Se detectó norovirus genogrupo I y genogrupo II en las muestras clínicas analizadas. Las tasas de ataque entre los residentes, trabajadores y convivientes domiciliarios de los trabajadores fueron del 23,49%, 46,22% y 22,87%, respectivamente. Los trabajadores más afectados fueron los gerocultores y el personal de limpieza. Ser conviviente domiciliario de un gerocultor afectado fue el factor que tuvo mayor influencia en la aparición de casos en los domicilios (odds ratio ajustada de 6,37).

Conclusión:

Los trabajadores de residencias geriátricas con un intenso contacto con los residentes tienen un mayor riesgo de infectarse durante un brote por norovirus. Los contactos domiciliarios de estos trabajadores también tienen un riesgo elevado de infectarse posteriormente.

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Original Paper

A foodborne norovirus outbreak in a nursing home and spread to staff and their household contacts

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Abstract

On 16 March 2018, a nursing home notified a possible acute gastroenteritis outbreak that affected 11 people. Descriptive and case–control studies and analysis of clinical and environmental samples were carried out to determine the characteristics of the outbreak, its aetiology, the transmission mechanism and the causal food. The extent of the outbreak in and outside the nursing home was determined and the staff factors influencing propagation were studied by multivariate analysis. A turkey dinner on March 14 was associated with the outbreak (OR 4.22, 95% CI 1.11–16.01). Norovirus genogroups I and II were identified in stool samples. The attack rates in residents, staff and household contacts of staff were 23.49%, 46.22% and 22.87%, respectively. Care assistants and cleaning staff were the staff most frequently affected. Cohabitation with an affected care assistant was the most important factor in the occurrence of cases in the home (adjusted OR 6.37, 95% CI 1.13–36.02). Our results show that staff in close contact with residents and their household contacts had a higher risk of infection during the norovirus outbreak.

Introduction

Noroviruses are non-enveloped RNA viruses of the *Caliciviridae* family which have six genogroups (GI to GVI), although only genogroups I, II and IV are human pathogens [1, 2]. Norovirus infection is usually characterised by nausea, vomiting and a self-limiting evolution of about 48–72 h [3].

The viral capsid provides specific resistance to the external environment, including high levels of chlorine [4], heat and cold [5], acidic pH and organic solvents [6, 7]. These characteristics mean norovirus is highly infective and can survive a long time in the environment [8]. There are different modes by which transmission may occur (direct person-to-person or indirect via faecal contamination in the environment or on foods) [9]. The low infectious dose (mean of 18 viral particles) [7], and the lack of long-term immunity in infected persons (the duration of immunity has been considered to be 6–24 months, although a recent study suggests that immunity lasts 4–9 years following infection) [10], means that norovirus often causes outbreaks in institutions and closed and semi-closed groups.

Norovirus is the main cause of viral acute gastroenteritis (AGE) outbreaks worldwide (>90% of AGE outbreaks are attributed to norovirus) [11], causing >1.8 million deaths in children aged <5 years annually [12].

As in other European countries [13], in Catalonia norovirus outbreaks are frequent in nursing homes: in 2010–2011 nearly 30% of norovirus outbreaks occurred in this type of setting [9], where older age and multiple comorbidities can result in hospital admission and even death [14]. The immediate introduction of infection control measures (hand hygiene, principles of food safety, disinfection of contaminated surfaces, isolation of ill persons and no return to work until 2 days after the absence of symptoms) decreases the duration of outbreaks and the attack rates in residents and staff [13].

In Spain, the institutionalised population increased by >90% between 2001 and 2011, and more than 60% of this population (2 70 000 people) are estimated to live in nursing homes [15].

Many studies have looked at the factors that cause outbreaks, but fewer have looked at transmission beyond the primary outbreak setting [16–19]. The objective of this study was

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to investigate a foodborne outbreak of norovirus in a nursing home and measure its impact on residents and the spread in staff and their household contacts.

Material and methods

Outbreak notification

On 16 March 2018, a nursing home notified a possible AGE outbreak that affected nine residents and two staff. The onset of AGE in the first cases occurred on the afternoon of March 15. The predominant symptoms were nausea, vomiting and watery diarrhoea without blood or fever. When the suspected outbreak was notified, some affected individuals were already recovering and no patient required hospitalisation

Outbreak setting

The care home had 166 residents on three floors. Residents with greater autonomy (116) lived on the first floor, semi-autonomous persons (27) on the second floor and those with the highest degree of dependency (23) on the third floor. The home had 119 staff: 42 on the first floor (three shifts of 14), 12 on the second floor (three shifts of 4) and 12 on the third floor (three shifts of 4). The remaining 53 staff (maintenance, laundry and cleaning, kitchen and medical) were not assigned to a specific floor.

The centre had a kitchen and dining room on the ground floor that was used by patients admitted to the first and second floors. On the third floor, there was a second dining room for patients although they could have meals in their rooms.

Various menus were prepared according to individual characteristics (normal diet, diabetic, easy chewing, soft foods and low calorie), although the menus were basically combinations of different dishes and only the soft food menu was markedly different.

The home had been in operation for some years; the structure and facilities were adequate for its stated activity and the number of residents was below the maximum capacity. Routine inspections had detected no deficiencies.

Definitions

A clinical case of norovirus infection was defined as a person with onset of diarrhoea and/or vomiting between March 15 and 31 who lived or worked in the nursing or was a household contact of a staff member. A confirmed case was defined as a clinical case that was positive for norovirus in faeces by real-time polymerase chain reaction (RT-PCR).

Study phases

The study was carried out in three phases: The first, carried out on March 17 and 18, was aimed at verifying the aetiology and mechanism of the outbreak. An epidemiological survey was designed to gather socio-demographic data, symptom onset and end and clinical data. A food survey was conducted to determine the type of menu and the specific dishes consumed. The questionnaire was filled in by the health staff of the centre. A case–control study was designed: cases were chosen from clinical cases in residents and controls were chosen from residents who did not become ill.

The second phase, carried out from March 19 to 25, was aimed at quantifying the size of the outbreak and monitoring its evolution, in addition to maintaining the control measures indicated. Information on the demographic and clinical variables of new cases was collected. The health personnel of the home collected the information. The food-handling facilities were inspected, including collecting environmental samples from the kitchen and common use areas. It was not possible to process samples of the food potentially implicated in the outbreak as our laboratory does not have approved International Organization for Standardization certification for the food that the case–control study showed was associated with the outbreak.

Between March 26 and April 1, the extent of the outbreak in household contacts of staff was quantified. We actively investigated how many staff were clinical cases using a survey that included age, sex, whether they had been infected (with date of onset and end), number of household contacts of staff (with age and sex), whether these were clinical cases and date of onset. The aim of this survey, which was self-completed by staff, was to determine the extent of illness in staff and their household contacts.

Laboratory analyses

Stool samples were collected on March 18. Samples were sent to the Vall Hebrón University Hospital microbiology laboratory for testing for norovirus (if negative, testing for rotavirus, adenovirus, astrovirus and sapovirus was made). Given the suspicion of viral AGE, bacterial tests were not carried out. The specific primers described by Kageyama *et al.* [20] for the detection of norovirus GI and GII were used for RT-PCR. Norovirus genogroup IV (GIV) was detected using a modification of the primers described by Farkas *et al.* [21] and Kageyama *et al.* [20]. For genotyping, the ORF1–ORF2 junction was sequenced from all samples laboratory-confirmed for norovirus. To amplify this region, a one-step RT-PCR assay was carried out using the One-Step RT-PCR kit (Qiagen, Hilden, Germany) with previously published primers GISKF, GISKR for GI NoV, and G2SKF and G2SKR primers for GII NoV [20, 22]. PCR products were then purified using Exo-SAP-IT (USB, Affymetrix Inc., Cleveland, Ohio, USA) and sequenced by the ABI Prism Big Dye Terminator cycle sequencing kit v3.1 on the ABI PRISME 3130XL sequencer (Applied Biosystems, Foster City, California, USA). Nucleotide sequences were assembled and edited using SeqMan 4.05 (Dnastar, Madison, Wisconsin, USA). Norovirus typing tool v2.0 was used to genotype norovirus.

Environmental samples were collected with polyester swabs. Total nucleic acids were extracted with BioMérieux NucliSENS easyMag system. The reagents, primers and cycling conditions of the real-time RT-PCR used for the detection of norovirus GI and GII were those indicated in ISO 15216-2:2013 [23].

The data were stored and subsequently debugged in a Microsoft Access database.

Statistical analysis

The mean age of clinical cases and controls was compared using the two-sample t test and percentages of the distribution of sexes using the χ^2 test. Univariate analysis was used to determine the associations between the consumption of each dish served and AGE, using the odds ratio (OR) and 95% confidence intervals (95% CI).

The risk of AGE in household contacts of staff who were clinical cases or not was calculated using the rate ratio (RR) and 95% CI.

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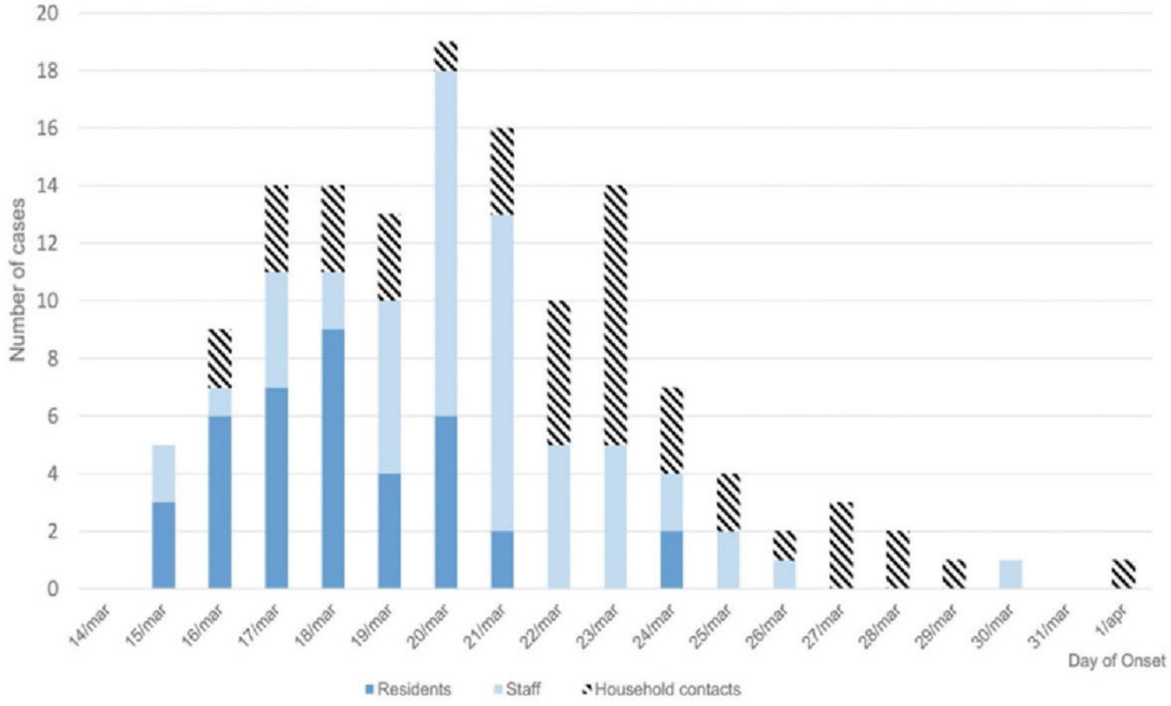

Fig. 1. Distribution of clinical cases by day of onset in residents, staff and household contacts.

In staff, the association between professional category and the risk of AGE was estimated by calculating the OR and 95% CI. In household contacts of staff that were clinical cases, the risk of infection and the determining factors were estimated by calculating the OR and 95% CI. Multivariate logistic regression was carried out to control for the influence of possible confounding variables: the variables age of household contact, sex of household contact, number of household contacts and the duration of illness in staff were included in the adjustment. The statistical analysis was made using the PASW Statistic 18.0.2 statistical package.

Results

Information was collected on 361 people (54 residents, 119 staff and 188 household contacts of staff). Seventy-nine persons (25 staff and 54 residents) were exhaustively surveyed, of whom 56 were infected and 23 were not. For the remaining 94 staff and 188 household contacts of staff, socio-demographic information, professional category and, in clinical cases, the date of onset, was collected.

The AGE outbreak affected 137 people (39 residents, 55 staff and 43 household contacts). The patients affected were mainly residents of the first and second floors and some staff, with no resident of the third floor being affected. The temporal grouping of the first reported cases suggested a common exposure that might have occurred at dinner on March 14, although breakfast on March 15 could not be ruled out. The clinical characteristics suggested norovirus as the cause of the outbreak.

Figure 1 shows the epidemic curve, and clinical cases in residents, staff and household contacts.

The attack rates in residents, staff and household contacts were 23.49%, 46.22% and 22.87%, respectively. The most frequent symptoms were vomiting (64.3%), diarrhoea (62.5%), abdominal pain (37.5%) and fever (14.3%). Symptom duration was approximately 24 h (median 1 day, range 1 h 55 min to 4 days).

Stool samples were collected from 28 people: 14 were clinical cases (10 residents and four caregivers) and 14 were not (six residents, three caregivers and five kitchen staff). Ten samples were positive for norovirus (two GI, six GII and two GI/GII). The genotype was identified in nine cases: seven were GII.17 and two GI.3. Six positive samples came from confirmed cases and four from asymptomatic persons. Eight environmental samples were collected (three from kitchen staff lavatories, two from the kitchen and three from common areas of the residence). No environmental sample was positive for norovirus.

The food consumption questionnaire was answered by 46 residents (25 clinical cases and 21 controls), including the type of diet and the food served during the two suspect meals. In four people, it was only possible to identify the type of diet followed. Twenty per cent of clinical cases and 19% of controls were male: the mean age was 78.8 years (S.D. 22.4) in clinical cases and 71.2 years (S.D. 26.2) in controls: the differences were not statistically significant. Table 1 shows the distribution of individuals who were clinical cases or not, according to the consumption of a specific food or diet. No significant differences were observed between different diets. However, when the consumption of each food was analysed, the turkey served on the night of March 14 was associated with being a clinical case (OR 4.22, 95% CI 1.11–16.01). In contrast, the alternative to turkey (cod fritters) showed an OR of 0.12 (95% CI 0.02–0.75).

Table 2 shows the attack rate in household contacts of staff that were clinical cases or not. The overall attack rate in household contacts was 22.87%, although the rates clearly differed between household contacts of staff with and without illness (38.09% *vs.*

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Table 1. Distribution of clinical cases and controls according to food consumption

	Clinical cases		Controls		
	Consumption	No consumption	Consumption	No consumption	OR (95% CI)
Food category					
Diabetic	5	20	4	17	1.06 (0.24–4.60)
Easy chewing	5	20	4	17	1.06 (0.24–4.60)
Low calorie	0	25	1	20	0 (0–15.96)
Normal	8	17	3	18	2.82 (0.64–12.44)
Soft food	3	22	1	20	2.73 (0.26–28.39)
Foods					
Sandwich	15	6	12	9	1.81 (0.52–6.29)
Coffee with milk	11	10	7	14	2.12 (0.63–7.14)
Milk	2	19	1	20	1.75 (0.21–14.55)
Biscuits	2	19	1	20	1.75 (0.21–14.55)
Soup	10	11	12	9	0.69 (0.21–2.28)
Turkey	11	10	4	17	4.22 (1.11–16.01)*
Cod fritters	1	20	8	13	0.12 (0.02–0.75)*
Mixed vegetables	2	19	0	21	5.51 (0.25–122.10)
Custard	1	20	0	21	3.15 (0.12–81.72)
Soft food	3	18	1	20	2.59 (0.34–19.36)

OR, odds ratio.
*Statistically significant values ($P < 0.05$).

3.61%), with an RR of 10.54 (95% CI 3.38–32.87; $P < 0.001$). Table 3 shows the risk of being a clinical case in each professional category, expressed as the OR and 95% CI. The administration and services categories had a protective effect (OR 0.07, 95% CI 0.01–0.53). Table 4 shows the risk of being a clinical case in household contacts in each staff category, expressed as the OR and 95% CI and the level of significance. Household contacts of a care assistant had a 5.5-fold higher risk of being a clinical case than household contacts of the remaining staff. The professional categories of the remaining staff did not influence the risk of their household contacts being a clinical case. In the multivariate analysis (Table 5), the administration and service categories were too small for separate analysis and were therefore grouped with the cleaning staff. After adjustment, the only factor significantly associated with the risk of being a clinical case was being a household contact of a care assistant (adjusted OR 6.37, 95% CI 1.13–36.02).

Discussion

Our results show that the AGE outbreak investigated originated from common exposure to food and was subsequently spread by person-to-person transmission. The epidemic curve showed the rapid emergence of cases, mostly in residents, and then a more pronounced peak including staff and spread to household contacts of staff. The distribution of clinical cases in residents was typical of the distribution of cases in a common source outbreak due to a specific exposure [24].

Reports have described outbreaks that begin with food poisoning and continue with person-to-person transmission in nursing centres and other institutions. Becker *et al.* described an AGE outbreak due to the consumption of turkey sandwiches in a football team and transmission to the opposing team [25]. Marsh *et al.* analysed three outbreaks of food poisoning in which secondary cases due to person-to-person transmission were found in household contacts of persons affected: 25% of the homes of those affected had secondary cases, with an attack rate of 20% among contacts [16].

The epidemic curve in staff showed a delay in the onset of illness compared with residents, which suggests residents were involved first and there was secondary transmission to staff. Cases in staff from the beginning of the outbreak suggest that, although the staff did not have dinner in the centre, some probably consumed the foods that triggered the outbreak. A possible reason for the absence of clinical cases in third floor residents may be explained by the fact that these persons did not consume the food that caused the outbreak and, due to limitations in movement, did not have physical contact with the other residents.

Norovirus GI and GII co-infection was detected in two patients. Co-infection by more than one genogroup during epidemic norovirus outbreaks is not exceptional. Ushijima *et al.* in a review of food poisoning due to norovirus in Japan found GI/GII co-infection in up to 10% of outbreaks [26]. Huang *et al.* analysed an outbreak that affected six groups of tourists and, of the 23 stool samples analysed, 22 were positive for norovirus GI and/or GII (six patients were co-infected by the two genogroups) [27]. The presence of more than one norovirus genogroup has also been observed when possible sources of infection are analysed. In an outbreak of AGE due to norovirus whose origin was bottled mineral water distributed among 925 Spanish companies, both

Table 2. Attack rates in household contacts of staff according to clinical case status in staff

	Number of household contacts	Number of household contacts who became clinical cases	Attack rate
Status of staff			
Clinical case	105	40	38.09%
Not clinical case	83	3	3.61%
Total	188	43	22.87%

Rate ratio (RR): 10.54 (95% CI 3.38–32.87; $P < 0.001$).

Table 3. Clinical cases according to professional category

	Clinical cases	Not clinical cases	OR (95% CI)[a]
Professional category			
Administration and services	1	15	0.07 (0.01–0.53)**
Health staff	7	5	1.96 (0.58–6.62)
Care assistants	32	31	2.06 (0.93–4.56)
Cleaning staff	7	10	0.89 (0.31–2.55)
Administration and services + cleaning staff	8	25	0.29 (0.12–0.74)**

OR, odds ratio.

[a] Comparing each professional category with the rest.

**Statistically significant values ($P < 0.05$).

the analysis of the water and the faeces of affected people were positive for norovirus GI and GII [28].

The attack rates in staff (46.22%) and residents (23.49%) are those expected in a norovirus outbreak due to food in a nursing home with these characteristics. A review by Lindsay *et al.* of norovirus infection in older people from middle- and high-income countries found the attack rate in outbreaks in nursing homes ranged from 3% to 45% [29]. Similar variability was found by Green *et al.* in a study of 20 AGE outbreaks between 1987 and 1988, with the attack rate ranging between 5% and 59% (median 27%) in residents of nursing homes and between 0.6% and 26% (median 9%) in staff [30]. Utsumi *et al.* in another review of outbreaks of infectious aetiology in long-stay centres between 1966 and 2008 found that, in norovirus outbreaks, the mean attack rate was 45% (range 13–100%) in residents and 42% (range 9–100%) in staff [31].

We found an attack rate in household contacts of clinical cases in staff of 22.87%, similar to the results of other studies. Marsh *et al.* described a secondary attack rate of 20% in household contacts of infected persons in three outbreaks of food poisoning [16]. In an outbreak of food poisoning due to norovirus related to the consumption of oysters in a restaurant in North Carolina in 2009, secondary cases were found in 20% of households included in the analysis and the attack rate was 14% in household contacts (who had not eaten in the restaurant) [17]. Heun *et al.* studied a 1984 outbreak of food poisoning in a school due to norovirus and, as in our study, the risk in household contacts of those affected was greater than the risk in household contacts of persons who did not become ill [18].

Although the association was not significant, we found the most frequently affected staff were care assistants (OR 2.06, 95% CI 0.93–4.56), who have the most direct, closest and longest contact with residents. The intensity of contact plays a fundamental role in the risk of infection during an AGE outbreak due to norovirus. Petrignani *et al.* reviewed norovirus outbreaks in care homes and found a higher intensity of contact between staff and residents was associated with higher attack rates [32]. Godoy *et al.* found attack rates of 56.5% in care assistants in an outbreak in an assisted living facility [33] and González Moran *et al.* found attack rates of 35.8% in health staff (mostly care assistants) in a nursing home [34]. These two studies also found that cleaning staff were frequently affected (attack rates of 55.6% and 40.0%, respectively). In our study, the attack rate in cleaning staff was 41.2%, similar to other studies [33, 35]: administration and service staff were the least affected (attack rate of 6.67%), and this category was a protective factor against infection (OR 0.07, 95% CI 0.01–0.53).

The greater involvement of care assistants could be due in part to the pressure of care to which they are subjected. According to a 2011 report by the Organization for Economic Cooperation and Development (OECD), the percentage of staff assigned to care for older people is below what is desirable, but in some countries, such as Spain, this is especially relevant. While Sweden allocates 3.6% of staff to this sector, Spain allocated just over 1% [36].

There were also AGE cases in household contacts of staff, with a higher attack rate in people who lived with infected staff than in those who did not (38.09% *vs.* 3.61%). This result is consistent with the study by Sukhrie *et al.* of five outbreaks of AGE due to norovirus in two care homes and a university hospital in Rotterdam, which described the role of health staff in the transmission of the virus, while noting the low importance of asymptomatic carriers [37]. There was a significant association between the risk of household contacts of care assistants becoming infected and the professional category of the worker (OR 5.50, 95% CI 1.72–17.61).

Multivariate analysis showed that household contacts of care assistants had an increased risk of infection (adjusted OR 6.37, 95% CI 1.13–36.02), independently of other variables such as the number of people in the home, the age or sex of household contacts and the duration of symptoms presented by staff.

The study had some limitations. First, we could not confirm the greater risk of illness among cleaning staff, probably due to a lack of statistical power. Second, the number of clinical samples did not allow to study the possible differences between people affected by GI, GII or co-infected by both genogroups. Third, we could not analyse the presence of norovirus in the foods involved in the outbreak, especially in the turkey dish served at dinner on March 15, which was statistically associated with the appearance of AGE. Fourth, clinical samples were not available for household contacts that became clinical cases; however, the attack rates of household contacts of ill and unaffected staff showed significant differences, suggesting that cases in household contacts of staff were related to the outbreak and not to other viruses circulating in the community.

In conclusion, our results suggest that the study of AGE outbreaks in nursing homes may be extended to the household

Table 4. Risk of becoming a clinical case in household contacts of clinical cases in staff

Professional category of clinical case staff	Number of household contacts of staff clinical cases: Household contacts who became clinical cases	Household contacts who did not become clinical cases	OR (95% CI)[a]	*P* value
Administration and services	0	5	0 (0–1.16)	0.069
Health personnel	2	6	0.46 (0.09–2.40)	0.293
Care assistants	35	35	5.50 (1.72–17.61)	0.005
Cleaning staff	2	11	0.23 (0.05–1.08)	0.091

OR, odds ratio.

[a] Comparing each professional category with the rest.

Table 5. Risk of becoming a clinical case in household contacts of the clinical cases in staff. Multivariate analysis

	OR (95% CI)	*P* value	aOR[a] (95% CI)	*P* value
Health staff	2.67 (0.30–23.42)	0.38	2.99 (0.23–39.54)	0.40
Care assistants	8.00 (1.71–37.42)	0.01	6.37 (1.13–36.02)	0.04
Administration and services/cleaners	Ref.		Ref.	

aOR, adjusted odds ratio; Ref., reference group.

[a] Adjusted for age of household contact, sex of household contact, number of household contacts and duration of illness in staff.

contacts of staff. Staff that have more intense contact with residents, such as care assistants, are more likely to generate secondary cases in household contacts, which has important health and economic consequences [38, 39]. Care assistants working in nursing home staff should receive additional training in the application of preventive protocols.

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Conflict of interest. None.

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7.2.- Artículo 2: Human astrovirus outbreak in a daycare center and propagation among household contacts.

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Título

Brote de astrovirus humano en una guardería y su propagación entre los contactos domiciliarios.

Resumen

Introducción:

Realizamos este estudio para investigar un brote de gastroenteritis aguda por astrovirus humano en una guardería, describiendo el mecanismo de transmisión, los grupos más afectados, los factores condicionantes y la extensión del brote entre los contactos domiciliarios de los asistentes a la guardería.

Material y método:

Los datos se recopilaron de las personas expuestas en la guardería y sus contactos en el hogar. Las muestras fecales de los asistentes a guarderías (afectados y no afectados) se analizaron para detectar presencia virus que causan gastroenteritis aguda mediante RT-PCR. Se calculó el porcentaje de viviendas afectadas y tasas de ataque. Las tasas de ataque se compararon mediante la razón de tasas con un intervalo de confianza del 95%.

Resultados:

Se obtuvo información de 245 personas (76 asistentes y 169 contactos) de los cuales 49 fueron casos clínicos. Se identificaron cinco astrovirus serotipo 4, dos astrovirus serotipo 8 y 3 astrovirus identificados no se pudieron serotipar. De los 10 astrovirus identificados, 6 correspondían a casos clínicos y 4 a personas infectadas asintomáticas. La tasa de ataque global fue del 20% (41,2% en niños menores de 2 años). Se obtuvieron datos de 67 hogares: 20 hogares de asistentes afectados y 47 de asistentes no afectados. Los contactos domiciliarios de los asistentes afectados tuvieron mayor tasa de ataque (74,3%) que los de los asistentes no afectados (2,4%).

Conclusiones:

Se observaron infecciones asintomáticas entre los asistentes a la guardería. La transmisión de astrovirus durante el brote no se limitó a la guardería, sino que se extendió a los contactos domiciliarios de los asistentes afectados y no afectados.

7.2.- Artículo 2: Human astrovirus outbreak in a daycare center and propagation among household contacts.

viruses

MDPI

Article

Human Astrovirus Outbreak in a Daycare Center and Propagation among Household Contacts

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Abstract: We investigated an outbreak of acute gastroenteritis due to human astrovirus in a daycare center, describing the transmission mechanism, the most affected age groups, conditioning factors and the extent of the outbreak among household contacts of the daycare center attenders. Data were collected from persons exposed at the daycare center and their home contacts. Fecal samples from affected and non-affected daycare center attenders were analyzed for viruses causing acute gastroenteritis by RT-PCR. The percentage of households affected and the attack rates (AR) were calculated. The attack rates were compared using the rate ratio (RR) with 95% confidence intervals. Information was obtained from 245 people (76 attenders and 169 contacts) of whom 49 were clinical cases. Five HAstV-4, two HAstV-8 and three non-typable HAstV cases were identified (six from clinical cases and four from asymptomatic infected people). The global AR was 20% (41.2% in children aged < 2 years). Data were obtained from 67 households: 20 households of affected attenders and 47 of non-affected attendees. Household contacts of affected attenders had a higher AR (74.3%) than that of non-affected attendees (2.4%). We found asymptomatic infections amongst daycare attendees. The transmission of HAstV during the outbreak was not limited to the daycare center but extended to household contacts of both affected and non-affected attenders.

Keywords: astrovirus; outbreak; acute gastroenteritis; daycare center; household contacts

1. Introduction

Human astroviruses (HAstV) are non-enveloped RNA viruses that cause up to 10% of sporadic cases of acute gastroenteritis (AGE) in children aged < 3 years [1–3] and are the third or fourth cause of viral AGE in children of this age [4–7].

Eight serotypes (HAstV-1 to HAstV-8) of HAstV are known to cause gastroenteritis. HAstV-1 is the most-frequently detected serotype worldwide [1,8,9], although other serotypes have occasionally been shown to be more prevalent in some geographical areas [4]. Infection with one serotype seems not to confer immunity against other serotypes [3].

Astroviruses are usually spread through direct person-to-person transmission via the fecal-oral route [10], although indirect transmission due to exposure to contaminated food or water has been described [11]. Astroviruses cause outbreaks, especially in healthcare [12,13] and daycare centers [14,15], mainly affecting small children.

Seroprevalence studies show that most children have been infected with HAstV by the age of 6 [16] and 75% of children aged 5–10 have antibodies [17]. More than 50% of children with diarrheal symptoms in whom HAstV are detected are aged < 2 years [6,14]. Asymptomatic infections are frequent [18], especially in daycare centers, and may represent more than 50% of persons infected [14]. To our knowledge, the involvement of home contacts in HAstV daycare center outbreaks has not been previously reported.

The objective of this study was to investigate an outbreak of AGE due to HAstV in a daycare center and determine the mode of transmission, the most-affected age groups and the spread to household contacts of attendees (children and workers) at the daycare center.

2. Materials and Methods

2.1. Study Design

On 9 March 2017, the Public Health Agency of Catalonia was notified of a possible AGE outbreak affecting three children who had initiated symptoms in the previous 48 h in a daycare center.

The center was attended by 110 children aged 0–3 years and 13 workers. Meals were served by an external catering company because the center had no kitchen of its own.

The epidemic period was defined as 1 February to 12 March 2017, and a clinical case was defined as any person in the daycare center or their household contacts who initiated symptoms of nausea, vomiting or diarrhea during the epidemic period. Confirmed cases were clinical cases in whom HAstV was detected by real time reverse transcription polymerase chain reaction (RT-PCR) in stool samples. A household contact was defined as an individual residing in the same home as a child or worker from the daycare center during the epidemic period.

An epidemiological survey was made that included demographic data, the date of onset and end of clinical manifestations and the symptoms presented, the number of people living in the home of each case, their age, whether they became ill and, if so, the date of symptom onset. Data were collected using a self-administered questionnaire that was completed by the parents or by the center workers.

A stool sample was collected from center attendees for microbiological study between March 13 and March 15 and sent to the Clinical Microbiology Laboratory of Vall d'Hebron Hospital. No samples were collected from household contacts.

According to clinical and epidemiological information, a viral etiology was suspected, and no bacteriological study was carried out. NucliSENS® easyMAG® (Biomerieux, Marcy-l'Étoile, France) was used to obtain genetic material. RT-PCR was used to identify the main viruses causing AGE (norovirus GI and GII, rotavirus, adenovirus, astrovirus and sapovirus) using the Allplex GI-Virus Assay (Seegene Inc. Seoul, Korea). Astrovirus genotyping was performed by sequencing the RT-PCR product according to a previously described protocol [19], and compared with Genbank sequences using Blastn analysis, which was also used to compare isolates within the same genotype.

2.2. Data Processing and Analysis

Data were collected using Microsoft Access (version 14.0) for cleansing and analysis with the PASW Statistic 18.2.0 software package and Epi Info™ for Windows.

The epidemic curve for cases at the daycare center and in home contacts was constructed to indicate the possible transmission mechanism.

The median age of affected and non-affected persons and the frequency of symptoms were calculated. The distribution of stool sample results by age groups was analyzed.

2.3. Statistical Analysis

The mean age of affected and non-affected persons in the daycare center and affected and non-affected household contacts was compared using the Student's *t*-test. Statistical significance was established as $p < 0.05$.

Attack rates (AR) were calculated for daycare center attenders (globally and by age groups) and their household contacts (globally, by affected or non-affected attenders and by age group of attenders). The AR of the categories in each group were compared using the rate ratio (RR) and 95% confidence intervals (CI).

3. Results

A total of 76 daycare center attenders of (71 children and 5 workers) responded to the survey, representing 61.8% of those exposed, and information was obtained from 169 household contacts. In total, information was obtained from 245 subjects, 49 of whom met the clinical-case definition (18 children, 2 workers and 29 household contacts). No affected person required hospital admission.

The first person (case 1) affected in the daycare center was a care assistant whose date of symptom onset was February 12. Her 6-year old son had initiated symptoms two days before. The care assistant's stool sample was negative.

Between the first and second case in the daycare center (case 4), two other people related with the center presented symptoms: the mother of a girl attending the daycare center (case 2) (from whom no stool sample was obtained) and the brother (case 3) of another girl from the same class. The second case at the center was a 2-year-old girl from the same class as the first case, who initiated symptoms on February 25; no stool sample was obtained (Figure 1). Nineteen stool samples were analyzed (13 from affected attenders and 6 from non-affected attenders).

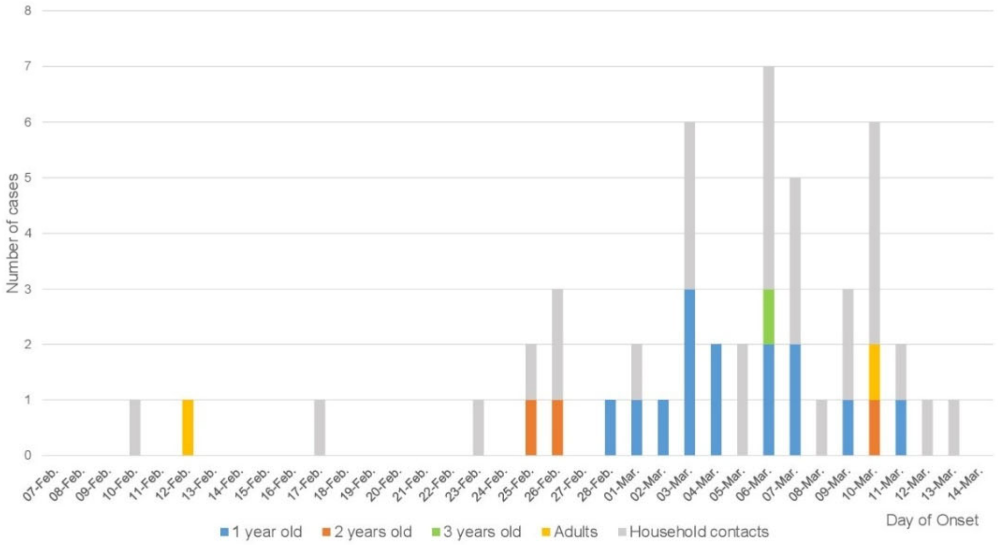

Figure 1. Epidemic curve of cases according to the day of symptom onset.

The overall AR was 26.3% (20/76) for daycare center attenders and 17.2% (29/169) in household contacts. Considering daycare center attenders and their household contacts jointly, the AR was 20% (49/245), 17.2% in males (22/128) and 23.1% in females (27/117) (RR 0.74 95 CI 0.45, 1.23).

The mean age was 1 year 237.6 days (SD 176.5) in affected child attenders at the daycare center and 2 years and 54.6 days (SD 287.4) in non-affected child attenders ($p = 0.002$).

The distribution of AR and RR according to age are shown in Table 1. The most affected age group in attenders were children aged 1 year. The ARs were lower in other groups and the differences were only The mean age was 26 years (SD 17 years) in affected contacts and 29.4 years (SD 13.9 years) in non-affected contacts (p = 0.25).

In household contacts the AR by age groups did not differ significantly.significant in children aged 2 years.

Table 1. Attack rates and risk ratio in day care center attenders and household contacts according to age.

	Age	Affected	Total	AR	RR (95% CI)
Daycare attenders	0 year	0	5	0%	NC
	1 year	14	29	48.3%	1
	2 years	3	28	10.7%	0.22 (0.07–0.69)
	3 years	1	9	11.1%	0.23 (0.03–1.52)
	Adults	2	5	40%	0.56 (0.26–1.23)
Household contacts	0–5	6	22	27.3%	0.82 (0.30–2.20)
	6–10 years	5	15	33.3%	1
	11–25 years	0	11	0%	NC
	26–35 years	5	42	11.9%	0.36 (0.12–1.06)
	36–45 years	13	72	18.1%	0.54 (0.23–1.29)
	46–55 years	0	5	0%	NC
	Total contacts *	29	169	17.2%	

* In two household contacts age was not recorded; NC: Not calculable.

All persons in the daycare center who were affected presented diarrhea, 20% vomiting, 15% abdominal pain and 15% fever. Symptoms lasted 3.8 days on average (SD 2.4 days).

All samples were negative for rotavirus, adenovirus, sapovirus and norovirus GII. Ten samples (six from affected children, three from non-affected children and one from non-affected childcare staff) were positive for astroviruses (five HAstV-4, two HAstV-8 and three non-typeable) (Table 2). Sequence comparison with HAstV reference strains showed a 98.52% and 98.52% nucleotide identity with EF138831.1 and MG970100.1, respectively. All sequences belonging to the same serotype were identical. Sequences showed the closest similarity to published sequences.

Table 2. Samples from exposed people with viruses identified by RT-PCR *.

Sample ID	Clinical Case (Yes/No)	Age	Virus	Date of Symptom onset
1	No	14 months	NoV GI	–
2	No	14 months	HAstV-8	–
3	No	9 months	HAstV-4	–
4	Yes	17 months	HAstV-4	28/2/17
5	Yes	16 months	HAstV-4	6/3/17
6	Yes	14 months	Astrovirus non-typeable	6/3/17
7	Yes	22 months	Astrovirus non-typeable	7/3/17
8	No	21 months	HAstV-4	–
9	Yes	21 months	HAstV-4	4/3/17
10	Yes	36 months	Astrovirus non-typeable	6/3/17
11	Yes	32 years	NoV GI	10/3/17
12	No	34 years	HAstV-8	–

* 7 samples were negative for all viruses screened.

Norovirus genogroup I was identified in two samples from daycare attenders (one from an affected person and one from a non-affected person). In household contacts of non-affected persons and in whom HAstV was detected in stools (two HAstV-4 and two HAstV-8) there were no symptoms of illness.

Sixty-seven of the 76 daycare attenders provided information about their household contacts: 44 household contacts of 20 affected attenders and 125 household contacts of 47 non-affected attenders.

There was a close association between clinical manifestations in attenders and household contacts (RR 30.95%; 95% CI 9.95–96.27) (Table 3) although clinical cases were also reported in household contacts of non-affected attenders (AR 2.4%)

Table 3. Attack rates in household contacts according to the clinical status of daycare attenders.

Status of Attenders	Household Contacts			
	Affected	Exposed	AR	RR (95% CI)
Affected	26	35 *	74.3%	30.95 (9.95–96.27)
Non-affected	3	125	2.4%	1

* No information was obtained about involvement in 9 household contacts of attenders affected in the daycare center.

There were no secondary clinical cases among the contacts of children aged < 1 year; the AR was 29.2% in contacts of 1-year-old children, 8.6% in contacts of 2-year-old children, 9.5% in contacts of 3-year-old children and 33.3% in contacts of workers (Table 4). Statistically significant differences were observed in the AR of household contacts of 1- and 2-year-old children (RR 3.04 95% CI 1.51–6.12 and RR 0.40 95% CI 0.16–0.99, respectively).

Table 4. Attack rates and risk ratios of household contacts by the age of child attenders.

Age of Daycare Center Attenders	Household Contacts			
	Affected	Total	AR	RR (95% CI) *
<1 year	0	16	0%	0.15 (0.01–2.40)
1 year	19	65	29.2%	3.04 (1.51–6.12)
2 years	5	58	8.6%	0.40 (0.16–0.99)
3 years	2	21	9.5%	0.52 (0.13–2.04)
Workers	3	9	33.3%	2.05 (0.76–5.51)

* Each category is compared with the other categories.

4. Discussion

According to published reports, this is the first descriptive study of an outbreak of AGE with circulation of several serotypes of HAstVs due to person-to-person transmission in a daycare center in which household contacts were also studied.

Astrovirus is responsible for 2–9% of non-bacterial cases of diarrhea [10]. Reports have collected the percentages of samples in which astrovirus was identified in children with AGE. A study in two Chinese provinces found 1.7% and 4% of positive samples in children aged < 5 years [20] while, in Russia, astrovirus was detected in 2.8% of samples from children aged < 3 years [21]. In France, the percentage of positive samples was 1.8% in children aged < 5 years [5] and an Italian study of children aged < 2 years found a rate of positive samples of 3.1% [22] while another Italian study found a rate of 18.9% in children aged < 3 years [23].

Two Spanish studies in Barcelona in children aged $\geq$ 5 years with AGE, the first in 1997–2000 and the second in 2016–2017, identified astrovirus in 4.9% and 7.6% of samples analyzed, respectively [3,24].

Several reports on AGE outbreaks of viral etiology in daycare centers with the involvement of HAstVs have been described [15,25,26], but they did not provide specific data on their extent among household contacts.

The AR observed in astrovirus outbreaks vary widely. We found a global AR of 20%, similar to the 17.6% (261/1479) described by Lui et al. [27] in an outbreak in schoolchildren in the Chinese province of Guangxi in 2017 and less than the 70% (7/10) in children aged 7–18 months described by Taylor et al. [26] in an outbreak in a South-African daycare center. Other reports have found smaller AR, ranging between 3.2% and 8.5% [28,29].

The long period between the first case and symptom onset of the other cases (more than one incubation period) in the daycare center might be explained by circulation of the virus among children's household contacts, as suggested by the two cases at the beginning of the outbreak, or because other members of the daycare center could have been infected asymptomatically.

No increase in the incidence of AGE in the municipality beyond those related to the childcare center was observed. In the first days of the outbreak there was a progressive onset of clinical cases in attenders of the daycare center and their household contacts. Other asymptomatic or symptomatic infections may have been propagated in the community and may have been underreported because healthcare was not required.

A progressive occurrence in cases, as shown by the epidemic curve, is typical of direct person-to-person propagation and suggests that this was the route of transmission [30]. However, fomite involvement cannot be ruled out [31,32].

To avoid the spread of HAstV infections in daycare centers the interruption of person-to-person transmission [33] is important and, therefore, training in infection control procedures including adequate hand hygiene and the cleanliness of surfaces of daycare providers and families of attenders should be promoted [34].

The highest AR was in children aged 1 year, similar to other reports. A study by Olortegui et al. that followed 2082 children from eight countries from 17 days to two years of age found that 26.4% of children had been infected at least once during the first year of life and 35.2% at two years of age [35].

In the outbreak described here there were four asymptomatic astrovirus infections (40% of HAstV detected), which is in agreement with other reports [36].

Norovirus GI was identified in two clinical samples (one in a child aged < 1 year and the other in an adult), which is not surprising given the high frequency of norovirus infection in the community. The prevalence of asymptomatic norovirus infection in children has sometimes surpassed 30% in some industrialized countries [37]. Viral coinfection has been described in isolated cases and outbreaks of AGE [25,38]. In neither of the two norovirus cases in this outbreak was there coinfection.

Our results suggest that the outbreak may be attributed to the circulation of more than one HAstV serotype. The fact that the two cases in which HAstV-8 was identified were asymptomatic and that HAstV-4 was identified in a patient with symptom onset in the initial phase of the outbreak supports the idea that the outbreak was caused by HAstV-4, but given the limited number of samples we cannot state this for sure.

Clinical cases occurred in 26.9% of the homes of both children and workers from the daycare center (16 household contacts of staff or children who become ill and 2 in non-affected people). Propagation of a childcare-associated outbreak in the community has been described for norovirus outbreaks [39,40], but not for astroviruses.

Our study has some limitations. First, no clinical samples were obtained from household contacts. However, the AR of household contacts of clinical cases were significantly higher than the AR of household contacts of non-affected attenders, suggesting that clinical cases in household contacts were related to the childcare-associated outbreak and not to community circulation of these astrovirus strains or other viruses. A second limitation is the long period between the first clinical case, which was not notified, and the start of the investigation, which probably made it difficult to identify other clinical cases. Thirdly, the mildness of the disease might have meant that some persons affected did not seek healthcare, which would have underestimated the overall impact of the illness in the community.

7.2.- Artículo 2: Human astrovirus outbreak in a daycare center and propagation among household contacts.

5. Conclusions

In conclusion, this outbreak of AGE due to astrovirus in a daycare center caused asymptomatic infections and person-to-person transmission that was not limited to the daycare center where the first clinical cases were notified but extended to household contacts of attenders. Education on hand hygiene and the cleanness of surfaces of daycare providers and family members of attenders should be reinforced when AGE outbreaks occur.

Author Contributions: I.P. and À.D. conceptualized and designed the study, carried out the initial analyses and interpretation of data and drafted the initial manuscript. À.D. was project administrator and acquired funding. E.P. scrubbed data, maintained research data and critically revised the manuscript. T.C.-S. performed the laboratory analyses and critically revised the manuscript. M.J., C.P., C.I. and S.G. contributed to the final analyses and interpretation of data and critically revised the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Commission of the University of Barcelona and was approved on 12 April 2016 (Institutional Review Board IRB00003099).

Informed Consent Statement: The authors declare that the Bioethics Committee of University of Barcelona approved the waiver for informed consent. All data used in the analysis were collected during routine public health surveillance activities as part of the legislated mandate of the Health Department of Catalonia, which is officially authorized to receive, treat and temporarily store personal data in cases of infectious disease. All data were fully anonymized. All study activities formed part of public health surveillance tasks. Law regulates these activities and informed consent should not be necessary.

Data Availability Statement: The datasets generated during the current study are available in the Mendeley Data repository (http://dx.doi.org/10.17632/pftbhzznd5.1).

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Conflicts of Interest: The authors declare no conflict of interest.

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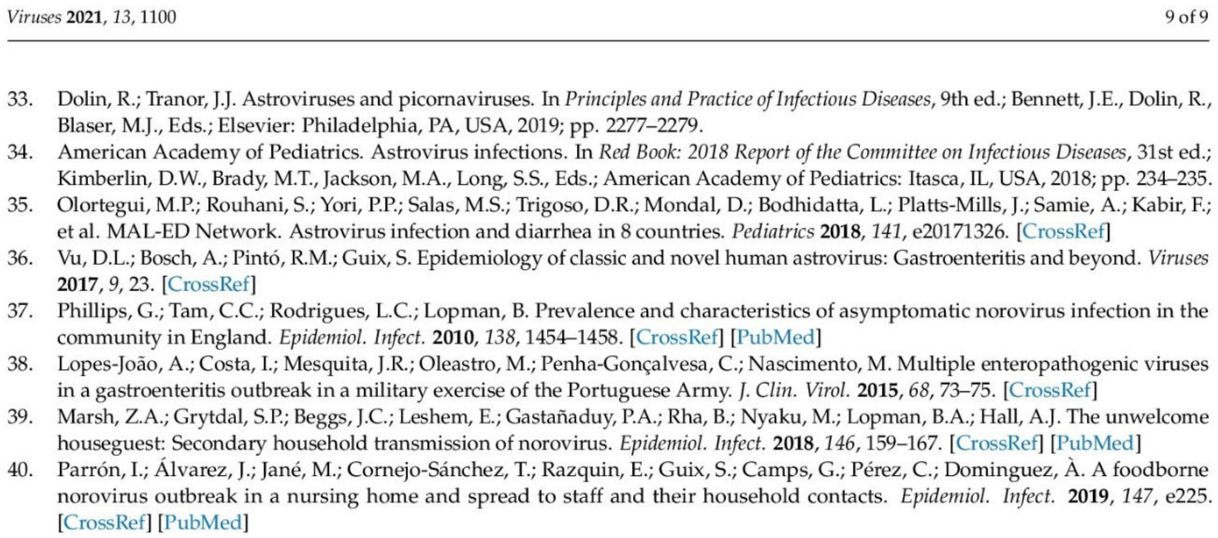

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7.3.- Artículo 3: Norovirus outbreaks in long-term care facilities in Catalonia from 2017 to 2018.

Parrón I, Barrabeig I, Alseda M, Rius C, Cornejo-Sánchez T, Jané M, Pérez C, Guix S, Domínguez À; Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. Norovirus outbreaks in long-term care facilities in Catalonia from 2017 to 2018. Sci Rep. 2021;11:23218.

Título

Brotes de norovirus en instituciones de larga estancia en Cataluña desde 2017 a 2018.

Resumen

Introducción:

Norovirus es la principal causa de brotes de gastroenteritis viral aguda y es responsable del 20% de los casos de diarrea por cualquier causa en todo el mundo. El objetivo del estudio fue investigar las características de los brotes en centros de larga estancia según diferentes variables (residentes vs trabajadores, capacidad del centro, tipo de transmisión, genogrupo y carga viral).

Material y método:

Se estudiaron los brotes en centros de larga estancia notificados entre 2017 y 2018 en Cataluña confirmados por RT-PCR.

Se analizaron las características de los centros, las personas expuestas y los genogrupos responsables. Se estimaron las cargas virales.

Para los diferentes factores estudiados se calculó la tasa de ataque, la razón de tasas o la odds ratio y sus intervalos de confianza al 95%. Las medias de los valores semicuantitativos de los umbrales de detección (Ct) de la RT-PCR se compararon mediante la prueba t para medias independientes.

Resultados:

Incluimos 30 brotes con 4631 personas expuestas. La tasa de ataque global fue del 25,9%. La razón de tasas de residentes vs. trabajadores fue 2,28 (IC 95% 2,0-2,6), 3,06 (IC 95% 2,63-3,63) en brotes con transmisión de persona a persona y 0,74 (IC 95% 0,57-0,96) en brotes con transmisión por vehículo común. Los residentes con dependencia grave fueron los más afectados. Las tasas de ataque fueron más altas en los centros más pequeños que en los más grandes (34,4 % frente a 19,5 % y razón de tasas 1,76; IC 95 % 1,60-1,94). El genogrupo II fue responsable del 70 % de los brotes y el 78,5 % de las identificaciones fueron de este genogrupo. Las cargas virales fueron más altas en los sintomáticos que en los sujetos asintomáticos (Ct 25,6 y 29,0, respectivamente; p=0,001).

Conclusiones:

Norovirus (especialmente genogrupo II) es un problema de salud importante en instituciones de larga estancia, especialmente entre las personas dependientes. Las cargas virales fueron más altas en los pacientes sintomáticos que en los asintomáticos.

7.3.- Artículo 3: Norovirus outbreaks in long-term care facilities in Catalonia from 2017 to 2018.

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Norovirus outbreaks in long-term care facilities in Catalonia from 2017 to 2018

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Norovirus is the leading cause of outbreaks of acute viral gastroenteritis. We carried out this study to investigate outbreaks in long-term care facilities reported in 2017 and 2018 in Catalonia (Spain). The characteristics of the centers, exposed persons and the genogroups responsible were analyzed. Viral loads were estimated. The attack rate (AR) of the outbreaks studied, and the rate ratio (RR) and the odds ratio (OR) and their 95% confidence intervals as measures of association were calculated. The mean cycle thresholds were compared using the t-test for independent means. We included 30 outbreaks (4631 exposed people). The global AR was 25.93%. The RR of residents vs. staff was 2.28 (95% CI 2.0–2.6). The RR between AR in residents with total or severe dependence vs. residents with moderate, low or no-dependence was 1.23 (95% CI 1.05–1.45). The AR were higher in smaller centers than in larger ones (38.47% vs. 19.25% and RR 2; 95% CI 1.82–2.2). GII was responsible for 70% of outbreaks. No association was found between the genogroup and presenting symptoms (OR 0.96; 95% CI 0.41–2.26). Viral loads were higher in symptomatic than in asymptomatic patients ($p = 0.001$).

Norovirus, an RNA virus of the *Caliciviridae* family with 10 genogroups, of which genogroup I (GI), genogroup II (GII) and genogroup IV (GIV) are human pathogens[1], usually produces symptoms of nausea, vomiting and diarrhea, with a self-limiting evolution of 48–72 h[2].

Norovirus is estimated to be responsible for 20% of cases of all-cause diarrhea worldwide[3] and may cause up to 90% of outbreaks of acute gastroenteritis (AGE) of viral etiology[4].

Inns et al., in a review of norovirus reports worldwide between 1995 and 2015, found an incidence of up to 60 cases per 1000 person-years and a hospitalization rate of up to 1.04 per 1000 person-years[5]. Kreidieh et al.[6] in a similar study in the Middle East and North Africa between 2000 and 2015 found that between 0.82% and 36.84% of AGE outbreaks in children aged < 5 years treated in hospital emergency rooms were caused by norovirus.

Of the more than 1000 outbreaks of AGE reported annually in 2009 and 2010 in the United States, norovirus was confirmed as the etiological agent in 86%, and 90% of norovirus-associated deaths occurred in people aged ≥ 65 years[7].

In long-term care facilities (LTCF), the attack rate of AGE outbreaks due to norovirus varies between 3% and 45%, with a case fatality rate ranging from 0.3% to 1.6%[8]. In these institutions, norovirus is the second leading cause of outbreaks, after the influenza virus[9]. In England, an incidence of 30 outbreaks per 100 LTCF per year was reported in 2014–2016[10]. In France in 2011, more than 70% of AGE outbreaks in LTCF were due to norovirus[11] and in the United States > 60% of norovirus outbreaks between 2009 and 2013 occurred in LTCF[12]. Although norovirus infection is usually mild, it may be more severe in older people. In developed countries, norovirus is responsible for between 10% and 20% of hospitalizations due to AGE in residents of LTCF and between 10% and 15% of deaths[8].

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GII is the most frequently identified genogroup of norovirus in outbreaks[13]. In Spain, this genogroup has also been the most prevalent in recent years[14].

Asymptomatic affected people may contribute to the transmission of norovirus[15] and to a longer duration of outbreaks, which has important repercussions for disease control.

Symptomatic persons have a higher viral load than asymptomatic ones[16], but no value has been established to predict the level of shedding associated with clinical manifestations[17].

In symptomatic patients, it has not been possible to associate the duration of symptoms with the viral load, although the duration of viral shedding has been shown to be longer in those with a higher load and in older people[18,19].

In patients with AGE, the viral load has been shown to be higher when symptoms are due to GII rather than GI, and the higher load of GII, which has been linked to increased ease of transmission[20], has also been observed in patients co-infected with GI and GII[16,18,21,22].

The objective of this study was to investigate attack rates in AGE outbreaks due to norovirus that occurred in LTCF and their association with the type of exposed person, the size of the center, the mode of transmission, the genogroup involved, and the viral load.

Materials and methods

A prospective study of outbreaks of AGE due to norovirus in LTCF reported between January 2017 and December 2018 was carried out in Catalonia, a region in Northeast Spain with a population of 7,496,276 in January 2017, of which 18.6% were aged ≥ 65 years[23] and 59,635 were residents of LTCF[24].

Outbreaks of any etiology must be reported to the Public Health Agency of Catalonia, which studies the causes and establishes control measures[25].

AGE was defined as sudden-onset diarrhea that may be accompanied by fever, nausea, vomiting, or abdominal pain. The involvement of ≥ 2 people with a common exposure (or possible person-to-person transmission) was considered as an outbreak of AGE. A confirmed outbreak of norovirus was defined as the identification of norovirus in stool samples by real-time semiquantitative reverse transcription polymerase chain reaction (RTqPCR).

Two periods were defined: the warm months, lasting from April through September, and the cool months, lasting from October through March of the following year.

Data collection.

All norovirus outbreaks occurring in LTCF confirmed by RTqPCR reported between January 2017 and December 2018 were included. The numbers of residents and staff (affected and unaffected), the capacity of the center and whether transmission was person-to-person or by a common vehicle were collected.

A survey was designed for exposed persons including sociodemographic data, the degree of dependence (estimated using the Barthel index[26]), the history of heart disease, diabetes mellitus, dementia, immunodeficiency and chronic obstructive pulmonary disease, the date and time of symptom onset, the symptoms presented, hospitalization and death. For staff, information on the type of work was collected.

Stool sample analysis.

Stool samples were collected to identify the cause of the outbreak. Norovirus was tested for using RTqPCR and identifying the genogroup detected. The semi-quantitative value given by the RTqPCR cycle of quantification (Cq) was used to measure the viral load in samples positive for norovirus. Stool samples were obtained from symptomatic and asymptomatic staff and residents and, in outbreaks where foodborne transmission was suspected, from food handlers.

The analyses were made in the microbiology laboratories of the Vall Hebrón University Hospital and the Public Health Agency of Barcelona. Allplex GI-Virus Assay, Seegene Inc, was used to detect norovirus GI and GII. Samples positive for norovirus were genotyped using the primers described by Kojima et al.[27]. After the sequences were obtained, the Norovirus Typing Tool Version 2.0 (https://www.rivm.nl/mpf/typingtool/norovirus/) was used to obtain the genotype.

Statistical analysis.

The attack rate (AR) by age group, sex, relation with the center (residents or staff), mode of transmission (person-to-person or foodborne), degree of dependency and, in staff, type of work activity were calculated. The rate ratio (RR) and 95% confidence intervals (CI) were calculated to estimate the risk of being affected globally and separately for sex, mode of transmission, the capacity of the center (<100 residents or ≥ 100 residents) and the level of dependence (residents with total or severe dependence vs. residents with moderate, low or no-dependence).

To assess the seasonality, we used a one-tailed Z-test to compare the proportion of outbreaks in the cool months (October to March) with a theoretical value of 50%. The correlation between the average temperature of each month in Barcelona[28] and the number of outbreaks in the month was estimated using Pearson's correlation coefficient and the p value with a t-Student test.

A one-tailed Fisher's exact test was used to compare the proportion of residents affected with specific underlying diseases (heart disease, diabetes mellitus, dementia, immunodeficiency and chronic obstructive pulmonary disease) with the proportion of residents without these underlying diseases. This test was also used to compare the proportion of residents and staff members who needed medical care. The association between genogroup and the presence of symptoms was assessed using the odds ratio (OR) and its 95% CI.

The mean Cq, as an approximation to the viral load, was compared in symptomatic and asymptomatic infected persons (in staff, residents and all attenders) using the t-test for independent means. The Student's t-test and its 95% CI was used to calculate the degree of significance of the difference between means. A Hartley's F_{max} test for variance homogeneity was used previously and if the p value of the F_{max} statistic was >0.05, the t-test was based on equal variance; otherwise, the t-test was based on unequal variance.

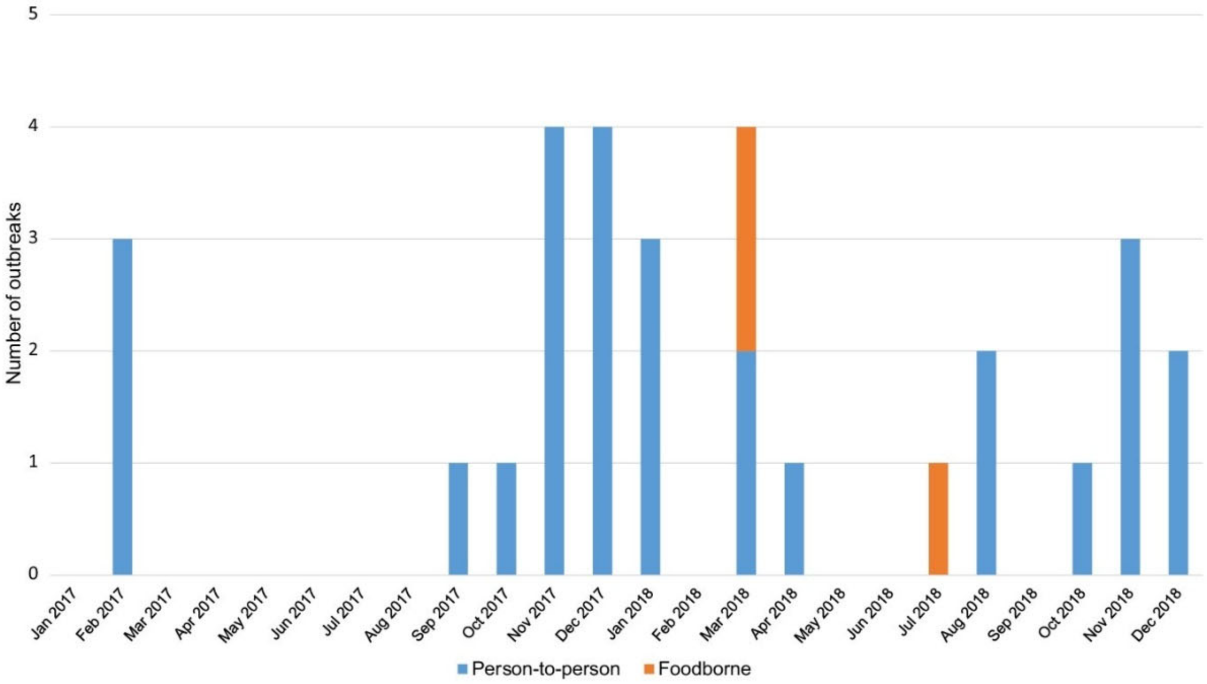

Figure 1. Number of acute gastroenteritis outbreaks due to norovirus in long-term care facilities according to the month of onset and mode of transmission.

Data collection and management was made using the MS-Office 2013 Access 12.0 database and the statistical analysis using the PASW Statistics 18.0.2 statistical package and Epi Info for Windows 7.2.

Ethics declarations and informed consent statement. The study was conducted according to the guidelines of the Declaration of Helsinki, regulations of the Public Health Agency of Catalonia and ethical protocols established. The study was approved by the University of Barcelona Bioethics Commission (ethics approval number IRB00003099) on April 12, 2016.

The authors declare that the Bioethics Committee of University of Barcelona approved the waiver for informed consent. All data used in the analysis were collected during routine public health surveillance activities as part of the legislated mandate of the Health Department of Catalonia, which is officially authorized to receive, treat and temporarily store personal data in the case of infectious disease. All data were fully anonymized. All study activities formed part of the public health surveillance tasks. The law regulates these activities and informed consent should not be necessary.

Results

Reported outbreaks. During the study period, 213 AGE outbreaks were reported to the Public Health Agency of Catalonia; 40 (18.78%) occurred in LTCF and norovirus was identified as the causal agent in 75% (30/40).

The transmission mode was person-to-person in 27 of the 30 outbreaks in LTCF and foodborne in the remaining three, although there was also subsequent person-to-person transmission in one foodborne outbreak. In these three foodborne outbreaks, four kitchen workers were affected (3 cooks and 1 kitchen assistant).

A total of 4631 persons were exposed and 1201 of these were affected (AR 25.93%); 3,034 exposed persons were LTCF residents, of whom 976 were affected (AR 32.17%). Of the 1597 exposed staff members, 225 were affected (AR 14.09%). The RR for residents vs. staff members was 2.28 (95% CI 2.0–2.61).

In person-to-person transmission outbreaks, 4170 persons were exposed and 1048 were affected (AR 25.13%). Among residents, the AR was 32.48% (2768 exposed and 899 affected) and among staff members it was 10.63% (1402 exposed and 149 affected) (RR 3.06 95% CI 2.60–3.59).

In foodborne outbreaks, the overall AR was 33.19% (461 exposed and 153 affected), and it was 28.95% (266 exposed and 77 affected) among residents and 38.97% (195 exposed and 76 affected) among staff (RR 0.74; 95% CI 0.57–0.96).

Twenty-five (83.33%) outbreaks occurred in the cool months and five (16.67%) in the warm months. There was a significantly higher proportion of outbreaks in the cool months (z-test = 3.61, $p < 0.001$). In addition, a Pearson's correlation coefficient of − 0.63 ($p = 0.03$) between the average temperature and the number of outbreaks per month was observed. Of the 27 outbreaks with person-to-person transmission, 13 (48.15%) occurred during the colder months (December to March) (Fig. 1).

	Centers with < 100 residents			Centers with ≥ 100 residents			
	Affected	Unaffected	Attack rate	Affected	Unaffected	Attack rate	RR (95% CI)
Residents	508	616	45.2	468	1442	24.5	1.85 (1.67–2.04)
Staff	111	374	22.89	114	998	10.25	2.23 (1.76–2.83)
Total exposed	619	990	38.47	582	2440	19.26	2 (1.82–2.2)

Table 1. Attack rates and rate ratio (RR) according to the capacity of the facility (separately for residents and staff and globally).

Degree of dependency	Affected residents	Unaffected residents	Attack rate (%)	RR (95% CI)
Total dependency (0–20)	45	13	77.59	
Severe dependency (21–60)	87	10	89.69	
Moderate dependency (61–90)	48	22	68.57	
Low dependency (91–99)	5	2	71.43	
Independent (100)	3	1	75	
Total + severe	132	23	85.16	1.23 (1.05–1.45)
Moderate + low + No dependence	56	25	69.13	1

Table 2. Degree of dependency (Barthel index) in affected and unaffected residents, attack rates and rate ratio (RR) in residents with total or severe dependence versus residents with moderate, low or no-dependence.

Of the centers where outbreaks occurred, 13 had a capacity of ≥ 100 residents and the AR was 19.26%. The remaining 17 centers had a capacity of < 100 residents and the AR was 38.47%. The RR, both globally and for staff or residents, indicated an increased risk of being affected in smaller centers (Table 1).

Results of the survey in exposed and affected persons in LTCF. A total of 495 exposed persons (365 residents and 130 staff) were interviewed, of whom 106 (21.41%) were male and 389 (78.59%) female. The average age was 86.20 years (SD 8.87) in residents and 40.14 years (SD 13.44) in staff. Nine residents aged < 65 years were affected (AR 88.89%), 64 aged 65–74 years (AR 84.21%), 158 aged 85–94 years (AR 82.29%) and 36 aged 95–105 years (AR 83.72%). In staff members, there were 38 affected persons aged 18–39 years (AR 65.52%) and 34 aged 40–65 years (AR 53.97%). The age was unknown in 20 residents and 9 staff members.

Of the total exposed people interviewed, 371 persons were affected (295 residents and 76 staff members) and 124 unaffected. Nine residents and 13 staff members needed medical care (3.05% of affected residents and 17.11% of affected staff members), with the difference being significant ($p < 0.001$). Four affected persons (1.08% of all affected people) were hospitalized: 3 residents (1.02% of residents affected) and 1 staff member (1.32% of staff members affected). The difference was not significant ($p = 0.60$). No deaths were recorded.

The AR was 72.64% in males (106 exposed and 77 affected) and 75.58% in females (389 exposed, 294 affected) (RR 0.96; 95% CI 0.84–1.09).

The AR was 76.55% in person-to-person outbreaks (388 exposed, 297 affected) and 69.16% (107 exposed, 74 affected) in foodborne outbreaks (RR 1.11; 95% CI 0.96–1.27).

Information on underlying diseases was obtained in 305 (83.56%) residents of whom 255 were affected. The proportion of residents with and without a specific underlying disease who were affected were compared: for heart disease these proportions were 86.33% and 81.33% ($p = 0.15$); for diabetes mellitus 82.89% and 64.21% ($p = 0.49$); for dementia 81.03% and 84.21% ($p = 0.34$); for immunodeficiency 81.82% and 83.67% ($p = 0.56$); for chronic obstructive pulmonary disease 76.74% and 84.73% ($p = 0.14$). The degree of dependence (measured by the Barthel index) was obtained in 236 residents (188 affected and 48 unaffected). The AR was 85.16% in residents with total or severe dependence and 69.13% in residents with moderate, low or no-dependence (RR 1.23; 95% CI 1.05–1.45) (Table 2).

Information on occupation was obtained for 123 staff members: 58 were maintenance personnel or kitchen staff (of whom 26 were affected) and 65 were healthcare staff or caregivers (of whom 49 were affected); in 7 staff members this information was not available. Kitchen staff and maintenance personnel had a lower risk of being affected than healthcare staff and caregivers (RR 0.59; 95% CI 0.43–0.82) (Table 3).

Of the 30 outbreaks studied, 5 were due to GI (16.67%), 21 to GII (70%) and the remaining 4 (13.33%) were due to mixed infection by GI and GII. We identified 35 genotypes: GII.4 was identified in 13 outbreaks, GII.17 in five, GI.6 in three, and GI.3, GI.4, GII.2 and GII.P16 in two outbreaks. The remaining genotypes (GI.2, GI.5, GI.P4, GI.P5, GII.P7, GII.P17 and GII.P31) were identified in one outbreak.

A total of 425 stool samples were collected and norovirus was identified by RTqPCR in 256. GI was identified in 53 samples (20.70%) and GII in 198 samples (77.34%). In 5 samples (1.95%) coinfection with GI and GII (2 symptomatic and 3 asymptomatic) was identified. The remaining 169 samples were negative for norovirus.

Occupation	Affected	Unaffected	Attack rate (%)	RR (95% CI)
Kitchen staff and service personnel	26	32	44.83	0.59 (0.43–0.82)
Health staff and caregivers	49	16	75.38	1
Total	75	48	60.98	

Table 3. Attack rates and rate ratio (RR) in long-term care facilities staff according to occupation.

Genogroup	Symptomatic	Asymptomatic	Total	OR (95% CI)
GI	45	8	53	0.96 (0.41–2.26)
GII	169	29	198	1

Table 4. Norovirus genogroup in symptomatic and asymptomatic infected long-term care facilities staff and residents.

	Symptomatic	Mean (SD)	Positive samples*	Difference between means of Cq (95% CI)	*p* Value
Staff	Yes	28.11 (5.54)	46	− 1.85 (− 4.87 to 1.17)	0.225
	No	29.96 (5.15)	18		
Residents	Yes	24.96 (5.87)	164	− 3.19 (− 5.82 to − 0.56)	0.018
	No	28.15 (5.84)	22		
All persons	Yes	25.65 (5.93)	210	− 3.35 (− 5.34 to − 1.35)	0.001
	No	29.00 (5.50)	40		

Table 5. Quantification cycle (Cq) values in symptomatic and asymptomatic infected staff and residents of long-term care facilities. *In 6 samples positive for GII norovirus, the Cq value could not be determined.

Table 4 shows the genogroup detected in symptomatic and asymptomatic infected LTCF staff and residents. No association was found between the genogroup and presenting symptoms (OR 0.96; 95% CI 0.41–2.26), indicating that the proportion of asymptomatic infections was similar for both genogroups.

With respect to the viral load in symptomatic and asymptomatic persons (Table 5), the difference between the means of the Cq was − 3.35 (95% CI − 5.34 to − 1.35), with a greater viral load found in symptomatic than in asymptomatic persons ($p = 0.001$).

Discussion

Our results show that 18.78% of AGE outbreaks occurred in LTCF, similar to the results obtained by Torner et al.[29] in a study carried out in Catalonia in 2010 and 2011.

Norovirus was identified as the cause of 75% of the 40 outbreaks occurring in LTCF, coinciding with results from other studies. Inns et al. described 566 AGE outbreaks in LTCF in Northeast England between 2016 and 2018 and norovirus was detected in 64% of outbreaks with an identified pathogen[30]. Steele et al.[31] studied 7094 norovirus outbreaks between 2009 and 2017 in the United States of which 5335 (75%) occurred in LTCF and Espenhain et al. found that 77% of norovirus outbreaks in Norway between 2005 and 2018 occurred in LTCF[32].

A seasonal distribution was observed, with most outbreaks occurring in the cool months. Our results are consistent with previous findings by other authors indicating a seasonality of norovirus disease[4,33].

The mode of transmission of the outbreaks studied showed there was person-to-person transmission in 90% and only 10% were foodborne. Similar results were found by Kroneman et al. in a study of norovirus outbreaks in 13 European countries between July 2001 and June 2006 (person-to-person transmission accounted for 88% of outbreaks, 10% were foodborne and 2% were food and waterborne)[34]. We found no outbreaks due to waterborne transmission. Chen et al. in a review of norovirus outbreaks in LTCF found person-to-person transmission in > 90% of outbreaks and linked this to close contact with other residents, shared facilities and contact with visitors and staff[35]. Lian et al.[4] in an analysis of norovirus outbreaks reported in China from 2014 to 2017 found that 77% were caused by person-to-person transmission, 6% foodborne, 4% waterborne and 13% by multiple transmission.

Hospitalization was required in 1.08% of affected people, coinciding with Espenhaim et al.[32] in a Norwegian study carried out during 2005–2018, who reported 0.91% of hospitalizations in affected people.

No deaths were reported in the norovirus outbreaks included in this study, coinciding with the results of the Lian et al. study carried out in China in a four-year period[4]. However, Espenhaim et al.[32] reported 0.67% of deaths in LTCF outbreaks in the above-mentioned period of 13 years.

Our results suggest that the closeness of contact between residents and staff may play an important role in the transmission, as staff who had greater contact with residents (healthcare staff and caregivers) had an increased risk of being affected than those who did not. A 2014 meta-analysis by Petrignani et al.[36] of 40 outbreaks in LTCF also found that the closeness of contact between staff and residents was related to the risk of staff being affected. The authors found that residents with medium or high dependence had a higher attack rate than those with low dependence. Our results showed that 70.21% of affected residents had total or severe dependence (Barthel score between 0 and 60) in agreement with the results obtained by these authors, suggesting that people with greater dependence require greater contact with caregivers.

Norovirus was identified by RTqPCR in the stool samples of 36.70% (40/109) of asymptomatic exposed persons, similar to the values estimated by Miura et al. in foodborne outbreaks in Japan from 2005 to 2006 in which they identified norovirus in 32.1% of asymptomatic persons[37].

We found that symptomatic infected persons had a higher viral load, with a mean Cq of 25.65, compared with 29.00 in asymptomatic infected persons. These results are similar to those obtained by Shioda et al., who studied 12,910 samples from outbreaks and isolated cases in the United States and Latin America with a mean Cq of 25.3 for symptomatic affected persons and 28.5 for asymptomatic affected persons[18].

We found that outbreaks in centers with a lower capacity had a higher attack rate than outbreaks in centers with greater capacity. A possible explanation is that, in smaller centers, the cleaning and disinfection protocols when outbreaks occur may be less developed than in large centers. Rosenthal et al. in a study conducted in Oregon between 2003 and 2006, found contrary results[38], with the differences possibly being due to characteristics of the centers that were not recorded.

A strength of the study is that all outbreaks reported in a region with the same surveillance system were included, and therefore the results should be homogeneous and reflect the real situation.

Our study has some limitations. First, the mild severity of AGE outbreaks due to norovirus means under-reporting may be greater in small centers than in large ones as, because there are fewer cases, contact with the health services and notification of the outbreak may be less likely. Secondly, we did not collect information about staff working closely with residents with different levels of dependence and, therefore, we could not analyze whether there was a relationship between these variables. Another limitation is that the number of samples from infected asymptomatic persons was low, meaning there was not sufficient statistical power to detect differences with symptomatic persons.

Conclusions

Norovirus caused the vast majority of AGE outbreaks in LTCF, with more residents than staff being affected, especially those with a high degree of dependence. Person-to-person transmission was the main mode of transmission and GII was the most prevalent causal agent. There was no outbreak caused by GIV.

Mean viral loads were higher in infected symptomatic persons than in infected asymptomatic persons, both globally and in residents. Because norovirus was detected in asymptomatic persons, control measures should be applied not only to people with symptoms but to all persons in LTCF where norovirus outbreaks occur.

Data availability

The datasets generated during the current study are available in the Mendeley Data repository (https://data.mendeley.com/datasets/58pjx5vpk2/1).

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Author contributions

I.P. and A.D. conceptualized and designed the study, carried out the initial analyses and interpretation of data and drafted the initial manuscript. M.A. and C.R.: designed the study and acquisition of data and revised critically the manuscript. T.C.-S. carried out analyses and interpretation of data, performed the laboratory analyses and drafted the initial manuscript. M.J., C.P., I.B. and S.G. contributed to the final analyses and interpretation of data and critically revised the final manuscript. The Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia contributed to the cencept and design of the the study, acquisition of data and critically revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Competing interests

The authors declare no competing interests.

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7.4.- Artículo 4: Involvement of workers in closed and semiclosed institutions in outbreaks of acute gastroenteritis due to norovirus.

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Título

Implicaciónde los trabajadores de instituciones cerradas y semicerradas en los brotes de gastroenteritis aguda por norovirus.

Resumen

Introducción:

Los brotes de norovirus ocurren con frecuencia en instituciones cerradas o semicerradas. Estudios recientes en Cataluña y varios países indican que en los brotes que se presentan estas instituciones, norovirus se detecta entre el 23% y el 60% de los trabajadores, y la prevalencia de infección en trabajadores asintomáticos implicados oscila entre el 17% y el 40%.

Material y método:

Se realizó un estudio prospectivo para investigar la participación de los trabajadores en instituciones cerradas y semicerradas durante los brotes. Se calcularon las tasas de ataque y las razones de tasas según el tipo de transmisión y categoría ocupacional. Se calcularon las razones de tasas y sus intervalos de confianza del 95% entre trabajadores y usuarios. Se compararon los valores medios del ciclo de cuantificación (Cq) según el genogrupo y la presencia de síntomas.

Resultados:

Las tasas de ataque fueron más altas en la transmisión de persona a persona que en los brotes de vehículos comunes, y el 38,8% de los trabajadores eran sintomáticos. La razón de tasas entre trabajadores y usuarios fue de 0,46 (IC 95% 0,41-0,52). Las tasas de ataque en los trabajadores fueron altas, particularmente en los trabajadores con mayor contacto con los usuarios. El Cq medio fue menor en enfermos que en infectados asintomáticos, aunque la diferencia solo fue significativa para el genogrupo I.

Conclusión:

La frecuencia de infectados asintomáticos sugiere que todos los trabajadores de los centros afectados deberían seguir cuidadosamente las medidas de higiene personal.

viruses MDPI

Article

Involvement of Workers in Closed and Semiclosed Institutions in Outbreaks of Acute Gastroenteritis Due to Norovirus

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Abstract: Norovirus outbreaks frequently occur in closed or semiclosed institutions. Recent studies in Catalonia and various countries indicate that, during outbreaks in these institutions, norovirus is detected in between 23% and 60% of workers, and the prevalence of infection in asymptomatic workers involved in outbreaks ranges from 17% to 40%. In this work, we carried out a prospective study to investigate the involvement of workers in closed and semiclosed institutions during outbreaks. The attack rates (ARs) and the rate ratios (RRs) were calculated according to the type of transmission and occupational category. The RRs and 95% confidence intervals (CIs) between workers and users were calculated. The mean cycle of quantification (Cq) values were compared according to the genogroup and the presence of symptoms. ARs were higher in person-to-person transmission than in common vehicle outbreaks, and 38.8% of workers were symptomatic. The RR between workers and users was 0.46 (95% CI 0.41–0.52). The ARs in workers were high, particularly in workers with closer contact with users. The mean Cq was lower in patients than in asymptomatic infected persons, although the difference was only significant for genogroup I (GI). The frequency of asymptomatic infected persons suggests that personal hygiene measures should be followed by all workers in the centers affected.

Keywords: norovirus; outbreak; acute gastroenteritis; closed institution; semiclosed institution; workers

1. Introduction

Most acute gastroenteritis (AGE) cases worldwide are caused by viruses. The causes include, in addition to norovirus, other enteric viruses such as adenovirus, astrovirus, rotavirus and sapovirus, although norovirus is the most common causal agent [1–3]. Noroviruses are nonenveloped RNA viruses of the *Caliciviridae* family. There are six genogroups (GI to GVI), although only genogroups I, II and IV are human pathogens [4,5].

The transmission mechanism is fecal–oral, and direct person-to-person contact is a very efficient mechanism. Transmission by food, water and fomites is common, and transmission by aerosols generated by vomiting has been described [6].

A systematic review estimated that human caliciviruses (including genogroup I and II norovirus and sapovirus) caused 71,000 deaths worldwide in children aged <5 years in 2011 [7].

Norovirus is highly resistant to high levels of chlorine [8], heat, cold [9], acidic pH and organic solvents [10,11], which allows its survival for long periods in the environment [12] and facilitates its transmission. The high transmissibility of norovirus is also facilitated by the short duration of immune protection [13], the low infectious dose and the frequency of asymptomatic infections and because the presence of norovirus viruses in feces may be prolonged in both symptomatic and asymptomatic infected persons.

The doses that cause infection in 50% of exposed people (ID_{50}) described to date range from 18 to 2934 viral genomes [14–17]. About 30% of people affected by norovirus have an asymptomatic infection [18].

AGE outbreaks due to norovirus frequently occur in closed or semiclosed institutions such as long-term care facilities, daycare centers, schools, nursing homes and hotels. According to 2006–2010 data, in Catalonia (Spain), >50% of norovirus outbreaks occurred in these types of institutions [19].

The measures to control outbreaks in Catalonia include the recommendation that workers with acute gastroenteritis due to norovirus do not return to work until >48 h after the end of symptoms [20].

Recent studies in Catalonia and various countries indicate that, during outbreaks in these institutions, norovirus in feces is detected in between 23% [21] and 60% [22] of workers and the prevalence of infection in asymptomatic workers involved in outbreaks ranges from 17% [21] to 40% [22].

Although few studies have investigated the involvement of workers in AGE outbreaks due to norovirus in closed and semiclosed institutions, the attack rate among workers may vary depending on the demographic characteristics and type of occupation, the type of institution and the type of transmission causing the outbreak.

The objective of the study was to investigate the involvement of workers in outbreaks due to norovirus in closed and semiclosed institutions according to type of center, type of transmission, genogroups involved and viral load.

2. Materials and Methods

2.1. Type of Study, Study Period and Study Population

This was a prospective study of AGE outbreaks due to norovirus reported in 2017–2019 to the Notifiable Diseases System of Catalonia [23], a region in the northeast of Spain with a population of 7,496,276 in January 2017 [24].

2.2. Outbreaks Included in the Study

All laboratory-confirmed AGE outbreaks due to norovirus that occurred in closed and semiclosed institutions during the study period were included.

A closed institution was defined as one in which users remained the whole day, including the night, although they might leave for short periods for exceptional reasons. Within this category, we included nursing homes, long-term care facilities and summer camps, among others. A semiclosed institution was defined as one carrying out social or educational activities (nonoccupational) in which users remained for >8 h per day, and in which most users consumed at least one meal. Daycare centers, preschool centers, schools and hotels were included in this category.

AGE was defined as sudden-onset diarrhea accompanied by nausea, vomiting, abdominal pain or fever. A norovirus outbreak was defined as AGE in ≥2 people with a common vehicle or person-to-person transmission with norovirus in stool samples identified by real-time reverse transcription polymerase chain reaction (RTqPCR) [25,26].

2.3. Data Collection

Data on the outbreaks included were collected by technicians of the Epidemiological Surveillance Services of the Public Health Agency of Catalonia and the Public Health Agency of Barcelona.

In all reported outbreaks, the type of institution, the number of users, the number of workers and the type of transmission (person-to-person or common vehicle) were recorded.

All persons exposed were questioned about sociodemographic variables (sex and date of birth) and their relationship with the institution (worker or user). Information on the type of occupation and the presence of clinical symptomatology was also collected in workers.

Samples of feces were collected from workers and users to identify norovirus genogroups I, II and IV by RTqPCR. Samples were analyzed in the Microbiology Laboratory of Vall d'Hebron University Hospital. The specific primers described by Kageyama et al. were used to detect norovirus GI and GII [25]. A modification of the primer described by Farkas et al. [26] and Kageyama et al. [25] was used to detect norovirus GIV.

2.4. Data Analysis and Management

The proportions of the study variables and their 95% confidence intervals (CIs) were calculated. The global attack rates, the attack rates and the rate ratios (RRs) and their 95% CIs were calculated for the mode of transmission (person-to-person or common vehicle), type of institution and type of work activity.

To estimate the risk of workers becoming ill with respect to users, the RRs and their 95% CIs were calculated.

To estimate the viral load, the mean cycle of quantification (Cq) values obtained by RTqPCR [27] were calculated. The means of Cq were compared using the Student's *t*-test. Statistical significance was established as $p < 0.05$. Data were collected and handled using Microsoft Access 12.0 database manager, and the PASW Statistics 18.0.2 statistical package was used for the statistical analysis.

3. Results

During the study period, 99 AGE outbreaks due to norovirus were detected in closed or semiclosed institutions (26 in 2017, 33 in 2018 and 40 in 2019): 49 in nursing homes (49.5%), 20 in summer camps (22.2%), 13 in schools (13.1%), 6 in daycare centers (6.1%), 6 in hotels (6.1%) and 5 in long-term care facilities (5.1%) (Figure 1).

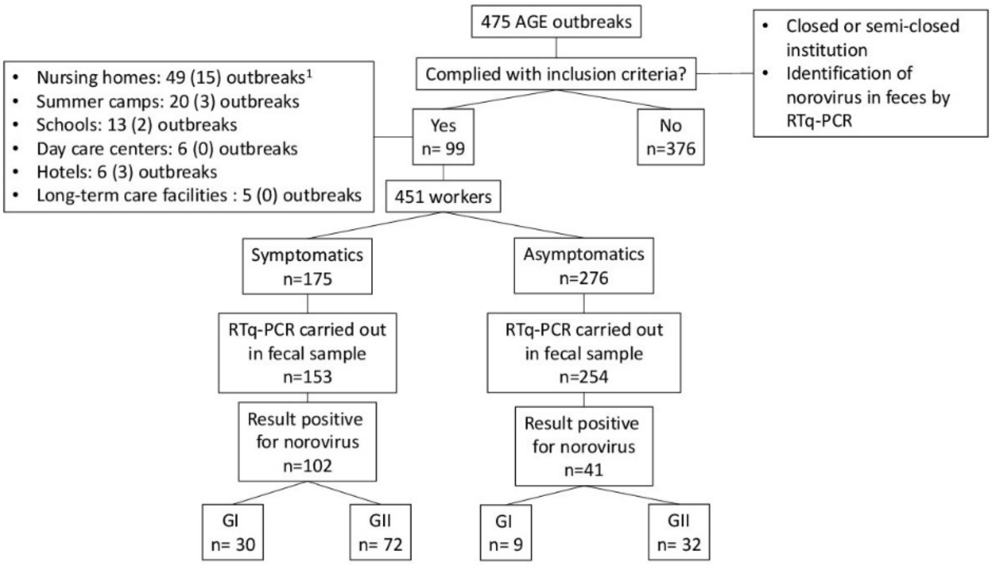

Figure 1. Study flowchart. [1] In brackets, the number of outbreaks in which the first case was a worker.

In 74 outbreaks (74.8%), transmission was person-to-person; in the remaining 25 (25.2%), it was due to a common vehicle.

The epidemiological survey was answered by 451 workers and 1015 users from the affected centers. Of the workers, 175 (38.8%) were symptomatic and 276 (61.2%) had no symptoms.

The attack rate was 32.03% in males and 41.49% in females. No significant differences in attack rates were found according to sex or age group (Table 1).

Table 1. Attack rates in workers by age and sex.

	Males			Females			
Age (Years)	**Symptomatic**	**Exposed**	**Attack Rate**	**Symptomatic**	**Exposed**	**Attack Rate**	***p*-Value**
17–25	10	17	58.82%	29	45	64.44%	0.69
26–35	11	38	28.95%	28	58	48.28%	0.06
36–45	10	27	37.04%	20	55	36.36%	0.95
46–55	5	24	20.83%	31	83	37.35%	0.13
56–65	4	14	28.57%	14	37	37.84%	0.54
NA	1	8	12.50%	12	45	26.67%	0.35
Total	41	128	32.03%	134	323	41.49%	0.06

NA: not available.

The attack rate in workers was 43.9% in person-to-person outbreaks and 32.6% in outbreaks with a common vehicle (Table 2). The risk of workers being symptomatic was higher in person-to-person outbreaks than in those with a common vehicle (RR 1.35; 95% CI 1.05–1.74). Analysis by type of institution showed the RR of attack rates was only significant for schools (RR 1.93; 95% CI 1.07–3.49).

Table 2. Attack rates and rate ratios (RRs) in workers according to type of institution and type of transmission.

Type of Institution (Total Affected) [1]	Symptomatic	Exposed	Attack Rate	RR (95% CI)
Summer camp				
Person-to-person (74)	1	11	9.09%	0.17 (0.02 to 1.30)
Common vehicle (105)	12	32	37.5%	1
Mixed transmission (4)	0	8	0%	NC
Total (183)	13	51	25.49%	
School				
Person-to-person (144)	15	56	26.79%	1.93 (1.07 to 3.49)
Common vehicle (24)	11	69	15.94%	1
Total (168)	26	125	20.80%	
Daycare center				
Person-to-person (69)	6	14	42.86%	NC
Common vehicle (0)	0	0	0%	
Total (69)	6	14	42.86%	
Hotel				
Person-to-person (14)	9	40	22.5%	0.93 (0.44 to 1.95)
Common vehicle (48)	5	21	23.81%	1
Total (62)	14	61	22.95%	
Nursing home				
Person-to-person (400)	77	133	57.89%	1.03 (0.78 to 1.38)
Common vehicle (93)	28	50	56.00%	1
Total (493)	105	183	57.37%	
Long-term care facility				
Person-to-person (55)	11	17	64.70%	NC
Common vehicle (0)	0	0	0%	
Total (55)	11	17	64.70%	
Total				
Person-to-person (756)	119	271	43.91%	1.35 (1.05 to 1.74)
Common vehicle (270)	56	172	32.56%	1
Mixed transmission (4)	0	8	0%	NC
Total (1030)	175	451	38.80%	

[1] The total number of affected persons, including users and workers, is shown in parentheses. NC: Not calculable.

A total of 1015 users responded to the epidemiological survey, of whom 854 were users who were symptomatic (attack rate 84.1%). The RR of attack rates between workers and users was 0.46 (95% CI 0.41–0.52). The lower risk of workers compared with users was also observed separately for each type of institution (Table 3).

Table 3. Attack rates and rate ratios (RRs) in workers and users according to type of institution.

Type of Institution	Symptomatic	Exposed	Attack Rate	RR (95% CI)
Summer camp				
Workers	13	51	25.49	0.30 (0. 19 to 0. 48)1
Users	170	201	84.58	
Total	183	252	72.62	
Schools				
Workers	26	125	20.80	0.25 (0.17 to 0.35)1
Users	141	167	84.43	
Total	167	292	57.19	

Table 3. *Cont.*

Type of Institution	Symptomatic	Exposed	Attack Rate	RR (95% CI)
Daycare center				
Workers	6	14	42.86	0.53 (0.28 to 0.97)1
Users	62	76	81.58	
Total	68	90	75.56	
Hotel				
Workers	14	61	22.95	0.26 (0.16 to 0.42)1
Users	48	55	87.27	
Total	62	116	34.83	
Nursing home				
Workers	105	183	57.38	0.69 (0.61 to 0.79)1
Users	388	468	82.91	
Total	493	651	75. 73	
Long-term care facility				
Workers	11	17	64.71	0.69 (0.48 to 0.99)1
Users	45	48	93.75	
Total	56	65	86.15	
Total				
Workers	175	451	38.80	0.46 (0.41 to 0.52)1
Users	854	1015	84.14	
Total	1029	1466	70.19	

The attack rates differed according to the type of institution; the highest rates were for long-term care facilities (86.15%) and nursing homes (75.73%), and the lowest rates were for hotels (34.83%). Globally, in all types of institutions, workers had significantly lower attack rates than users.

With respect to the type of occupation, caregivers in nursing homes and healthcare workers had an increased risk of becoming ill, while being a kitchen worker was a protective factor against infection (Table 4).

Table 4. Attack rates and rate ratios (RRs) in workers according to type of occupation.

Type of Occupation	Attack Rate	RR [1] (95% CI)
Cook	9.8%	0.26 (0.12.0.56)
Kitchen assistant	15.0%	0.36 (0.23.0.59)
Waiter	37.5%	1.13 (0.45. 2.79)
Dining monitor	27.8%	0.79 (0.54. 1.14)
Caregiver or healthcare worker	71.6%	3.18 (2.32. 4.35)
Global attack rate	38.8%	

[1] Workers in each type of occupation compared with all other workers.

The most common genogroup was norovirus GII with 66 outbreaks (66.7%); 26 were due to GI (26.3%), and the etiology was mixed in 7 (7.1%). No outbreak due to GIV was detected. GII was more frequently involved in all types of institutions than GI (Figure 2).

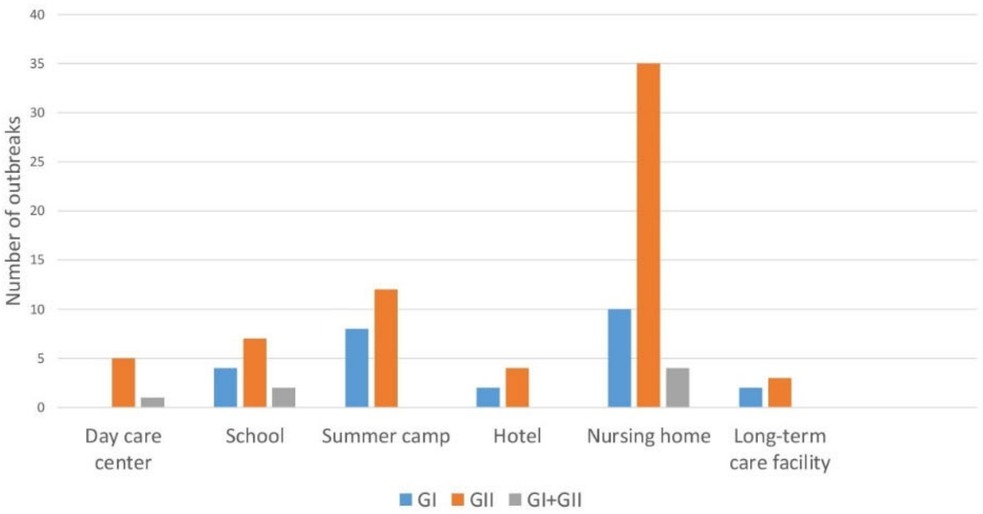

Figure 2. Outbreaks according to type of center and genogroup.

Norovirus was detected by RTqPCR in 143 workers (102 symptomatic and 41 asymptomatic) and 687 users (603 symptomatic and 84 asymptomatic). Norovirus GI was detected in 30 symptomatic workers, 9 asymptomatic workers, 144 symptomatic users and 19 asymptomatic users. Norovirus GI was detected in 72 symptomatic workers, 32 asymptomatic workers, 459 symptomatic users and 65 asymptomatic users. The mean viral load, measured indirectly by Cq, was 27.69 for GI and 31.61 for GII, although the values are not comparable as the measurements were made using different tests.

Mean Cq was lower in symptomatic persons than in asymptomatic infected persons, with a higher viral load in symptomatic persons, for genogroups GI and GII. For GI, a mean Cq of 36.97 (SD 5.13) was observed in asymptomatic infected persons and a mean Cq of 30.01 (SD 5.51) was observed in symptomatic persons ($p = 0.002$). Although the Cq was also higher for GII in asymptomatic compared with symptomatic persons (29.19; SD 5.26 vs. 27.01; SD 5.84), the differences were not statistically significant ($p = 0.07$) (Table 5).

Table 5. Difference in viral load between symptomatic and infected asymptomatic persons according to genogroup.

Genogroup	Symptomatic	N	Mean Cq	SD	*p*-Value
GI	Yes	30	30.01	5.51	0.002
	No	9	36.97	5.13	
GII	Yes	72	27.01	5.84	0.07
	No	32	29.19	5.26	

4. Discussion

The attack rate in workers in institutions where outbreaks included in the study occurred was 38.8%, higher than the 10.45% found by Wu et al. in workers involved in norovirus outbreaks in Shanghai between 2015 and 2017 [21] and the 30% described by Sabria et al. in food handlers and healthcare workers in outbreaks in Catalonia between 2010 and 2012 [22]. The higher attack found in our study may be because the studies mentioned were not limited to closed or semiclosed institutions, in which transmission occurs more easily than in other types of institutions.

We found that 16.14% of asymptomatic workers in AGE outbreaks due to norovirus in closed and semiclosed institutions were infected.

Asymptomatic norovirus infection is common, even among people without known exposure. Qi et al., in a meta-analysis of published studies on asymptomatic norovirus infection, found a prevalence of infection of 7% worldwide in the general population [28]. Yu et al. found that 3.3% of food handlers unrelated to outbreaks were asymptomatically infected [29], and Okabayashi et al. found a rate of asymptomatic infections of up to 12% in workers in institutions [30].

Wang et al. found norovirus infections in 4.04% of the inhabitants of municipalities related to oyster cultivation, with no differences between workers in oyster farms and the rest of the population [31].

Other studies of workers involved in AGE outbreaks due to norovirus have found very similar rates to those described in our study. Wu et al. found an infection rate of 17% in asymptomatic workers in institutions where outbreaks occurred [21], a rate very similar to ours. Qi et al. found the prevalence of asymptomatic infected people to be 18% in workers related to outbreaks [28]. Our results, in common with other reports [32], found no significant differences in attack rates between male and female workers.

The main transmission route of norovirus is direct person-to-person contact, and its dissemination is facilitated by the conditions in which the outbreak occurs.

Increased personal contact between individuals, such as in nursing homes, schools and daycare centers, is likely to facilitate greater transmission [33,34]. Godoy et al. found an increased risk in workers in an AGE outbreak due to norovirus in a nursing home when workers had more direct contact with residents [35].

Our results showed the greatest risk of transmission was direct person-to-person transmission rather than transmission by a common vehicle (RR 1.35 95%; CI 1.05 to 1.74) and that the greatest risk was in caregivers in nursing homes and healthcare workers, whose occupational activity involves closer and longer-lasting contact with users. In contrast, kitchen workers, who have less direct contact with users, had a lower risk of being symptomatic.

GII was the most frequently identified genogroup, both in symptomatic and asymptomatic infected persons. The predominance of the GII genogroup, both in isolated cases of AGE and in outbreaks or asymptomatic infections, has been described by various authors. Yu et al. identified GII in 65% of food handlers in elementary schools in the Incheon region (Korea) [29]. Park et al. identified GII in 75% of positive samples from workers in nursing homes with norovirus outbreaks [36].

Likewise, 90.5% of asymptomatic children studied by Qi et al. in a nursery in Changzhou, China, were infected by genogroup GII [37], and in the United States between 2009 and 2015, 81% of norovirus outbreaks were caused by GII [38].

The viral load was higher in symptomatic persons than in asymptomatic infected persons in all cases. While differences in the mean Cq of symptomatic persons vs. asymptomatic infected persons were statistically significant for GI ($p = 0.002$), no significant differences were found for GII ($p = 0.073$).

Teunis et al. found no differences in the viral load between symptomatic persons and asymptomatic infected persons in workers and users of nursing homes and hospitals involved in GII outbreaks [39]. Kabue et al. found that, in children in rural South Africa, the viral load of GII was higher in symptomatic than in asymptomatic persons, but there were no significant differences for GI [40].

The interpretation of the significance of these differences is difficult, given the discrepancies in the results obtained in different studies, but in all cases, viral loads were detected in asymptomatic infected persons, indicating the potential of these people to act as sources of contagion during outbreaks.

The study has some limitations. Firstly, 12.6% of symptomatic workers and 8% of asymptomatic workers were not analyzed using RTqPCR, which could bias the results. However, the differences between these percentages were not significant ($p = 0.11$), and therefore the comparisons between the two categories of infected persons are valid.

Secondly, the fact that in some studies significant differences in relation to an increased viral load in symptomatic versus asymptomatic persons were observed for genogroup GII compared with genogroup GI may be explained by the sample size or confounding factors, and this could be the subject of further studies.

Thirdly, our study was carried out using the surveys answered, and it may be that people who became ill were more willing to collaborate in responding to the survey than those who did not present symptoms, which could have resulted in an overestimate of the attack rates.

Fourthly, some data were not available, such as the theoretical total capacity of the affected centers, the density of occupancy at the time of the outbreak or the ratio between workers and users, so the possible influence of these factors on the attack rates observed could not be analyzed.

The main strength of our study was that it was carried out in the context of epidemiological surveillance, and therefore the study coverage was universal in the target population.

5. Conclusions

The attack rate in workers in closed and semiclosed institutions was high and was related to the type of activity, being higher in workers with closer contact with users.

The frequency of asymptomatic infected persons suggests that in an AGE outbreak due to norovirus, personal hygiene measures should be followed by all workers in the institution where the outbreak occurred.

Although the genogroup I viral load in symptomatic persons was significantly higher than that in asymptomatic infected persons, the viral loads of asymptomatic infected persons were high for both genogroups GI and GII, indicating the potential of these asymptomatic people as a source of infection.

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7.5.- Articulo 5: Outbreaks of gastroenteritis due to norovirus in schools and summer camps in Catalonia, 2017-2019.

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Título

Brotes de gastroenteritis por norovirus en escuelas y casas de colonias en Cataluña, 2017-2019.

Resumen

Introducción:

Norovirus es una frecuente causa de brotes en casas de colonias. Las actividades de estos centros de colonias y la deficitaria infraestructura en las instalaciones de agua potable, así como la falta de educación sanitaria de usuarios y trabajadores pueden ser factores que faciliten la aparición de estos brotes.

Norovirus también es una causa frecuente de brotes en escuelas.

El objetivo del estudio fue analizar los brotes de gastroenteritis aguda por norovirus en escuelas y colonias de verano en Cataluña 2017-2019 e identificar los factores asociados a su aparición y extensión.

Material y métodos:

Estudio prospectivo de los brotes notificados en Cataluña de 2017 a 2019. Para cada variable independiente se calcularon las tasas de ataque, las razones de tasas, la odds ratio y sus intervalos de confianza del 95%. Para estimar la odds ratio ajustada se realizó un análisis multivariado.

La correlación entre el retraso en la notificación y la duración del brote y la correlación entre el tamaño del grupo afectado y la tasa de ataque se estimaron utilizando el coeficiente de correlación de Pearson.

Resultados:

La tasa de ataque global fue del 33,42% en casas de colonias y del 21,27% en colegios (razón de tasas 1,57 [IC 95% 1,43-1,73]) y el 52,63% de brotes se produjeron en meses fríos y el 47,37% en meses cálidos. El retraso medio en la notificación del brote fue de 5,61 días (desviación estándar 5,58 días) y la duración media del brote de 6,11 días (desviación estándar 6,08 días), con un coeficiente de correlación de Pearson de 0,84 (p<0,001) entre estas variables. En los brotes con transmisión de persona a persona, la odds ratio ajustada fue mayor cuanto mayor fue la demora en informar: 3,07 (IC 95% 1,21 - 7,81) cuando la demora fue de 5-9 días y 3,81 (IC 95% 1,42 – 10,23) cuando fue > 9 días.

Conclusiones:

Las variables independientes mostraron distinta asociación con el riesgo de enfermar en brotes de transmisión de persona a persona y brotes por vehículo común, lo que sugiere la conveniencia de analizar sepradamente los brotes según el tipo de transmisión. Debe considerarse la posible existencia de subgrupos con niveles de exposición no homogéneos, ya que la tasa de ataque global no refleja la tasa de ataque de los diferentes subgrupos.

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RESEARCH ARTICLE

Outbreaks of Gastroenteritis Due to Norovirus in Schools and Summer Camps in Catalonia, 2017–2019

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ABSTRACT We studied outbreaks of acute gastroenteritis due to norovirus in schools and summer camps during 2017–2019 in Catalonia (Spain). The overall attack rate was 21.27% in schools and 33.42% in summer camps (RR 0.64 [95% CI 0.58–0.70]) and 52.63% of outbreaks occurred in cold months and 47.37% in warm months. The mean delay in reporting was 5.61 days (SD 5.58 days) and the mean duration was 6.11 days (SD 6.08 days), with a Pearson correlation coefficient of 0.84 ($P < 0.001$) between these variables. In outbreaks with person-to-person transmission, the aOR was higher the longer the delay in reporting: 3.07 (95% CI 1.21–7.81) when the delay was 5–8 days and 3.81 when it was >9 days (95% CI 1.42–10.23). The cold months posed a higher risk than the warm months. In common source outbreaks the risk was higher in children in secondary-higher education and in summer camps.

IMPORTANCE Norovirus is the main cause of viral acute gastroenteritis outbreaks worldwide. The low infectious dose and the lack of long-term immunity in infected persons means that norovirus often causes outbreaks in institutions and closed and semiclosed centers. Norovirus gastroenteritis are usually mild, with no complications, but occasionally can result in hospital admission. Understanding the risk factors involved in a norovirus outbreak can reduce the spread, severity, and duration of the outbreak and, when a vaccine becomes available, this understanding would help us identify the population groups need to get vaccinated. Here, we show the outbreaks due to norovirus in schools and summer camps, the correlation between the delay in reporting and duration of outbreaks and the relationship of the attack rate and the size of the groups.

KEYWORDS norovirus, outbreak, summer camps, schools, calicivirus infection, delayed reporting, size of the group, seasonality

Acute gastroenteritis (AGE) is the second leading cause of death from infectious diseases in children aged < 5 years worldwide (1, 2). The agents most frequently involved are viruses, mainly of the *Caliciviridae* family. In 2011, human caliciviruses caused 71,000 deaths worldwide in children aged < 5 years (3). In 2019, the World Health Organization (WHO) estimated that norovirus was the most frequent cause of foodborne illness in the European Region with 15 million of cases (4). In the United States, norovirus is responsible for $> 60\%$ of cases of AGE (5) and is estimated to be the cause of 90% of all viral AGE outbreaks worldwide (6). AGE norovirus outbreaks often occur in closed or semiclosed institutions such as nursing homes, summer camps, nurseries, schools, and cruise

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ships, where transmission is easy, either person-to-person through close contact or from exposure to contaminated food, water, or surfaces (6, 7).

In the United States, 14 million children and adults attend summer camps annually (8). AGE norovirus outbreaks are frequently reported in US summer camp users. Between 2009 and 2016, 63% of AGE outbreaks in summer camps were due to norovirus (9). The activities and accommodation of summer camps, infrastructure deficits in the supply of drinking water and the lack of health education of users and staff may be facilitating factors (9).

Norovirus is also a common cause of outbreaks in schools. In England from 2014 to 2019, 18.4% of all suspected or confirmed norovirus outbreaks occurred in educational settings (10). In Shanghai in 2016 and 2017 there were 215 outbreaks of norovirus reported, 87.91% (189 outbreaks) of them occurred in schools and kindergartens (11). In the United States between 2013 and 2018 there were 1409 norovirus outbreaks in schools and universities affecting 83,669 people (12). In 2017, in Catalonia (Spain), school was the setting for 35% of outbreaks occurred in closed and semiclosed facilities (13).

Norovirus infections occur at any time of the year, although it is reported that the incidence of isolated cases and outbreaks is higher during the cold months (14). The low infective dose (mean of 18 viral particles) and the lack of long-term immunity in infected people mean that noroviruses frequently cause epidemic outbreaks (6). Water and food control, hand hygiene and surface disinfection are common preventive measures (15). However, the high resistance of the virus to chlorine (16), heat (17), organic solvents and acidic pH (7, 17) limit the effectiveness of these measures.

The objective was to analyze outbreaks of AGE due to norovirus that occurred in schools and summer camps in 2017–2019 in Catalonia and identify the factors associated with their appearance and extent.

RESULTS

In the study period, 101 outbreaks in which norovirus was identified as the causative agent were reported: 37.6% (38 outbreaks) occurred in schools or summer camps, with 5,165 exposed people and 1,295 affected people (AR 25.07%). There were 12 outbreaks in 2017 with 2,120 exposed people and 598 affected people (AR 28.21%),11 outbreaks in 2018 with 1026 exposed people and 271 affected people (AR 26.41%) and 15 outbreaks in 2019 with 1,927 exposed people and 417 affected people (AR 21.64%). Thus, there was a downward trend in attack rates during the study period (Chi square of linear trend 22.82; $P < 0.001$).

Of the 38 outbreaks, 19 occurred in schools with 3,549 exposed people and 755 affected people (AR 21.27%) and the remaining 19 in summer camps with 1,616 exposed people and 540 affected people (AR 33.42%). The ratio of these rates (RR) was 0.64 (95%CI 0.58–0.70); $P < 0.001$. Person-to-person transmission occurred in 23 outbreaks (60.53%) and common source transmission in 15 (39.47%), of which transmission was due to well water in one outbreak and was foodborne in the rest.

The AR was 19.19% (591/3079) in outbreaks of person-to-person transmission and 33.75% (704/2086) in outbreaks of common source transmission (RR 0.57 [95% CI 0.52–0.62]; $P < 0.001$). Twenty outbreaks occurred in the cold autumn or winter seasons (52.63%) and 18 in the warm spring or summer seasons (47.37%). The time from symptom onset in the first case to its reporting to the Epidemiological Surveillance unit ranged from 0 to 25 days (mean 5.61 days, SD 5.58 days). The duration of outbreaks from symptom onset of the first case to symptom onset of the last case ranged from 1 to 14 days (mean 6.11 days, SD 6.08 days). The delay in reporting and the duration of the outbreak showed a linear correlation (Pearson's correlation coefficient 0.84; 95% CI 0.72–0.92; $P < 0.001$) for all outbreaks and for outbreaks with person-to-person transmission (Pearson's correlation coefficient 0.86 [95% CI 0.69–0.93]; $P < 0.001$), but not for common source transmission (Pearson's correlation coefficient −0.05 [95% CI −0.57 to 0.49]; $P = 0.86$). (Fig. 1).

The mean number of exposures was 135.9 people per outbreak (SD 217.6) and the overall attack rate was 41.65%. The mean number of people affected per outbreak was 34.1 (SD

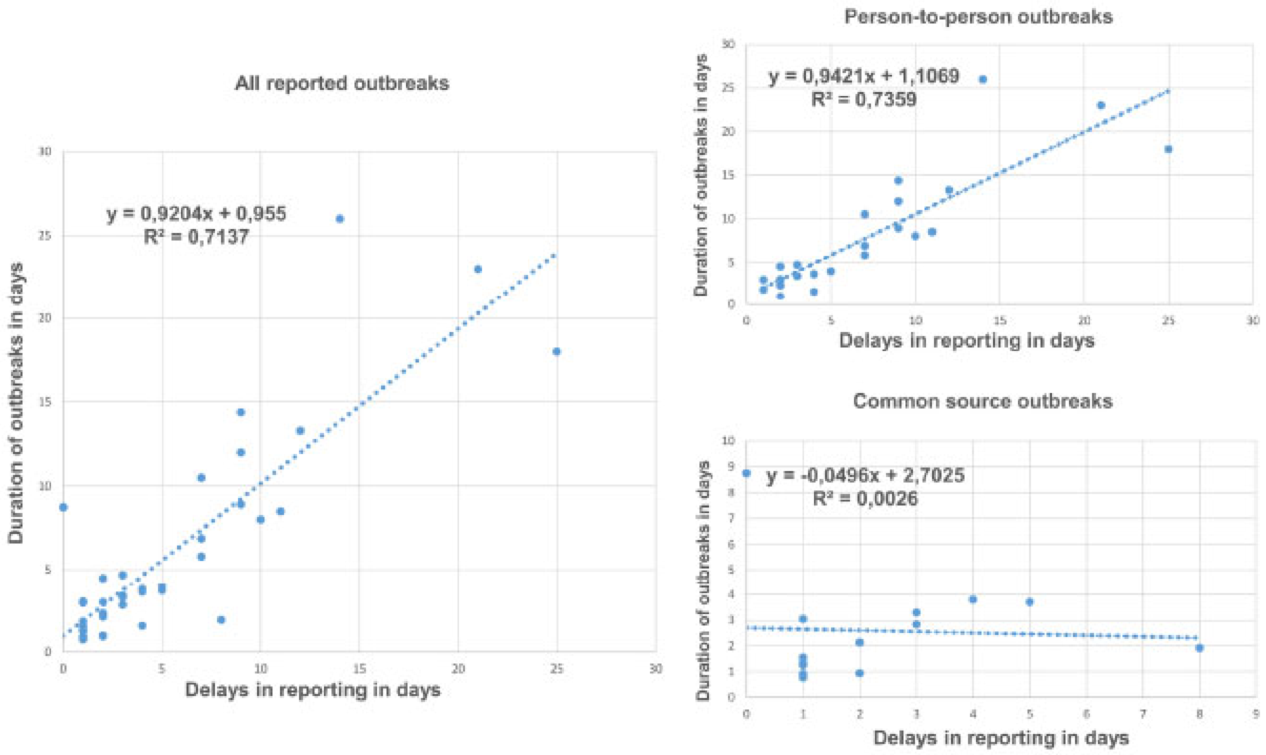

FIG 1 Correlation between delay in reporting and duration of outbreaks in total outbreaks and by type of transmission.

46.2), with the number of people affected ranging from 3 to 293. Considering all outbreaks together, a decreasing exponential relationship was observed between the number of people exposed and the attack rate (Fig. 2) and in the log-log model the Pearson correlation coefficient was −0.6713 (95% CI −0.8159 to −0.4477; $P < 0.001$) and −0.6825 (95% CI −0.8544 to −0.3761; $P < 0.001$) in outbreaks with person-to-person transmission and −0.7568 (95% CI −0.9186 to −0.3781; $P = 0.002$) in common source outbreaks.

We surveyed 636 exposed persons, of whom 420 were affected (66.04%). Table 1 shows the raw and adjusted ORs according to sex, educational level, type of center, season of the year, delay in reporting and type of transmission in the subjects surveyed. We detected an interaction between the type of transmission and the type of center and the season of the year, so common source and person-to-person transmission outbreaks were analyzed separately.

In outbreaks with person-to-person transmission (Table 2) the risk of becoming ill was associated with being a primary school child, the outbreak occurring in a summer camp during the cold months of the year, and delayed reporting. The association between delayed reporting and the risk of becoming ill showed a dose-response relationship. Taking as a reference outbreaks reported in ≥ 2 days, the aOR was 3.07 (95% CI 1.21–7.81) for 5 to 9 days and 3.81 (95% CI 1.42–10. 23) for > 9 days.

In common source outbreaks (Table 3) there was an association between the risk of becoming ill and seasonality, outbreaks in summer camps, being a secondary education or higher student and a delay in reporting of 3 to 4 days. No dose-response was observed between delayed reporting and the risk of becoming ill.

Norovirus GI produced 28.9% of outbreaks (AR 21.57%), 63.2% were due to GII (AR 25.92%) and in 7.9% the etiology was mixed GI/GII (AR 31.22%). Genogroup IV was not detected in any outbreak. The rate ratio (RR) between GI and GII outbreaks was 0.83 (95% CI 0.7–0.94). In outbreaks with person-to-person transmission produced by GI the AR was 15.49% and in those produced by GII the AR was 19.56% (RR 0.79 [95% CI

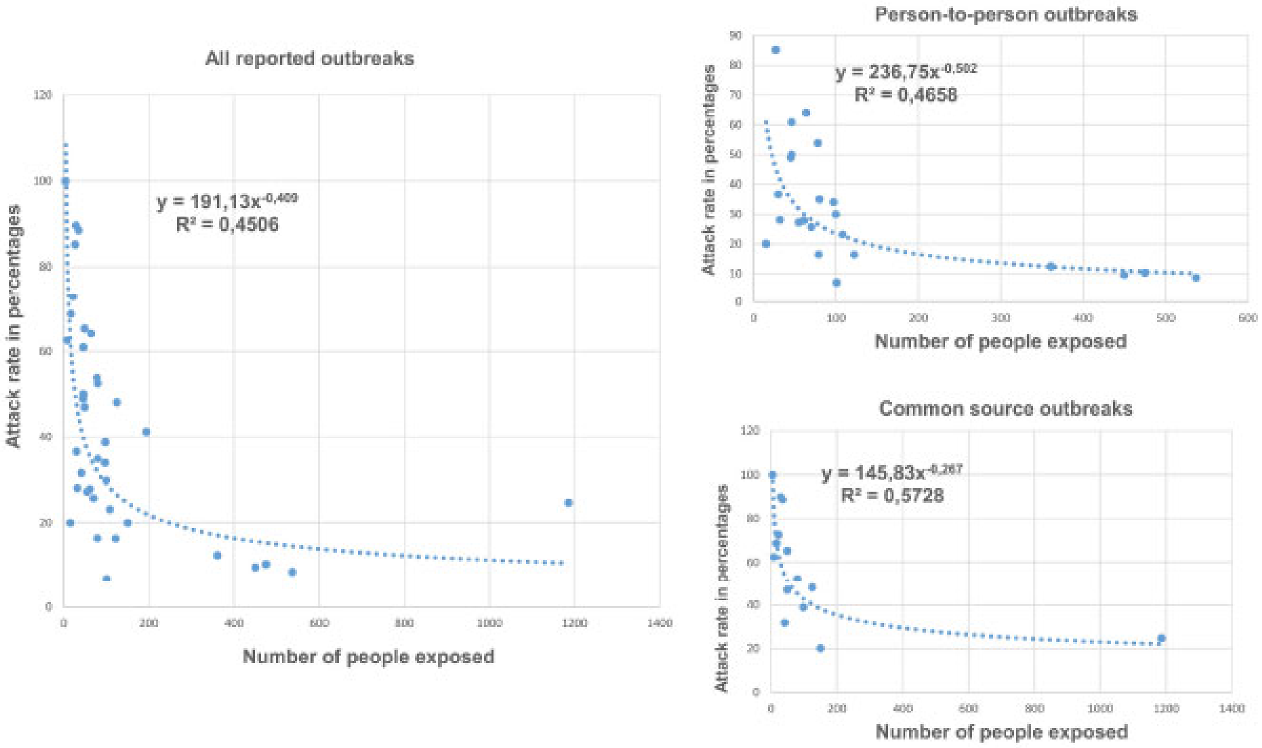

FIG 2 Correlation between number of exposures per outbreak and the attack rate observed in all outbreaks together and by type of transmission.

0.6–0.95]). When transmission was by common source the AR of the outbreaks produced by GI was 35.14% and in those caused by GII was 32.43% (RR 1.08 [95% CI 0. 93–1.26]).

The most frequent genotypes for GI were GI.3 (45.5%), GI.4 (27.3%) and GI.1 (18.2%) and for GII were GII.2 (24%), GII.4 (24%), GII.3 (12%), and GII.6 (12%); in 3 outbreaks (2 of GII and 1 of GI) the genotype was not identified.

DISCUSSION

The proportion of AGE outbreaks due to norovirus in schools and summer camps found in our study (37.6%) is similar to that found by other authors. In England, 22% of norovirus outbreaks between 2015 and 2020 occurred in educational establishments (18). As this study includes data from 2020, in which activity in schools decreased significantly due to the COVID-19 pandemic, this could explain the differences observed between the studies.

The AR we observed in outbreaks of person-to-person transmission (19.19%) is also similar to that described by other authors. Steele et al. studied norovirus outbreaks reported in the United States. between 2009 and 2017 with person-to-person transmission and found an attack rate of 22% (19) and Matthews et al. in a review of 902 outbreaks reported between 1993 and 2011 found a mean rate of 27% in outbreaks of person-to-person transmission (20).

In our study only one outbreak was waterborne with an attack rate of 64.1% (21), clearly larger that the attack rate in all common source transmission outbreaks (33.42%) or in all studied outbreaks (25.7%). Waterborne outbreaks reported by other authors also show a large number of affected people (22, 23).

We found a higher proportion of women were affected (55.37%). Wikswo et al. in a 2009–2010 United States study found that 71% of persons affected by norovirus outbreaks were women (24). However, other studies did not find women were more affected. Thus, Wang et al. studied norovirus outbreaks in schoolchildren in Shanghai

TABLE 1 Raw and adjusted odds ratios of the association between the variables considered and the risk of becoming ill in all outbreaks

Variable	Ill	Not ill	Total	OR[a]	95% CI	aOR[b]	95% CI
Sex							
Male	188	61	249	2.06	1.45–2.93	1.67	1.10–2.52
Female	232	155	387	1		1	
School level							
Infant (>2 and <7 yrs)	183	70	253	1		1	
Primary (≥7 and <13 yrs)	106	6	112	6.76	2.84–16.09	6.95	2.69–17.94
Secondary and higher (≥13 and <19 yrs)	63	17	80	1.42	0.78–2.59	1.51	0.71–3.22
Adults (≥19 yrs)	68	123	191	0.21	0.14–0.32	0.23	0.14–0.37
Center							
School	237	147	384	1		1	
Summer camp	183	69	252	1.18	1.06–1.31	3.55	1.96–6.44
Season							
Warm months	164	108	272	1		1	
Cold months	256	108	364	1.56	1.12–2.17	1.67	1.09–2.56
Reporting delay							
≤ 2 days	143	99	242	1		1	
>2 days and ≤4 days	74	44	118	1.16	0.74–1.83	3.08	1.60–5.92
>4 days and ≤9 days	119	46	165	1.79	1.17–2.74	5.25	2.88–9.58
>9 days	84	27	111	2.15	1.30–3.56	7.11	3.62-13.95
Type of transmission							
Common source	133	90	223	0.65	0.46–0.91		
Person-person	287	126	413	1			

[a] OR, raw odds ratios.

[b] aOR, adjusted odds ratios.

in 2017 and reported that 52.1% of those affected were male (25). Despite the higher percentage of women affected in our study, when we analyzed the risk of becoming ill by sex, we found that the risk was significantly higher in men than in women in all outbreaks. The differences between the sexes could be explained by the fact that

TABLE 2 Raw and adjusted odds ratios of the association between the different variables considered and the risk or of becoming ill in outbreaks of person-to-person transmission

Variable	Ill	Not ill	Total	OR[a]	95% CI	aOR[b]	95% CI
Sex							
Male	121	37	158	1.75	1.12–2.75	1.28	0.77–2.13
Female	166	89	255	1		1	
School level							
Infant (>2 and <7 yrs)	171	40	211	1		1	
Primary (≥7 and <13 yrs)	46	6	52	1.79	0.72–4.49	2.16	0.79–5.93
Secondary and Higher (≥13 and <19 yrs)	37	15	52	0.58	0.29–1.15	0.83	0.32–2.19
Adults (≥19 yrs)	33	65	98	0.12	0.07–0.20	0.14	0.08–0.26
Center							
School	213	88	301	1		1	
Summer camp	74	38	112	0.93	0.80–1.09	1.41	0.54–3.64
Season							
Warm months	83	59	142	1		1	
Cold months	204	67	271	2.16	1.40–3.34	2.13	1.23–3.67
Reporting delay							
≤ 2 days	57	37	94	1		1	
>2 days and ≤4 days	39	19	58	1.33	0.67–2.65	1.95	0.68–5.57
>4 days and ≤9 days	107	43	150	1.61	0.94–2.78	3.07	1.21–7.81
>9 days	84	27	111	2.02	1.11–3.68	3.81	1.42–10.23

[a] OR, raw odds ratios.

[b] aOR, adjusted odds ratios.

TABLE 3 Raw and adjusted odds ratios of the association between the different variables considered and the risk of getting ill in common source outbreaks

Variable	Ill	Not ill	Total	OR[a]	95% CI	aOR[b]	95% CI
Sex							
Male	67	24	91	2.79	1.57–4.97	2.17	0.94–5.01
Female	66	66	132	1		1	
School level							
Infant (>2 and <7 yrs)	12	30	42	1		1	
Primary (≥7 and <13 yrs)	60	0	60	–[c]	–	–	–
Secondary and higher (≥13 and <19 yrs)	26	2	28	32.50	6.65–158.80	44.88	6.70–300.73
Adults (≥19 yrs)	35	58	93	1.51	0.68–3.32	2.11	0.66–6.67
Center							
School	24	59	83	1		1	
Summer camp	109	31	140	2.70	1.9–3.82	9.75	3.35–28.33
Season							
Warm months	81	49	130	1		1	
Cold months	52	41	93	0.77	0.45–1.32	7.42	2.31–23.84
Reporting delay							
≤ 2 days	86	62	148	1		1	
>2 days and ≤4 days	35	25	60	1.01	0.55–1.85	5.38	1.74–16.64
>4 days and ≤9 days	12	3	15	2.88	0.78–10.65	0.40	0.06–2.69
>9 days	0	0					

[a] OR, raw odds ratios.
[b] aOR, adjusted odds ratios.
[c] –, no calculable.

unaffected women responded to the survey more frequently than men, although other authors have found that men are less susceptible than women to norovirus (26). When common source outbreaks and person-to-person transmission outbreaks are analyzed separately, this association was not maintained.

The mean number of people affected in each outbreak (34 people per outbreak) is in line with the results found by Lian et al. in a study conducted between 2014 and 2017 in China (mean of 34 people per outbreak) (27) and is not very different from that described by Wikswo et al. in outbreaks of person-to-person transmission in the US in 2009–2010 (mean of 44 people per outbreak) (24) or from the mean of 40 people per outbreak observed by the same authors in 2009–2013 (28). He et al. found a median of 16 persons affected by outbreaks (range 5 to 148) in Shanghai schoolchildren between June 2016 and December 2017 (11).

In our study, the highest proportion of persons affected was observed in early-years education (43.57%), coinciding with the results found by other authors. O'Brien et al. in a study during 2008 and 2009 in the United Kingdom found a higher incidence in children aged < 5 years than in the other age groups (29). Inaida et al. in a study conducted between 2006 and 2009 in Tokyo observed that the group most affected was children aged 0 to 4 years, followed by those aged 5 to 9 years (30), which seems logical, since young children have greater contact with each other, and hand hygiene is worse. However, in studies of outbreaks in schools and summer camps, other studies have found that primary school children were the most affected group. Lian et al. in China between 2014 and 2017 found that 47.98% of affected schoolchildren were in primary school versus 16.86% in early childhood education (27). Wang et al. found that 54.9% of affected children in Shanghai in 2017 were primary school children compared with 39.73% in early childhood education (25) and He et al. in Shanghai between June 2016 and December 2017 found that 61.08% of affected children were in primary school (11).

In common source outbreaks the risk of becoming ill was higher in summer camps than in schools, which may be explained by the fact that in summer camps all meals are made in common and also consume the same water.

In our study, 52.63% of outbreaks occurred in the fall and winter months (October to March), similar to the 68% found by Steele et al. in outbreaks in the US in 2009 (21). Ahmed et al. in a meta-analysis of 293 studies found that 71% of outbreaks occurred in the cold months of the year (October to March in the Northern hemisphere and April to September in the Southern hemisphere) (14). Other studies have also found this seasonal effect (24, 27).

Outbreaks lasted between 1 and 23 days with a mean of 6.11 days (SD 6.08). Lian et al. found a duration of 7.4 days in outbreaks in secondary schools (27). Cheek et al. describe several outbreaks in summer camps lasting 4 to 9 days (31) and Nygård et al. found a duration of 10 days in a waterborne outbreak in a camp in Norway (32).

The delay in reporting was linearly correlated with a longer duration of outbreaks, a result consistent with that observed by He et al. in the study conducted in Shanghai in 2016 and 2017 (11). The fact that the later the outbreak is reported the longer it lasts suggests that rapid action should be taken to control the spread of outbreaks and shorten their duration. Friesema et al. found that rapid adoption of control measures in norovirus outbreaks in nursing homes decreased both the rate of attack and the duration (33).

In our study, the attack rate showed a decreasing exponential relationship with the size of the groups. A similar phenomenon was observed by Tsang et al., who found that in outbreaks that affected households with fewer cohabitants, attack rates were higher than in outbreaks that affected households with more cohabitants and attributed these results to the fact that contact between cohabitants is lower in homes with a greater number of members (26). Brinkhues et al. studied the association between social groups and the prevalence of some infectious diseases, including AGE. They found that large groups with close relationships (such as groups of friends) had a high prevalence of AGE, while in groups in whom the relationship was not so close, the prevalence of AGE was lower (34). Potter et al. observed the same phenomenon when studying the transmission of influenza in schoolchildren, a disease in which contact between people plays an important role, and indicated that groups of schoolchildren are not homogeneous, so it cannot be assumed that contacts are random (35). The lower attack rate found in our study in larger groups can be explained by the segmentation that occurs in large groups, with the risk being heterogeneous in the different subgroups, something that does not occur in small groups whose members have a similar level of exposure.

Norovirus GII was the most frequently identified genogroup. Although the differences were not statistically significant, the risk of being affected by GI was higher than that of being affected by GII in outbreaks with common source transmission, in contrast to what occurred in outbreaks with person-to-person transmission. These results coincide with those found by other authors. Matthews et al. in a review of norovirus outbreaks between 1983 and 2010 found that 76% of outbreaks in day care centers and schools were caused by GII and that GI was more frequently associated with outbreaks with common source transmission than with those with person-to-person transmission (20).

Our study has limitations. First, possible selection bias among the individuals completing the epidemiological survey, since the number of unaffected respondents is low compared to the number of affected persons (216 and 420, respectively). Second, by conducting separate analyses of outbreaks of person-to-person transmission and common source outbreaks, the number of persons included in the analysis was reduced, with the consequent loss of statistical power.

A strength of the study was that it was an analysis in real conditions of epidemiological surveillance covering all Catalonia over a period of several years.

Conclusions. School level, the type of center and delayed reporting showed distinct associations with the risk of becoming ill in outbreaks of person-to-person transmission and common source outbreaks, suggesting the desirability of analyzing norovirus AGE outbreaks depending on the type of transmission. In outbreaks with a large number of exposed persons, the possible existence of subgroups with nonhomogenous exposure levels should be considered, because the overall attack rate does not reflect the attack rate of the different subgroups. A noteworthy aspect of this study carried out in schools and summer camps is that the delay in reporting was associated with a longer duration of outbreaks, which reinforces the importance of early reporting in this type of centers.

MATERIALS AND METHODS

Prospective study of outbreaks reported in Catalonia from January 2017 to December 2019 in schools and summer camps. The clustering of ≥ 2 cases of AGE in schools or summer camps in which norovirus was identified in clinical samples by real-time semiquantitative reverse transcription PCR (RTqPCR) was considered an outbreak.

Feces were collected to identify norovirus genogroups I, II and IV by RTqPCR. Samples were analyzed at the Microbiology Laboratory, Vall d'Hebron Hospital and Public Health Agency of Barcelona laboratory. The specific primers described by Kageyama et al. were used to detect norovirus GI and GII (36). A modification of the primer described by Farkas et al. (37) and Kageyama et al. (36) was used to detect norovirus GIV.

For each outbreak, the number of exposed and affected people, the type of transmission (person-to-person or by common source), the date of onset and the end of the outbreak and the date when the Epidemiological Surveillance unit was notified were collected. Percentages were compared using a linear trend chi-square test. Attack rates (AR) were calculated considering the total number of affected and exposed persons in the outbreaks and according to the causal genogroup; the rate ratio (RR) and 95% confidence intervals (CI) globally and according to type of transmission were calculated.

To study the associations between sex, age, type of center, delay in reporting and season and the risk of becoming ill, crude odds ratios (OR) and adjusted odds ratios (aOR) were estimated with their 95% CI. To estimate the aOR, multivariate analysis was performed by logistic regression, adjusting using the backward stepwise procedure with a cutoff point of <0.2.

The correlation between the delay in reporting and the duration of the outbreak was estimated using Pearson's correlation coefficient and 95% CI. For studied the relation between the size of the affected group and the attack rate was used a model of logarithmic transformation both the dependent and independent variables (log-log model) and the correlation between the transformed variables was estimated using Pearson's correlation coefficient and 95% IC.

The study was conducted according to the guidelines of the Declaration of Helsinki, regulations of the Public Health Agency of Catalonia and ethical protocols established.

The study was approved by the University of Barcelona Bioethics Commission (Institutional Review Board IRB00003099) on April 12, 2016.

We declare that the Bioethics Committee of University of Barcelona approved the waiver for informed consent.

All data used in the analysis were collected during routine public health surveillance activities as part of the legislated mandate of the Health Department of Catalonia, which is officially authorized to receive, treat and temporarily store personal data in the case of infectious disease. All data were fully anonymized. All study activities formed part of the public health surveillance tasks. Law regulates these activities and informed consent should not be necessary.

Data availability. The data sets generated during the current study are available in the Mendeley Data repository, https://data.mendeley.com/datasets/3ktswshxgv/1.

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7.6.- Articulo 6 (enviado a International Journal of Infectious Diseases): Impact of the COVID-19 pandemic on the incidence of acute gastroenteritis outbreaks in Catalonia (Spain).

Parrón I, Carol M, Bes N, Izquierdo C, Godoy P, Barrabeig I, Sala MR, Minguell S, Ferras J, Rius C, Martínez A, Domínguez A, Working Group for the Study of Outbreaks of Acute Gastroenteritis in Catalonia. Impact of the COVID-19 pandemic on the incidence of acute gastroenteritis outbreaks in Catalonia (Spain)

Título

Impacto de la pandemia de COVID-19 en la incidencia de brotes de gastroenteritis aguda en Cataluña (España).

Resumen

Introducción:

El 11 de marzo de 2020, la Organización Mundial de la Salud declaró la enfermedad causada por SARS-CoV-2 como una pandemia. Al principio de esta pandemia hubo incertezas sobre los mecanismos de transmisión del virus. Se pensaba que la transmisión era por contacto persona a persona, por gotas respiratorias y por fómites y se realizaron recomendaciones para evitar la propagación del virus por estas vías. Estas recomendaciones podían influir en la transmisión de otras enfermedades como las gastroenteritis agudas.

El objetivo de este estudio fue comparar la incidencia y características de los brotes de gastroenteritis aguda en Cataluña de los años 2015 a 2019 (período prepandémico) con las de los brotes de los años 2020 y 2021 (período pandémico)

Material y métodos:

Estudio retrospectivo de los brotes de gastroenteritis aguda notificados entre el 1 de enero de 2015 y el 31 de diciembre de 2021 en Cataluña, una región del noreste de España con una población de 7.543.825 habitantes en enero de 2018.

Resultados:

Observamos una mayor tasa de incidencia de brotes durante el período prepandémico (16,89 brotes/1.000.000 personas-año) que durante el período pandémico (6,96 brotes/1.000.000 personas-año) (razón de tasas 0,41; IC95% 0,34 a 0,51). Al comparar las tasas de incidencia de brote en los períodos prepandémico y pandémico según la etiología del brote, las de etiología viral oscilaron entre 7,82 y 3,38 brotes/1.000.000 personas-año (razón de tasas 2,31; IC95% 1,72 a 3,12) y las de etiología bacteriana entre 5,01 y 2,78 brotes/1.000.000 personas-año (razón de tasas 1,80;IC 95% 1,29 a 2,52).

El modo de transmisión más frecuente fue por vehículo común y la proporción fue mayor en el período pandémico que en el prepandémico (59,05% de brotes por vehículo común en período pandémico vs 54,63% en el período prepandémico, p=0,46). Considerando solo los brotes de etiología viral o bacteriana, la proporción de brotes transmitidos por vehículo común también fue mayor en el período pandémico que en el prepandémico (odds ratio 1,26; IC95% 0,80 a 1,97)

Conclusiones:

Durante la pandemia de COVID-19 se ha producido una gran reducción de brotes de gastroenteritis aguda en Cataluña. Esta reducción puede deberse al efecto de las medidas no farmacológicas aplicadas para reducir la transmisión del SARS-CoV-2, pero también puede haber influido el colapso tanto del sistema sanitario como de los servicios de vigilancia epidemiológica.

TITLE PAGE

Category: Original Article

Impact of the COVID-19 pandemic on the incidence of acute gastroenteritis outbreaks in Catalonia (Spain)

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ABSTRACT

Objective: To compare the incidence of AGE outbreaks in Catalonia from 2015 to 2019 (pre-pandemic period) with that observed during 2020 and 2021 (pandemic period)

Methods: A retrospective study of AGE outbreaks reported between January 1, 2015 and December 31, 2021 in Catalonia, an area in the Northeast of Spain with a population of 7,543,825 inhabitants as of January 2018.

Results: We observed a higher incidence rate of outbreaks during the pre-pandemic period (16.89 outbreaks/1,000,000 person-years) than during the pandemic period (6.96 outbreaks/1,000,000 person-years) (RR 0.41; 95%CI 0.34 to 0.51). When comparing the incidence rates for the pre-pandemic and pandemic periods according to the etiology of the outbreak, those of viral etiology ranged from 7.82 to 3.38 outbreaks/1,000,000 person-years (RR 2.31; 95%CI 1.72 to 3.12) and those of bacterial etiology from 5.01 to 2.78 outbreaks/ 1,000,000 person-year (RR 1.80 95%CI 1.29 to 2.52).

The most frequent mode of transmission was by common vehicle and the proportion was higher in the pandemic period than in the pre-pandemic period (59.05% of outbreaks by common vehicle in pandemic period vs 54.63% in the pre-pandemic period, p=0.46). Considering only the outbreaks of viral or bacterial etiology, the proportion of common vehicle outbreaks was also higher in the pandemic period (OR 1.26; IC95% 0.80 to 1.97)

Conclusions: During the COVID-19 pandemic, there has been a great reduction of AGE outbreaks in Catalonia. This reduction may be due to the effect of the non-pharmaceutical measures applied to reduce the transmission of SARS-CoV-2, but the collapse of the healthcare system and epidemiological surveillance services may also have had a strong influence.

Keywords: SARS-CoV-2, COVID-19, non- pharmacological intervention, social distancing, acute gastroenteritis outbreak, norovirus, enteric pathogen, pandemic

TEXT

On March 11th, 2020, the World Health Organization (WHO) declared the disease caused by the SARS-CoV-2 virus as the COVID-19 pandemic. At the beginning of the pandemic, there was great uncertainty about transmission mechanisms of the virus. Person-to-person contact, respiratory droplet transmission, and fomite-mediated transmission were recognized as the most common routes of SARS-CoV-2 virus spread. It was considered that the disease could be transmitted through aerosols generated during the manipulation of patients' airways [1], although this mode of transmission was considered of little relevance beyond clinical settings [2]. Based on these data, the main WHO recommendation was the implementation of use droplet and contact transmission prevention measures, including hand hygiene [2]. Subsequently, outbreaks of COVID-19 were described in restaurants [3] and gyms [4], which led to various authors to state the possibility aerosol transmission [5,6].

It was expected that measures to prevent transmission by contact would also have an impact on other diseases that share this route of transmission, such as acute gastroenteritis (AGE). The measures to control COVID-19 had already shown a very early impact on the flu season in the southern cone (from February to September 2020) [7] and in other diseases as varicella or mumps [8]

The decrease in the notification was not only observed in those that share transmission mechanisms with SARS-CoV-2. Lai et al. [9] described a 54.8% reduction in the notification of vector-borne diseases in Taiwan between 2019 and 2000.

Globally, in 2019, diarrhea was the eighth leading cause of death in all ages [10]. In 2017, there were approximately 1.7 billion cases of AGE in children [11]. In 2018, one in nine deaths in children was due to diarrhea [12] and in 2019 AGE caused 370,000 deaths in children under 5 years of age, being the second cause of death in this age group [13]. Viral etiology is the main cause of AGE and it is estimated that norovirus has been responsible for 90% of viral gastroenteritis outbreaks [14].

The main mode of transmission of viral AGE is person-to-person, although transmission by common vehicle (food or water) is also feasible [15].

Direct contact between an infected person and a susceptible person plays an important role in the transmission of norovirus [16].

All of this justifies investigating whether the COVID-19 pandemic and preventive measures adopted to curb the transmission of SARS-CoV-2 may have influenced the incidence of AGE outbreaks.

The objective of this study is to compare the incidence of AGE outbreaks in Catalonia from 2015 to 2019 (pre-pandemic period) with that observed during 2020 and 2021 (pandemic period).

MATERIAL AND METHOD

Type of study, period considered and target population

A retrospective study of AGE outbreaks reported between January 1, 2015 and December 31, 2021 in Catalonia, an area in the northeast of Spain with a population of 7,543,825 inhabitants as of January 2018 [17].

Epidemic outbreaks of any etiology must be reported to the Public Health Agency of Catalonia, which carries out an epidemiological study to determine the causes and establish control measures. In the city of Barcelona, these activities are carried out by the Barcelona Public Health Agency.

Definitions

AGE was defined as a sudden onset of diarrhea that may also present nausea, vomiting, abdominal pain, or fever. An AGE outbreak was defined as the involvement of two or more epidemiologically related people, either by person-to-person contact or by a common exposure.

Two periods were considered: a first period from 2015 to 2019 (pre-pandemic period) and a second period from 2020 and 2021 (pandemic period).

Data collection and management

Data were collected by technicians from the Epidemiological Surveillance Services of the Catalan Public Health Agency and the Barcelona Public Health Agency.

Data collection and cleansing were performed using the Access 12.0 database manager of the MS-Office 2013 software package and statistical analysis using the PASW Statistics 18.0.2 statistical package.

Etiological confirmation

The etiological confirmation of outbreaks was carried out by collecting stool samples and their subsequent microbiological analysis at the Vall d'Hebron University Hospital laboratory or at the Barcelona Public Health Agency laboratory. In outbreaks in which viral etiology was suspected, real-time RT-PCR was performed to identify the presence of enteric viruses.

Statistical analysis

The incidence rate of outbreaks per 1,000,000 person-years was calculated for the pre-pandemic and pandemic period. This rate was also calculated for lockdown weeks (from March 14 to June 24, 2020) and for the same weeks from 2015 to 2019 and according to viral or bacterial etiology. Rate ratio (RR) and its 95% confidence interval were calculated to compare the incidence rates of outbreaks.

A chi-square test with Yates correction was used to compare the frequency and transmission mode of outbreaks in the periods studied (overall, according to mode of transmission and according to setting), the odds ratio (OR) and their 95% confidence intervals were calculated assuming a p value <0.05 as the limit of statistical significance. The risk of getting sick in exposed people according to the period (globally, by mode of transmission and in closed and semi-closed institutions) was calculated using the rate ratio (RR) and its 95% confidence interval.

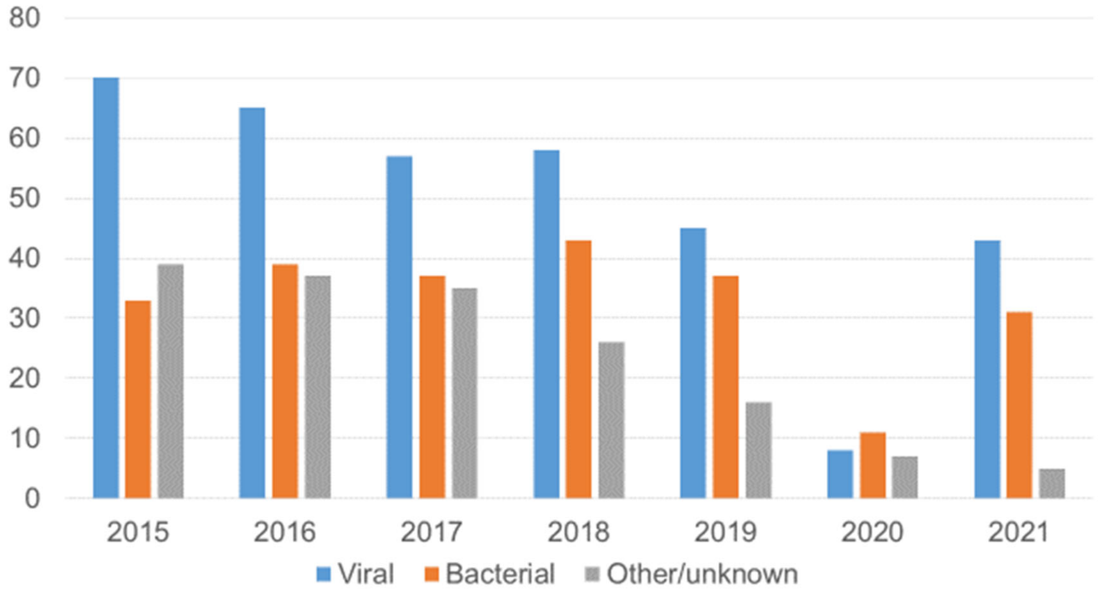
The mean number of people exposed in outbreaks of viral etiology and in those of bacterial etiology that occurred in the pre-pandemic period was compared with the mean number of people exposed in the pandemic period by calculating Student's t-test for the difference between means and its 95% confidence interval.

RESULTS

During the study period, 742 AGE outbreaks were reported: 637 outbreaks in the pre-pandemic period, from 2015 to 2019, (16.88 outbreaks/1,000,000 person-years) and 105 outbreaks in the pandemic period, from 2020 to 2021, (6.96 outbreaks/1,000,000 person-years) [RR of 2.43 (95%CI95: 2.25 to 2.62)] for the pre-pandemic period vs. the pandemic period.

In 346 outbreaks, the etiology was determined to be viral (97.4% norovirus, 2.02% rotarivus, and 0.58% other viruses); 231 outbreaks were of bacterial etiology (44.59% *Salmonella*, 13.85% *Shigella*, 11.69% *Clostridium perfringens*, 10% *Staphylococcus aureus*, 4.32% *Bacillus cereus*, 4.76% *Campylobacter* and 10.82% other bacterial etiologies); 47 outbreaks were due to neither bacterial nor viral causes and in 118 outbreaks etiology was not identified (Figure 1)

Figure 1 Outbreaks of AGE declared according to etiology. Catalonia, 2015 to 2021.



During the entire period studied, norovirus was the most frequently identified etiological agent, causing 54.01% (337/624) of the outbreaks with known etiology (Table 1).

Table 1. Reported outbreaks according to etiology and year of notification.

Year	Norovirus (%)		Rotavirus (%)		Other viruses (%)		Bacterial etiology (%)		Other etiology /Unknown (%)		All
2015	66	46.48	4	2.82	0	0.00	33	23.24	39	27.46	142
2016	65	45.77	0	0.00	0	0.00	39	27.46	37	26.06	142
2017	55	42.64	1	0.78	1	0.78	37	28.68	35	27.13	129
2018	56	44.09	1	0.79	1	0.79	43	33.86	26	20.47	127
2019	44	44.90	1	1.02	0	0.00	37	37.76	16	16.33	98
2020	8	30.77	0	0.00	0	0.00	11	42.31	7	26.92	26
2021	43	54.43	0	0,00	0	0.00	31	39.24	5	6.33	79
Total	337	45.42	7	0,94	2	0.27	232	31.27	165	22.24	742
2015-19	286	44.83	7	1.10	2	0.31	189	29.62	153	23.98	638
2020-21	51	48.57	0	0.00	0	0,00	42	40.00	12	11.43	105

The decrease in incidence rate of outbreaks during the pandemic period compared to the pre-pandemic period was observed for all etiologies. Among those of viral etiology, 295 occurred in the pre-pandemic period (7.82 outbreaks/1,000,000 person-years) and 51 in the pandemic period (3.38 outbreaks/1,000,000 person-years) [RR= 2.31 (95%CI: 1.72 to 3.12)]. Among those of bacterial etiology, 189 outbreaks occurred in the pre-pandemic period (5.01 outbreaks/1,000,000 person-years) and 42 outbreaks in the pandemic period (2.78 outbreaks/1,000,000 person-years [RR=1.80 (95%CI: 1.29 to 2.52)].

During the pandemic period, transmission by common vehicle accounted for 59.05% of the outbreaks (62 of 105 outbreaks), and during the pre-pandemic period this mode of transmission was 54.63% (348 of 637 outbreaks) (p=0.46). During lockdown weeks, from March 14 to June 24, 2020, only 3 common vehicle outbreaks were reported. During the same weeks from 2015 to 2019, 208 outbreaks were reported, 109 (52.40%) by common vehicle and 99 (47.60 %) by person-to-person transmission (p=0.37) (Table 2).

Table 2. AGE outbreaks according to mode of transmission in the pre-pandemic and pandemic periods and during lockdown weeks (March 14 to June 24, 2020).

Period of outbreak occurrence	Common vehicle		Person to person		Total	
	Number of outbreaks	Rate*	Number of outbreaks	Rate*	Number of outbreaks	Rate*
2020 - 2021	62	4.11	43	2.85	105	6.96
2015 -2019	348	9.23	289	7.66	637	16.89
RR (CI95%)**	0.45 (0.34 – 0.58)		0.37 (0.27 – 0.51)		0.41 (0.34 – 0.51)	
From March 14th to 24th of June, 2020	3	1.42	0	NC	3	1.42
From March 14th to 24th of June, 2015-2019	109	10.34	99	9.39	208	19.73
RR (CI95%)**	0.14 (0.04 – 0.43)		NC		0.07 (0.02 – 0.23)	

* Outbreak rate per 1,000,000 persons-year; ** Reference: 2015 to 2019 values; N.C: Not calculable

Outbreak rates per 1,000,000 person-years on the overall and by mode of transmission were higher during the pre-pandemic period than during the pandemic period. The same occurred when lockdown weeks were compared to the same weeks of 2015 to 2019.

When analyzing the outbreaks of viral and bacterial etiology jointly (Table 3), no significant differences were found in the frequency of outbreaks observed according mode of transmission and pre-pandemic o pandemic period (OR 1.26; 95%CI 0.80 to 1.97).

Table 3. Outbreaks of AGE with viral and bacterial etiology according to mode of transmission. Catalonia 2015 to 2021.

	Common vehicle		Person to person		Total	
Year	Number of outbreaks	%	Number of outbreaks	%	Number of outbreaks	OR (95%CI)*
2015	44	42.72	59	57.28	103	0.66 (0.43 to 1.02)
2016	53	50.96	51	49.04	104	0.99 (0.65 to 1.52)
2017	48	51.06	46	48.94	94	1.00 (0.64 to 1.55)
2018	56	55.45	45	44.55	101	1.23 (0.80 to 1.9)
2019	42	51.22	40	48.78	82	1.00 (0.63 to 1.60)
2020	14	73.68	5	26.32	19	2.76 (0.98 to 7.77)
2021	38	51.35	36	48.65	74	1.01 (0.62 to 1.65)
TOTAL	295	51.13	282	48.87	577	
Total 2020-21	52	55.91	41	44.09	93	1.26 (0.80 to 1.97)
Total 2015-19	243	50.21	241	49.79	484	Ref

* Each year is compared to the rest of years. OR: Odds ratio

No significant differences were observed among different years of the study according to the mode of transmission. In the pandemic period (2020 and 2021), transmission by common vehicle was more frequent than during the pre-pandemic period.

In the pre-pandemic period, 225 outbreaks were reported in closed and semi-closed institutions (nursing homes, health institutions, schools and nursing homes) and 412 outbreaks in other areas, while in the pandemic period the number of outbreaks was 56 and 49, respectively (p<0.001).

In closed or semi-closed institutions, the risk of getting sick among exposed people was lower during the pre-pandemic period than in the pandemic period (RR 0.74; 95%CI: 0.71 to 0.77). In the community, the risk was higher during the pre-pandemic period than in the pandemic period in common vehicle outbreaks (RR 1.66; 95%CI1.50 to 1.84) (Table 4).

Table 4. Attack rate and Rate Ratio in the pre-pandemic and pandemic periods according to setting and mode of transmission of the outbreak.

Setting	Mode of transmission	Period	Number of outbreaks	Affected	Unaffected	All	AR	RR (95%CI)
Closed and semi-closed facilities*	Person to person	Pandemic	37	1220	2227	3447	35.39	1
		Pre-pandemic	171	5122	16518	21640	23.67	0.67 (0.64 to 0.70)
	Common vehicle	Pandemic	19	897	1777	2674	33.55	1
		Pre-pandemic	54	2165	4828	6993	30.96	0.92 (0.87 to 0.98)
	All	Pandemic	56	2117	4004	6121	34.59	1
		Pre-pandemic	225	7287	21346	28633	25.45	0.74 (0.71 to 0.77)
Other settings	Person to person**	Pandemic	6	23	23	46	50.00	1
		Pre-pandemic	118	1172	5107	6279	18.67	0.37 (0.28 to 0.50)
	Common vehicle	Pandemic	43	289	722	1011	28.59	1
		Pre-pandemic	294	3181	3509	6690	47.55	1.66 (1.50 to 1.84)
	All	Pandemic	49	312	745	1057	29.52	1
		Pre-pandemic	412	4353	8616	12969	33.56	1.14 (1.03 to 1.25)
All	Person to person	Pandemic	43	1243	2250	3493	35.59	1
		Pre-pandemic	289	6294	21625	27919	22.54	0.63 (0.60 to 0.67)
	Common vehicle	Pandemic	62	1186	2499	3685	32.18	1
		Pre-pandemic	348	5346	8337	13683	39.07	1.21 (1.15 to 1.28)
	All	Pandemic	105	2429	4749	7178	33.84	1
		Pre-pandemic	637	11640	29962	41602	28.00	0.83 (0.80 to 0.86)

*Nursing homes, health institutions, schools and summer camps

The mean number of people exposed in outbreaks of viral etiology during the pre-pandemic period was 100.65 people per outbreak, while in 2020-21 it was 88.22 people per outbreak, being the difference not statistically significant (difference between means of 12.43; 95%CI: -23.93 to 48.79). For bacterial outbreaks, the mean number of exposed was 39.94 in the pre-pandemic period and 68.03 in the pandemic period (difference between means of -28.09; 95%CI: -71.48 to 15.30).

In both viral and bacterial outbreaks, the risk of becoming ill was higher for exposed people during the pandemic period than during the pre-pandemic period (Table 5).

Table 5. Attack rate and Rate Ratio according to mode of transmission in outbreaks of viral and bacterial etiology in the pre-pandemic and pandemic periods.

	Viral etiology			Bacterial etiology		
	Common vehicle	Person to person	All outbreaks	Common vehicle	Person to person	All outbreaks
Period	AR (Affected/Exposed)	AR (Affected/Exposed)	AR (Affected/Exposed)	AR (Affected/Exposed)	AR (Affected/Exposed)	AR (Affected/Exposed)
2020-21	27.05 (267/987)	35.46 (1183/3336)	33.54 (1450/4323)	33.18 (882/2658)	36.51 (23/63)	33.26 (905/2721)
2015-19	42.63 (3192/7487)	22.19 (4460/20997)	27.57 (7852/28484)	33.29 (1448/4350)	21.65 (632/2919)	28.61 (2080/7269)
RT (95%CI)*	0.63 (0.57 to 0.71)	1.60 (1.52 to 1.68)	1.22 (1.16 to 1.27)	1.00 (0.93 to 1.07)	1.69 (1.21 to 2.35)	1.16 (1.09 to 1.24)

AR: Attack rate RR: Rate Ratio *Reference: 2015 a 2019 values

Viral etiology outbreaks was 27.57% in pre-pandemic period and 33.54% in pandemic period (5.97 percentage points) and in bacterial etiology outbreaks was 28.61% in pre-pandemic period and 33.26% in pandemic period (4.65 percentage points) (P<0.001)

DISCUSSION

In our study, we observed a decrease in the notification of AGE outbreaks during the COVID-19 pandemic in Catalonia. The rate decreased from 16.89 outbreaks/1,000,000 person-years in the pre-pandemic period to a rate of 6.96 outbreaks/1,000,000 person-years in the pandemic period. (RR 0.41; 95%CI 0.34 to 0.51). We also observed this decrease in outbreaks during the pandemic period when analyzing separately outbreaks transmitted by a common vehicle (RR 0.45; 95%CI 0.34 to 0.58) or those of person-to-person transmission (RR 0.37; 95%CI 0.27 to 0.51), as well as when comparing lockdown weeks with the same weeks of the pre-pandemic years (RR 0.07; 95%CI 0.02 to 0.23). Other authors have also observed a decrease in the incidence of AGE. Hatun et al. [18] studied the incidence of AGE in children aged 0 to 17 years in Massachusetts from week 13 to 18 of 2019 and in the same weeks of 2020 (weeks after the indications of social distancing measures), observing a decrease in incidence, from 15/100,000 children in 2019 to 1.8/100,000 children in 2020. The authors considered that this decrease could be attributed both to a real decrease in incidence and to a lower frequency of consultations with health services. Armstead et al. [19] examined outpatient visits for AGE in Colorado, finding 9,064 visits in 2020 versus an annual mean of 18,784 from 2017 to 2019. Douglas et al. [20] observed a decrease of 85% in the notification of norovirus to the systems that monitors the activity of this virus in England during the pandemic period and pointed out the risk that a decrease in surveillance conditions pose, especially for a lack of detection of possible replacements of virus variants. Lennon et al. [21]

compared the months of lockdown in the United States in 2020 with the same months in 2019 and observed a reduction in norovirus outbreaks of 90.57%.

The decrease in the rate of AGE outbreaks, both in common vehicle outbreaks and in person to person outbreaks, seems logical, since during the pandemic period there was a significant decrease in social events and less use of collective catering services.

In our study, we observe significant differences between the reduction of outbreaks of viral etiology and the reduction of outbreaks of bacterial etiology. Ondrikova et al. [22] in England between 2015 and 2020 describe that the decrease in notification was greater in norovirus than in *Campylobacter*, indicating as possible causes the prioritization of the analysis of bacterial AGE samples and that the greater severity of the Campylobacter infection entails a greater frequency of consultation with health services. Wang et al. [23] observed in China in the period from 2012 to 2019 an annual average of 5,521 cases of AGE and only 1,772 cases in 2020, with this decrease occurring in all viral and bacterial AGE except those caused by non-typhoid Salmonella and Campylobacter, in those that describe an increase of 66.53% and 90.48%, respectively. Mark et al. [24] in Germany observed a significant decrease in positive samples in patients with viral AGE but not in those with bacterial AGE. In the mentioned study, the decrease in mean samples tested for viruses in the pandemic period compared to the pre-pandemic period was 40.6%, while in samples tested for bacteria the decrease was 27.1%. One possible explanation is that, since viral etiology symptoms are usually less severe than bacterial, during the pandemic period people with viral symptoms either did not consult the health system or were studied less than in the pre-pandemic period**.**

The attack rate was lower in the pre-pandemic period than in the pandemic for all outbreaks (RR 0.83; 95%CI 0.80 to 0.86); in outbreaks of person-to-person transmission, the RR was 0.63 (95%CI 0.60 to 0.67) and in those of transmission by common vehicle, the RR was 1.21 (95%CI 1.15 to 1.28). We have also observed a lower attack rate in the pre-pandemic period than in the pandemic period, both in outbreaks of viral etiology (RT 1.22; 95%CI 1.16 to 1.27) and in those of bacterial etiology (RT 1.16; 95%CI 1.09 to 1.24), particularly in person to person outbreaks. This suggests that, despite the non-pharmacological preventive measures adopted to reduce the transmission of SARS-CoV-2, there were circumstances during the pandemic period that favored a higher risk of transmission of AGE by direct contact, especially in those of viral etiology, that need a lower infective dose (a mean of 18 viral particles for norovirus [25] vs 106 particles in Salmonella [26]).

Our work has some limitations. We cannot rule out that the differences observed in the attack rates were due to the fact that during the pandemic, epidemiology services had less capacity to study outbreaks and determine the number of exposed than before the pandemic. In addition, the situation of collapse in the health services during the pandemic period allows us to assume that outbreaks that in other circumstances would have been notified to the epidemiological surveillance system were not detected nor reported.

CONCLUSION.

The COVID-19 pandemic brought a significant reduction in notifications of AGE outbreaks. This reduction can be attributed to a lower incidence due to the non-pharmaceutical measures applied to prevent the transmission of SARS- CoV-2 or to a reduction in the diagnosis and notification of outbreaks to the epidemiological surveillance services.

It is also possible that affected people did not consult overloaded health services. The lower capacity of surveillance services to investigate reported outbreaks, prioritizing larger outbreaks, may explain the higher attack rate during the pandemic period. Diminished surveillance of the etiological agents causing AGE can have a negative impact on the health of the population, since emerging agents with a special impact on vulnerable groups can go undetected.

DECLARATIONS

Ethics approval and consent to participate

- **Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, regulations of the Public Health Agency of Catalonia and ethical protocols established. The study was approved by the University of Barcelona Bioethics Commission (Institutional Review Board IRB00003099) on April 12, 2016.
- **Informed Consent Statement:** The authors declare that the Bioethics Committee of University of Barcelona approved the waiver for informed consent.

  All data used in the analysis were collected during routine public health surveillance activities as part of the legislated mandate of the Health Department of Catalonia, which is officially authorized to receive, treat and temporarily store personal data in the case of infectious disease. All data were fully anonymized. All study activities formed part of the public health surveillance tasks. Law regulates these activities and informed consent should not be necessary.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions

I.P. and A.D. conceptualized and designed the study, carried out the initial analyses and interpretation of data and drafted the initial manuscript.

M.C., A.M. and C.I. contribued to final analyses and interpretation of data and revised critically the final manuscript.

N.B., P.G., I.B., M.R.S., S.M., J.F. and C.R. carried out acquisition of data and revised critically the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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8.- Discusión

En esta tesis se ha abordado la problemática de los brotes de GEA vírica en diferentes tipos de instituciones cerradas y semicerradas tales como centros geriátricos, guarderías, escuelas y casas de colonias.

El periodo de estudio fue del año 2017 al 2021, si bien los periodos considerados para los diferentes análisis han variado. El análisis de los brotes de GEA víricas en residencias geriátricas se circunscribió a los años 2017 y 2018; para los brotes en centros escolares y casas de colonias se analizaron los años 2017 a 2019 y este mismo periodo fue el seleccionado para el estudio de la afectación de los trabajadores de instituciones en las que se presentó un brote de GEA.

Hemos estudiado más detalladamente dos de los brotes de GEA que se produjeron durante el periodo de estudio, uno en una residencia geriátrica, en el que la etiología fue norovirus, y otro en una guardería, en el que la causa del brote fue astrovirus.

Durante los meses de realización de la tesis se produjo la pandemia de COVID-19 y sus consecuencias fueron muy importantes en instituciones cerradas y semicerradas, especialmente en residencias geriátricas, centros de día y residencias para personas con diversidad funcional, lo que nos llevó a incluir un análisis de la repercusión de la pandemia de COVID-19 en la notificación de brotes de GEA. Para estudiar la posible repercusión el periodo analizado fue el de 2015 a 2021.

El agente etiológico más identificado en los brotes analizados fue norovirus. En residencias geriátricas norovirus fue la causa del 75% de los brotes de GEA. Diversos autores identifican norovirus como el agente causal más frecuente de brotes de GEA vírica en otros países. Se estima que norovirus es la causa del 90% de los brotes de GEA de etiología vírica a nivel mundial (168). De los brotes de GEA notificados en Estados Unidos en los años 2009 y 2010 el 86% fueron por norovirus (169). Inns et al. analizaron 566 brotes de GEA en residencias geriátricas en el noroeste de Inglaterra entre 2016 y 2018, identificando a este agente en el 64% de los brotes (170). En Estados Unidos, de los 7.094 brotes por norovirus entre 2009 y 2017 analizados (69), el 75% ocurrieron en residencias geriátricas y en Noruega se observó que el 77% de los brotes por norovirus entre 2005 y 2008 se presentaron en residencias geriátricas (171).

En centros escolares y casas de colonias sólo un brote de GEA vírica no estuvo ocasionado por norovirus, siendo astrovirus el agente causal. En Estados Unidos, donde se estima que anualmente más de 14 millones de niños son usuarios de casas de colonias y están atendidos por más de un millón y medio de trabajadores (172), el 63% de todos los brotes de GEA en casas de colonias fueron causados por norovirus (157) y en Shanghai en 2016 y 2017 el 87,9% de los brotes por norovirus notificados se produjeron en guarderías y escuelas (159).

El genogrupo de norovirus más identificado fue GII, responsable del 63,2% de los brotes por norovirus en casas de colonias y escuelas y del 70% de los brotes en centros geriátricos. La mayor frecuencia de afectación por genogrupo GII tanto en casos aislados como en brotes o en expuestos asintomáticos ha sido descrita por varios autores. Yu et al. en Incheon (Corea) identificaron GII en el 65% de los manipuladores de alimentos de las escuelas elementales (173). Park et al. observaron que el 75% de las muestras de trabajadores de centros geriátricos donde se habían producido brotes por norovirus eran positivas a GII (174). En un brote en una guardería en Changzhou (China) el 90,5% de los niños asintomáticos estaban infectados por genogrupo GII (175) y de los brotes causados por norovirus en Estados Unidos entre 2009 y 2015, el 81% fueron causados por genogrupo GII (176).

La coinfección por más de un genogrupo en los brotes de GEA no es excepcional y nosotros la hemos detectado en el 13,3% de los brotes en centros geriátricos y en el 7,9% de los brotes en escuelas y casas de colonias. Ushijima et al. en una revisión de brotes por norovirus mediados por alimentos describieron la coinfección por GI/GII en más del 10% de los brotes (177). La presencia de más de un genogrupo de norovirus también se ha detectado en las posibles fuentes de infección de brotes. En Cataluña, en un brote de norovirus originado por consumo de un agua mineral embotellada, norovirus GI y GII fueron identificados tanto en el agua como en las heces de los afectados (178).

Durante el periodo de estudio no se detectó ningún brote por norovirus GIV. Los brotes por norovirus GIV son poco frecuentes, habiendo sido notificados en Holanda en 1998 (179), en Estados Unidos en 1999 y en 2016 (180,181) y en Australia en 2010 (182). Estudios de seroprevalencia en Italia, Estados Unidos y Holanda muestran que se detectan anticuerpos contra este genogupo en frecuencias que van del 19% al 31% de la población (183-185). Posibles explicaciones serían que este genogrupo ocasiona frecuentemente infecciones asintomáticas o que, si bien en los brotes estudiados en esta tesis si se ha investigado la posible etiología de norovirus GIV, algunos laboratorios no estudian con la misma frecuencia este genogrupo que GI o GII (184).

En nuestro estudio los dos genotipos de GII más frecuentes en escuelas y casas de colonias fueron GII.4 y GII.2, aunque también detectamos la presencia de otros genotipos como GII.3 y GII.6. En el brote ocurrido en la residencia geriátrica se identificó GII.17.

En la primera década del 2000 el genotipo GII.4 presentó un importante aumento de actividad a nivel mundial, siendo identificado como agente etiológico de brotes en Australia, Holanda, Japón y Taiwán en 2004 y 2005 (186); desde entonces GII.4 ha causado la mayoría de los brotes de norovirus a nivel mundial, aunque en los últimos años otros genotipos como GII.17 y GII.2 han reemplazado temporalmente a GII.4 en algunas regiones (187,188). En un estudio realizado en Seúl (Corea del Sur) durante el periodo 2014 a 2016 se observó que en casos esporádicos de GEA el genogrupo más frecuente fue GII (93,7% de las muestras analizadas) y de estos GII.4 representó el 54,5% de las muestras, GII.17 el 19,4% y GII.3 el 10,7% (189). En un estudio realizado por Moreno et al. en brotes por norovirus en residencias geriátricas de Barcelona entre 2010 y 2012 se observó que el genotipo más frecuentemente identificado era GII.4 (190)

Norovirus GI, aunque en menor frecuencia, también fue identificado en los brotes en escuelas y casas de colonias, así como en el brote que describimos en una residencia geriátrica. El genotipo más frecuente fue GI.3 (que también fue el identificado en el brote que afectó a la residencia geriátrica). Con menor frecuencia también se identificaron GI.4 y GI1. La infección por genotipo GI.3 generalmente ocurre en niños de guarderías y educación primaria (191)

El genotipo GI.3 ha sido observado también por otros autores como el GI más frecuente. En Taiwán entre 2015 y 2019 se notificaron 2121 brotes de GEA de los cuales el 16,5% fueron producidos por norovirus GI, siendo GI.3 el más frecuente (el 36,8% de los GI), seguido de GI.4 (21,7%) y GI.2 (18,5%); el incremento de identificaciones de GI.3 en este país fue especialmente importante en 2018 (191).

En los brotes cuyo modo de transmisión fue por vehículo común, el riesgo de enfermar entre los expuestos fue mayor si el brote fue por norovirus GI que si lo fue por GII. Estos resultados coinciden con lo encontrado por otros autores. Matthews et al., en una revisión de brotes de norovirus entre 1983 y 2010 observó que GI se asociaba más frecuentemente a los brotes transmitidos por vehículo común que a los transmitidos de persona a persona (192)

En el año 2017 nos fue notificado un brote de GEA de posible etiología vírica en una guardería, identificándose astrovirus tanto en las heces de asistentes sintomáticos como en las de asistentes asintomáticos. Los serotipos identificados fueron HAstV-4 y HAstV-8. Es conocida la importancia de astrovirus como etiología de casos esporádicos de GEA en niños pequeños. Un estudio sobre casos de gastroenteritis en niños menores de 5 años en zonas rurales de China identificó astrovirus entre el 1,7% y el 4% de las muestras positivas (193). Similar proporción (1,8%) se observó en Francia en niños de la misma edad hospitalizados por GEA esporádica (98). En un estudio realizado en Italia se identificó astrovirus en el 18,9% de los niños de 3 años de edad hospitalizados por GEA adquiridas en la comunidad (194).

Los brotes por astrovirus se presentan con menor frecuencia que los producidos por norovirus. En un estudio sobre brotes de GEA en diferentes ciudades españolas realizado por Buesa et al. (51) entre 2000 y 2001 observaron que el 56% de los brotes fueron de etiología vírica, siendo todos ellos causados por norovirus. En otro estudio realizado por Torner et al. (195) de brotes de GEA notificados en Cataluña entre 2010 y 2011 se observó que norovirus fue la causa del 98% de los brotes con etiología, conocida mientras que ninguno pudo atribuir a astrovirus.

En todos los ámbitos en que se han producido brotes de GEA hemos observado unas altas tasas de ataque entre las personas expuestas que oscilaban entre el 25,1% en escuelas y casas de colonias y el 38,8% en trabajadores considerados globalmente.

En el brote por norovirus que estudiamos en una residencia geriátrica, las tasas de ataque fueron muy elevadas, tanto globalmente (33%) como entre usuarios (23,5%) y entre trabajadores (46,2%). Lindsay et al. (150), en una revisión sistemática de brotes por norovirus en residencias geriátricas de países de ingresos medianos y altos observaron unas tasas de ataque de hasta el 45%. Otros autores también han descrito tasas de ataque elevadas en trabajadores y usuarios de residencias geriátricas. Green et al. (196) estudiaron 20 brotes de GEA por norovirus en residencias geriátricas de Maryland y encontraron tasas de hasta el 59% entre residentes y de hasta el 26% entre trabajadores y Utsumi et al. (197) en una revisión de brotes en centros geriátricos describieron medias de tasas de ataque del 45% en residentes y del 42% en trabajadores.

En esta tesis hemos analizado el conjunto de brotes de GEA ocurridos en centros geriátricos notificados a la Agència de Salut Pública de Catalunya durante los años 2017 y 2018. De dichos brotes, el mayor porcentaje (75%) fueron de etiología vírica, siendo norovirus el agente identificado en todos ellos. Inns et al. (170) estudiaron 566 brotes de GEA en residencias geriátricas del noreste de Inglaterra entre 2016 y 2018, observando que en el 71,1% de los brotes con etiología conocida el agente causal identificado era un virus. Davis et al. (198) estudiaron 60 brotes de GEA en 2008 en centros residenciales de Queensland (Australia) y detectaron etiología vírica en el 70,1% de ellos, de los que norovirus supuso el 97,5%.

En nuestro estudio, la tasa de ataque global observada fue del 25,9% y las específicas para usuarios y para trabajadores del 32,2% y del 14,1%, respectivamente. Sin embargo, cuando analizamos separadamente las residencias de menos de 100 usuarios y las de 100 o más usuarios observamos que tanto la tasa de ataque global como la de residentes y trabajadores separadamente fueron mayores en las residencias pequeñas. Esta relación inversa entre tasa de ataque y tamaño del centro también la observamos en brotes de GEA en escuelas y casas de colonias. Una posible explicación de las menores tasas observadas cuando se estudian en conjunto todas las residencias puede ser que en los brotes en residencias grandes no se comunican todos los casos incidentes sino solo aquellos con mayor sintomatología, lo que no ocurriría en las residencias pequeñas en las que los cuidadores serian más propensos a comunicar todos los casos incidentes. Otra posible explicación, tanto en relación al tamaño de la residencia como al de las escuelas y casas de colonias, es que en grupos muy grandes pueden existir subgrupos con diferente grado de afectación; en dichas circunstancias la tasa de ataque del total del grupo puede ser baja, pero al calcular las tasas de ataque en subgrupos específicos pueden aumentar. Así, Li et al. (199) en 2017, en un brote que afectó a un centro escolar de 3.053 escolares de 6 a 15 años de edad de la ciudad de Shenzen (China), observaron una tasa de ataque de 3,2%, pero la afectación fue muy diferente entre las diversas clases, observando tasas de ataque del 28,1% y de 23,8% en algunos subgrupos específicos (los contactos de casos sintomáticos y las personas que tocaron el grifo de agua del centro, respectivamente).

En el brote por astrovirus que afectó a una guardería observamos una tasa de ataque global del 20,0%, siendo del 48,3% en los niños de 1 año de edad. El número de trabajos publicados sobre brotes por astrovirus en guarderías es limitado. Mitchell et al. (104) estudiaron las muestras de heces de niños de 6 a 30 meses de edad procedentes de 70 brotes de GEA en guarderías en los que no se había identificado previamente el agente causal, detectando astrovirus en 73 de las 217 muestras (34%) analizadas. Konno et al. (200) describieron un brote en un centro de enseñanza primaria que afectó a escolares de 5 y 6 años en Japón, siendo la tasa de ataque del 54,2%. En escolares de enseñanza secundaria Lui et al. (201) describen un brote en China en 2017 con una tasa de ataque de 17,6%. En la misma ciudad y el mismo año, Tan et al. (108) estudiaron un brote en la ciudad de Wuzhou (China) en el que enfermaron 125 niños de un total de1.476 escolares expuestos, lo que supone una tasa de ataque de 8,5%. También en 2017, Li et al. (199) observaron una tasa de ataque del 3,2% en un brote que afectó a escolares de 6 a 15 años de edad de la ciudad de Shenzen (China). Hwang et al. (109) describen un brote en un campamento de policías en Corea en el que participaban 98 personas, siendo la tasa de ataque del 10%.

La edad de las personas expuestas sin duda es relevante, ya que astrovirus en la comunidad afecta principalmente a niños pequeños y es lógico que las tasas de ataque sean mayores en aquellos colectivos de menor edad.

También estudiamos los brotes de GEA víricas ocurridos en Cataluña en centros escolares y casas de colonias en el periodo 2017-2019, siendo la etiología norovirus en todos los brotes excepto en el brote por astrovirus mencionado anteriormente. El 37,6% del total de brotes de GEA vírica se produjeron en este tipo de instituciones y la proporción es similar a la descrita en otras fuentes. Así, en Inglaterra el 22% de los brotes por norovirus entre 2015 y 2020 se produjeron en centros educativos (202) y probablemente esta proporción habría sido mayor si no incluyera los datos de 2020, año en el que la pandemia por SARS-CoV-2 redujo significativamente la actividad escolar.

De los brotes analizados, 19 ocurrieron en escuelas y otros 19 en casas de colonias. La tasa de ataque global fue de 25,1% y las tasas de ataque observadas fueron elevadas tanto en escuelas (21,2%) como en casas de colonias (33,4%), pero inferiores a las observadas en residencias geriátricas. Estas tasas de ataque son similares a las observadas por otros autores. Matthews et al. (192) en una revisión sistemática de publicaciones sobre brotes por norovirus entre 1993 y 2011 observaron una tasa de ataque del 28% en brotes en escuelas y guarderías.

En esta tesis también hemos estudiado específicamente la afectación de los trabajadores de centros geriátricos (49.5% de los brotes), casas de colonias (el 22,2% de los brotes), escuelas (el 13,1% de los brotes), guarderías (el 6,1% de los brotes) y centros de larga estancia (el 5,1% de los brotes). La tasa de ataque global en los trabajadores fue de 38,8%, aunque fue más elevada en los trabajadores de centros de larga estancia, centros geriátricos y guarderías que en los trabajadores de hoteles, casas de colonias y escuelas. Estas tasas son más elevadas que la de 10,5% descrita por Wu et al. (153) en un estudio en Shanghai en trabajadores de establecimientos en los que se habían producido brotes de norovirus entre 2015 y 2017. Sabrià et al. (203) en un estudio entre manipuladores de alimentos y trabajadores sanitarios en Cataluña entre 2010 y 2012 observan una tasa de ataque global del 22,3%, siendo del 15,8% en manipuladores de alimentos y del 49,3% en trabajadores sanitarios.

Las m tasas de ataque mas elevadas observadas en nuestro estudio pueden atribuirse a que los estudios citados no se limitaban a instituciones cerradas o semicerradas, donde la transmisión puede ocurrir más fácilmente que en otros tipos de instituciones.

En nuestro estudio hemos observado que las tasas de ataque en mujeres fueron superiores que en hombres. Pudimos observarlo en el análisis del brote por astrovirus en una guardería, en el de los trabajadores de las instituciones estudiadas y en el de los brotes por norovirus en residencias geriátricas, aunque en ninguno de estos estudios las diferencias en las tasas de ataque entre sexos fueron estadísticamente significativas. Sin embargo, en el estudio en centros escolares y casas de colonias las tasas de ataque fueron superiores en hombres que en mujeres, siendo en esta ocasión las diferencias estadísticamente significativas, si bien la asociación desapareció al analizar separadamente los brotes de transmisión persona a persona y los de transmisión por vehículo común, lo que sugiere que el tipo de transmisión puede actuar como variable de confusión. Diversos autores observan mayor afectación de mujeres que hombres. Wikswo et al. (204) en un estudio en Estados Unidos en 2009 y 2010 observaron que el 71% de las personas afectadas en brotes por norovirus eran mujeres. Sarvikivi et al. (205) en un brote de GEA por norovirus que afectó a escolares de diferentes centros educativos de Finlandia obtienen una mayor tasa de ataque estadísticamente significativa en mujeres que en hombres. Igualmente, Tsang et al. (206) en un brote por norovirus en un área semi-urbana del sur de China describen tasas de ataque más elevadas en las mujeres que en los hombres.

Por el contrario, Wang et al. (207) en un estudio de brotes por norovirus en escuelas de Shanghai en 2017 observaron que el 52,1% de los afectados eran hombres, si bien en dicho estudio no se aportan las tasas de ataque por sexo. La disparidad de resultados tanto en nuestros estudios como en los estudios realizados por otros autores indican que influencia del sexo en las tasas de ataque ha de ser objeto de nuevos estudios.

En los diferentes estudios que conforman esta tesis el análisis de la influencia de la edad está limitado por el hecho de que esta variable viene condicionada por el tipo de institución estudiada.

Tan solo en el brote por astrovirus en que investigamos la afectación de los contactos domiciliarios de los usuarios y trabajadores pudimos analizar grupos de expuestos de todas las edades, tanto niños como adultos. En este brote el grupo de edad más afectado fue el de 1 año de edad y entre los contactos domiciliarios los grupos de edad más afectados fueron los de 0 a 5 años y de 6 a 10 años; las tasas de ataque entre los adultos fueron inferiores a las observadas en estos dos grupos.

Astrovirus suele afectar a niños menores de 3 años (83,86,93) y son la tercera causa de GEA vírica en este grupo de edad (94,97). En el estudio de Olortegui et al. (208) en el que siguieron a 2082 niños de 17 días a 2 años de edad en ocho países observaron que el 26,4% de los niños se infectaban al menos una vez por astrovirus en su primer año de vida y el 35,2% a los 2 dos años de edad.

Un aspecto a destacar de esta tesis es que tanto en el brote que se produjo en un centro geriátrico como en el que se produjo en una guardería se estudió la aparición de casos secundarios entre los convivientes domiciliarios de los trabajadores y de los niños de la guardería. El estudio del brote en la guardería, hasta donde sabemos, fue el primer estudio publicado con circulación de varios serotipos de HAstVs de transmisión de persona a persona en el que se invetigó la extensión del brote a los contactos domiciliarios.

La transmisión secundaria entre los convivientes domiciliarios de brotes en guarderías había sido descrita para norovirus (209), pero no para astrovirus. Un 26,9% de los hogares de los niños y trabajadores de la guardería presentaron casos secundarios y la tasa de ataque global entre los contactos domiciliarios de los asistentes a la guardería fue del 17,2%, siendo las tasas de ataque más elevadas las de los contactos domiciliarios de trabajadores y de niños de 1 año de edad (33,3% y 29,2%, respectivamente).

Entre los contactos domiciliarios de trabajadores de centros geriátricos las tasas de ataque fueron del 22,9%.

Las tasas de ataque obtenidas en contactos domiciliarios, tanto de trabajadores como de asistentes a la guardería, revelan una gran transmisión de los virus más allá de la institución.

Las personas que presentan síntomas tienen un mayor riesgo de producir una transmisión secundaria del virus entre sus contactos domiciliarios. Entre los contactos domiciliarios de las personas asistentes a la guardería que enfermaron se observó una tasa de ataque del 74,3%, mientras que en los contactos domiciliarios de las personas del centro que no enfermaron la tasa de ataque fue del 2,4%. En el estudio del brote ocurrido en la residencia geriátrica la tasa de ataque en los contactos domiciliarios de los residentes que enfermaron fue de 38,1%, mientras que fue del 3,6% en los contactos domiciliarios de los residentes que no enfermaron.

Alfano-Sobsey et al. (210), en un brote por consumo de ostras en un restaurante describieron una tasa de ataque del 14% entre los contactos domiciliarios de las personas que presentaron un cuadro clínico de GEA compatible con norovirus. En el estudio de Matsuyama et al. (211) en los contactos domiciliarios de casos relacionados con un brote de GEA por norovirus la tasa de ataque global fue del 13,8% y del 25,8% en el grupo de edad de 0 a 14 años de edad.

Las diferencias en las tasas de ataque entre los contactos domiciliarios de trabajadores que enfermaron respecto a los que no enfermaron también se ha observado en otros estudios publicados. Sukhrie et al. (212) estudiaron cinco brotes de GEA por norovirus en dos centros geriátricos y un hospital de Rotterdam y observaron que los trabajadores asintomáticos tenían un escaso papel en la transmisión a pesar de encontrar altas cargas virales.

El modo de transmisión más frecuente en los brotes analizados ha sido el de persona a persona. En los brotes en residencias geriátricas se presentó en el 90% de los brotes y sólo el 10% fue por vehículo común. Estos resultados son similares los observados por Kroneman et al. (213) en un estudio de brotes por norovirus en 13 países europeos entre julio de 2001 y junio de 2006 en el que la transmisión persona a persona se produjo en el 88% de los brotes, siendo el 10% vehiculados por alimentos y el 2% por alimentos y agua. Lian et al. (168), en un análisis de brotes por norovirus en China de 2014 a 2017 observaron que el 77% de los brotes fueron por transmisión de persona a persona, el 6% por alimentos, el 4% por agua y el 13% tuvieron múltiples modos de transmisión. Chen et al. (214) en una revisión de brotes en centros geriátricos observaron que la transmisión persona a persona se produjo en el 90% de los brotes y relacionaron esta alta proporción con el estrecho contacto que se produce en este tipo de instituciones entre residentes, trabajadores y visitantes.

En esta tesis hemos observado que en centros escolares y casas de colonias el modo más frecuente de transmisión ha sido el de persona a persona (el 60,5% de los brotes). Estos resultados coinciden con los de Kambhampati et al. queanalizaron 229 brotes de GEA en este tipo de instituciones notificados al National Outbreak Reporting System de Estados Unidos (de los que el 63% fueron por norovirus), observando que en el 53% de todos los brotes la transmisión fue de persona a persona (157).

En esta tesis en los brotes en centros geriátricos en que la transmisión fue de persona a persona la tasa de ataque global fue de 25,1% (32,5% en residentes y 10,6% en trabajadores) y en los brotes por vehículo común la tasa de ataque global fue del 33,2% (29% entre residentes y 39% entre trabajadores). En centros educativos y casas de colonias la tasa de ataque fue de 19,2% en los brotes de transmisión persona a persona y de 33,8% en los brotes por vehículo común. Estas tasas son similares a las descritas por otros autores. Matthews et al. (192) en una revisión de 902 brotes notificados entre 1993 y 2011, tanto de ámbito comunitario como en instituciones cerradas y semicerradas, observaron unas tasas de ataque en los brotes de transmisión de persona a persona del 27% (similar a la descrita por nosotros en centros geriátricos) pero del 50% en los brotes transmitidos por vehículo común; Jiang et al. (215) observaron una tasa de ataque de 39,7% en un brote vehiculado por alimentos que afectó a 541 estudiantes de cuatro institutos en Taiwan.

En el único brote con transmisión hídrica detectado en nuestro estudio, que se presentó en una casa de colonias, la tasa de ataque fue del 64,1% (216), claramente superior a las observadas en los brotes con transmisión de persona a persona o vehiculados por alimentos. Los brotes de transmisión hídrica comunicados por otros autores también presentaron un importante número de afectados (217,218) y elevadas tasas de ataque. Ter Waarbeek et al. (219) describen un brote por norovirus GI y GII en una casa de colonias relacionado con el consumo de agua de un pozo en el que se observó una tasa de ataque del 85% y Arévalo et al. (220) describieron un brote de norovirus GI en un grupo de 119 niños durante una excursión en el que la tasa de ataque fue del 77%.

La comorbilidad y el grado de dependencia de los expuestos en residencias geriátricas también se ha analizado en esta tesis. Hemos observado que las personas con dependencia total o grave, medida mediante el índice de Barthel, presentaron una tasa de ataque de 85,16% mientras que en aquellos con dependencia moderada, baja o no-dependencia fue de 69,13% (RR 1,23; IC95% de 1,05 a 1,45). Petrignani et al. (154) en un metaanálisis realizado en 2014 de 40 brotes de GEA por norovirus en residencias geriátricas observan que los residentes con niveles de dependencia altos y medios presentan mayores tasas de ataque que el resto de residentes, aunque las diferencias no fueron estadísticamente significativas.

De lo observado en los tres puntos anteriores (modo de transmisión, afectación según actividad laboral e influencia del grado de dependencia en el riesgo de enfermar) se puede interpretar que un contacto directo, prolongado y próximo entre los expuestos en un brote implica un mayor riesgo de transmisión; incluso en aquellos brotes en que originariamente la transmisión fue por vehículo común se presentaron casos secundarios en personas no expuestas debido a una posterior transmisión de persona a persona.

Los trabajadores de las instituciones estudiadas presentaron mayor riesgo de enfermar cuando el brote se transmitió de persona a persona que cuando lo hizo por vehículo común (RR 1,93; IC95% de 1,07 a 3,49). En el estudio del brote en una residencia geriátrica (articulo 1 de esta tesis) los trabajadores sanitarios y gerocultores de residencias geriátricas presentaron un riesgo de enfermar significativamente superior al del resto de trabajadores, mientras que ser trabajador de cocina se comportó como factor de protección. Petrignani et al. (154) en un metaanálisis sobre brotes por norovirus en residencias geriátricas también observaron que los trabajadores con mayor contacto con los usuarios presentaban mayores tasas de ataque. La tasa de ataque en gerocultores en nuestro estudio ltores fue de 50,79%, inferior a la observada por Godoy et al. (221) en un brote en una residencia asistida en que dicha tasa del 56,5% y superior a la de 35,8% observada por González Moran et al. (222), si bien en este último estudio no se diferencia entre gerocultores y el resto de trabajadores sanitarios. En nuestro estudio los trabajadores de la limpieza presentaron una tasa de ataque de 41,18% y en los estudios de Godoy et al. y de González Morán et al. las tasas de ataque en este colectivo de trabajadores fueron de 55,5% y 40,0%, respectivamente.

Un aspecto a destacar de esta tesis es la posible influencia del tamaño del grupo de expuestos en la magnitud de la tasa de ataque. Hemos observado en el quinto estudio de esta tesis una relación entre el tamaño del grupo y las tasas de ataque, siendo esta relación inversa y exponencial (cuanto mayor es el tamaño del grupo, menor es la tasa observada). Hemos podido encontrar pocos estudios de brotes de GEA vírica que analicen este aspecto. Tsang et al. (206) observaron en un brote por norovirus que las tasas de ataque en domicilios con pocos contactos eran superiores a las observadas en domicilios con más contactos y atribuyeron el resultado al hecho de que la interrelación entre los contactos de los domicilios con más convivientes es menor a la existente en los domicilios con menos convivientes. Brinkhues et al. (223) estudiaron el efecto entre las relaciones sociales y determinadas enfermedades infecciosas, incluidas las GEA, observando que los grupos grandes con contactos estrechos entre sus miembros (como los grupos de amigos) tienen altas prevalencias de GEA, mientras que en los grupos con relaciones sociales no tan estrechas las prevalencias son menores. Petter et al. (224) observan un fenómeno similar en la transmisión de la gripe en escolares y lo atribuyen a que los grandes grupos no son homogéneos y a que los contactos entre sus miembros no son aleatorios. Las menores tasas de ataque observadas en los grupos grandes podrían estar enmascarada por la existencia de subgrupos con elevadas tasas y subgrupos con tasas pequeñas o nulas.

Estas diferencias en las tasas de ataque no se presentarían en los grupos pequeños, donde todos los miembros están expuestos a niveles similares de exposición.

En los brotes estudiados hemos identificado el virus responsable del brote también en muestras de heces procedentes de personas asintomáticas. La presencia del virus en asintomáticos es común, incluso entre personas en las que no hay una exposición conocida. Qi et al., en un metaanálisis de publicaciones sobre infecciones asintomáticas por norovirus, obtuvieron una prevalencia de la infección del 7% en la población general (225). Yu et al. observan que el 3,3% de los manipuladores de alimentos no relacionados con brotes presentan infección asintomática (173) y Okabayashi et al. describen con una frecuencia superior al 12% las infecciones asintomáticas detectadas entre trabajadores (226).

La presencia del virus en las heces de personas asintomáticas, y que por lo tanto seguirán realizando sus actividades habituales, puede tener una gran importancia en la expansión de los brotes en las instituciones afectadas. La proporción de personas con infección asintomática puede ser elevado entre los trabajadores de las instituciones afectadas por brotes de GEA. Wu et al. observaron que el 17% de los trabajadores donde se habían producido brotes estaban infectados (153) y en el trabajo de Qi et al. (225) dicha proporción fue del 18%.

La presencia de personas asintomáticas en las que se identificó el agente causal en sus heces no se limitó a los brotes por norovirus; en el brote por astrovirus que estudiamos en una guardería también se identificó el virus en muestras de heces de personas asintomáticas.

El análisis de la carga viral de los brotes en que este dato estuvo disponible mostró que la carga fue superior en las personas que presentaban síntomas que en las personas asintomáticas, lo que concuerda con lo observado por otros autores. Costantini et al. (227) en un estudio prospectivo en 43 centros geriátricos en Orengon (Estados Unidos) entre 2009 y 2013 observaron en todos los brotes en que se analizó la carga viral un mayor número de copias de RNA vírico en las heces de los casos sintomáticos que en los infectados asintomáticos y Kabue et al. (228) observan el mismo fenómeno para norovirus GII en casos aislados de GEA en una comunidad rural en Sudáfrica en los años 2014 y 2015, aunque no encontraron diferencias significativas en la carga viral de los sintomáticos y los asintomáticos en los que se detectó GI. Shioda et al. (229) en el estudio de muestras procedentes de brotes de GEA en América Latina y Estados Unidos, observaron que las cargas virales en los brotes por norovirus GII eran superiores en sintomáticos que en asintomáticos, pero las diferencias no fueron significativas en los brotes por norovirus GI.

Hay que destacar que en personas asintomáticas también se detecta presencia de virus, por lo que se considera fundamental establecer recomendaciones de extremar las medidas de higiene personal y ambiental a todos los trabajadores y residentes de las instituciones, independientemente de que presenten manifestaciones clínicas o no.

Finalmente, en nuestro último artículo hemos analizado como la pandemia de COVID-19 ha repercutido sobre los brotes de gastroenteritis víricas comparando los brotes de GEA notificados durante los años 2020 y 2021 con los de los años 2015 a 2019. Puesto que ha habido una importante disminución del número de brotes notificados durante el periodo pandémico respecto al periodo prepandémico, no nos hemos limitado a los brotes aparecidos en instituciones cerradas y semicerradas, sino que hemos analizado todos los brotes de GEA que se han producido en Cataluña en cualquier ámbito.

Las tasas de incidencia han disminuido con la pandemia, pasando de 16,89 brotes/1.000.000 personas-año en el periodo 2015-2019 a 6,96 brotes/1.000.000 personas-año en 2020-2021 (RR 0,41; IC95% 0,34 a 0,51). Otros autores han descrito disminuciones en el mismo sentido. Hatoun et al. observaron una disminución de la incidencia de GEA en Massachusetts en niños de 0 a 17 años, pasando del 15/100.000 niños en 2019 a 1,8/100.000 niños en 2020 (230). Douglas et al. observaron una disminución del 85% en las notificaciones de GEA por norovirus en Inglaterra durante el periodo pandémico respecto a los 5 años previos (231).

A pesar de las medidas adoptadas para evitar la transmisión de SARS-CoV-2 en instituciones cerradas y semicerradas en Cataluña (232,233), el riesgo de enfermar entre las personas expuestas en los brotes de GEA fue mayor durante el periodo pandémico que durante el periodo prepandémico (RR 0,74; 95%CI 0,71 a 0,77). Por el contrario, en los brotes que se produjeron fuera de las instituciones el riesgo de enfermar en Cataluña fue mayor en el periodo prepandémico que en periodo pandémico (RR 1,66; IC95% 1,50 a 1,84). Es lógico este mayor riesgo en la comunidad durante el periodo prepandémico, ya que las restricciones que se indicaron durante la pandemia supusieron una disminución de las interacciones sociales. Es más difícil de interpretar el mayor riesgo en periodo pandémico en las instituciones cerradas y semicerradas, ya que las medidas adoptadas para evitar la transmisión de SARS-CoV-2 deberían haber disminuido también la transmisión de virus productores de GEA y repercutido no solo en una disminución del número de brotes sino en una disminución de las tasas de ataque. Es posible que durante la pandemia las instituciones que tuvieron un mayor riesgo de presentar un brote de GEA fueran aquellas con personal gerocultor insuficientemente formado o desbordado por las circunstancias.

En nuestro estudio hemos observado una mayor reducción en el periodo pandémico respecto al periodo prepandémico en los brotes de etiología vírica que en los de etiología bacteriana. Otros autores también han descrito este fenómeno. Ondrikova et al. (234) en Inglaterra entre 2015 y 2020 describen una mayor disminución de notificaciones de casos por norovirus en 2020 que de casos por *Campylobacter*. Wang et al. (235) en un estudio realizado en China compararon la incidencia de GEA en el año 2020 respecto al periodo de 2012 a 2019 y observaron una disminución tanto de las bacterianas como de las víricas, excepto para *Salmonella* no tifoidea y *Campylobacter,* que aumentaron un 66,5% y un 90,5% respectivamente. Una posible explicación sería que, puesto que las GEA víricas suelen ser menos graves que las bacterianas, durante la pandemia las personas con GEA de etiología vírica o no consultaron al sistema sanitario o fueron estudiadas menos que en el periodo prepandémico.

Los datos que se aportan en esta tesis indican que la pandemia de COVID-19 ha producido una reducción significativa de la notificación de brotes de GEA. Esta reducción puede ser debida a múltiples factores. La adopción y seguimiento de medidas no farmacológicas para prevenir la transmisión de SARS-CoV-2 sin duda ha incrementado la higiene de manos y ha limitado los contactos interpersonales, pero también puede haberse producido una disminución en el diagnóstico y en la notificación de los brotes a los servicios de vigilancia epidemiológica, así como una disminución de las consultas por cuadros clínicos de GEA, especialmente de aquellos pacientes que presentaban una clínica más leve.

En la realización de esta tesis nos hemos encontrado con algunas limitaciones.

Los datos para la realización del artículo sobre el impacto de la pandemia de COVID-19 en la notificación de brotes de GEA provienen de los informes realizados por los servicios de vigilancia epidemiológica de la Agència de Salut

Pública de Catalunya y de la Agència de Salut Pública de Barcelona. La utilización de informes, que es una herramienta descriptiva del brote, no está pensada para la obtención de variables uniformes y tabuladas de todos los brotes, lo que implica que en ocasiones las variables objeto de estudio o no están recogidas o lo están de forma parcial.

Otra limitación para el estudio de la transmisión secundaria en los domicilios de las personas asistentes a una institución donde ha habido un brote es que no hemos tenido la posibilidad de obtener muestras de heces de los contactos domiciliarios, por lo que la relación de los afectados en los domicilios con el brote en estudio se ha realizado por vínculo epidemiológico, sin que se haya podido tener una confirmación microbiológica de dicha relación.

También hemos de considerar una limitación el hecho de que en los informes de brotes utilizados se recoge de una manera más exhaustiva la información sobre las personas sintomáticas que sobre las asintomáticas, lo cual dificulta la comparación de ambos colectivos.

Una última limitación es que muchos de los cuadros clínicos de gastroenteritis producidos por virus son de escasa gravedad, lo que puede provocar que los afectados no consulten al sistema sanitario y que, por lo tanto, o bien el brote no llegue a detectarse o bien el número de afectados contabilizados sea menor que el real.

La principal fortaleza del este trabajo es que los datos provienen de un sistema de vigilancia que cubre toda la población de Cataluña y en el que los médicos asistenciales deben comunicar de forma obligatoria cualquier sospecha de brote de GEA que se detecte.

En el futuro se ha de profundizar en el estudio de las características de las personas expuestas que las hacen especialmente susceptibles a desarrollar la enfermedad y, en especial, los factores de riesgo relacionados con la aparición de formas graves de enfermedad. A partir de estos conocimientos se podrá determinar que grupos han de ser especialmente protegidos en situación de brote en una institución cerada o semicerrada y determinar que personas serían candidatas para la aplicación de determinadas medidas preventivas como son las vacunas, tanto las ya existentes frente a rotavirus como también las vacunas de las que podamos disponer en un futuro próximo frente a otros agentes como norovirus.

9.- Conclusiones.

1. Los brotes de gastroenteritis vírica son un importante problema de salud en instituciones cerradas y semicerradas, tanto por su nivel de incidencia como por las altas tasas de ataque que ocasionan y la vulnerabilidad de las personas usuarias de algunas de estas instituciones.

2. El agente causal identificado con mayor frecuencia en los brotes de gastroenteritis vírica en instituciones cerradas y semicerradas ha sido norovirus y, en especial, norovirus genogrupo II. Durante el periodo de estudio hemos detectado un brote por astrovirus, pero no hemos identificado ningún brote por sapovirus, rotavirus o adenovirus.

3. En los brotes de gastroenteritis por norovirus y astrovirus en que se ha estudiado la afectación entre los contactos domiciliarios se han identificado casos secundarios en los domicilios, tanto de los enfermos como de los expuestos asintomáticos. El estudio de los brotes de gastroenteritis vírica en instituciones cerradas y semicerradas no debería limitarse a las personas de la institución, sino que debería extenderse a sus contactos domiciliarios.

4. El modo de transmisión más frecuente en los brotes de gastroenteritis vírica en instituciones cerradas y semicerradas ha sido el contacto de persona a persona. Los trabajadores de instituciones cerradas y semicerradas, especialmente aquellos que tienen un contacto más directo con los usuarios, pueden contribuir a la extensión de los brotes, por lo que es importante su formación en materia de higiene personal y ambiental.

5. En los brotes de gastroenteritis vírica que se presentan en instituciones cerradas y semicerradas se han detectado personas infectadas asintomáticamente que, aunque presentan menor excreción del virus en heces que las personas que presentan síntomas, pueden actuar como propagadores del agente causal en la institución.

6. La transmisión hídrica ha sido poco frecuente en los brotes de gastroenteritis vírica ocurridos en instituciones cerradas y semicerradas, pero cuando se ha producido dicha transmisión la tasa de ataque ha sido muy superior a la de los brotes con otros modos de transmisión.

7. Cuando los brotes de gastroenteritis vírica en instituciones cerradas y semicerradas se presentan en grupos grandes debería estudiarse si existen subgrupos con diferentes grados de exposición y realizar el análisis individualizado de dichos subgrupos para conocer la magnitud real de su afectación.

8. Durante la pandemia de COVID-19 se ha producido una importante reducción en la notificación de brotes de gastroenteritis aguda, que puede ser debida al efecto de las medidas no farmacológicas aplicadas para evitar la transmisión de SARS-CoV-2 pero también a la falta de detección y notificación por parte de unos servicios sanitarios colapsados o a la insuficiencia de recursos de vigilancia epidemiológica.

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