Epidemiologia de les infeccions respiratòries víriques en pacients pediàtrics a Manhiça, una zona rural de Moçambic

Cristina O'Callaghan Gordo

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Tesi Doctoral

Epidemiologia de les infeccions respiratòries víriques en pacients pediàtrics a Manhiça, una zona rural de Moçambic

Memòria presentada per **Cristina O'Callaghan Gordo** per optar al títol de Doctora per la Universitat de Barcelona.

Directora de tesi: Dra. **Anna Roca i Aparicio**
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La Dra. Anna Roca Aparicio certifica que la tesi titulada "**Epidemiologia de les infeccions respiratòries víriques en pacients pediàtrics a Manhiça, una zona rural de Moçambic**" presentada per Cristina O'Callaghan Gordo ha estat realitzada sota la seva direcció, i compleix tots els requisits que dicta la normativa vigent per a la presentació de tesis doctorals com a compendi d'articles a la Facultat de Medicina de la Universitat de Barcelona.

Dra. Anna Roca

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ARTICLES QUE CONSTITUEIXEN AQUESTA TESI

Viral acute respiratory infections among infants visited in a rural hospital of southern Mozambique

Cristina O'Callaghan-Gordo, Núria Díez-Padrisa, Fatima Abacassamo, Pilar Pérez-Breña, Inmaculada Casas, Pedro L. Alonso, Anna Roca

Tropical Medicine and International Health, 2011; 79 (9): 1054-1060

Factor d'Impacte (2010): 2,841

Quartil: 1 (Tropical medicine)

Etiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique. A malaria endemic area with high prevalence of human immunodeficiency virus

Cristina O'Callaghan-Gordo, Quique Bassat, Luis Morais, Núria Díez-Padrisa, Sónia Machevo, Tacilta Nhampossa, Delino Nhalungo, Sergi Sanz, Llorenç Quintó, Pedro L. Alonso, Anna Roca

The Pediatric Infectious Disease Journal, 2011; 30(1): 39-44

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Quartil: 1 (pediatrics)

The global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis

Harish Nair, W Abdullah Brooks, Mark Katz, Anna Roca, James A Berkley, Shabir A Madhi, James Mark Simmerman, Aubree Gordon, Masatoki Sato, Stephen Howie, Anand Krishnan, Maurice Ope, Kim A Lindblade, Phyllis Carosone-Link, Marilla

Theodoratou , Malinee Chittaganpitch, Osaretin Chimah, Angel Balmaseda, Philippe Buchy, Eva Harris, Valerie Evans, Masahiko Katayose; Bharti Gaur; Cristina O'Callaghan-Gordo , Doli Goswami, Wences Arvelo, Marietjie Venter, Thomas Briese, Rafal Tokarz, Marc-Alain Widdowson, Anthony W Mounts, Robert F Breiman, Daniel R Feikin, Keith P Klugman, Sonja J Olsen, Bradford D Gessner, Peter F Wright, Igor Rudan, Shobha Broor, Eric A F Simões, Harry Campbell

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Quartil: 1 (Medicine, general & internal)

Human rhinovirus and wheezing: Do lower respiratory tract infections associated with rhinovirus during infancy increase rates of wheezing during childhood?

Cristina O'Callaghan-Gordo, Quique Bassat, Núria Díez-Padrisa, Luis Morais, Sónia Machevo, Tacilta Nhampossa, Llorenç Quintó, Pedro L. Alonso, Anna Roca

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Ús de terminologia científica en català segons el model TERMCAT - Centre de Terminologia (http://www.termcat.cat), Institut d'Estudis Catalans, 2011.

En les abreviacions dels noms dels virus s'ha mantingut l'ordre de les sigles utilitzat en anglès.

Als meus pares

ÍNDEX

ÍNDEX

ACRÒNIMS

ACRÒNIMS

ADN	àcid desoxiribonucleic
ADV	Adenovirus
ANF	Aspirat nasofaringi
ARI	Infecció respiratòria aguda (*acute respiratory infection*)
ARN	àcid ribonucleic
CISM	Centre de recerca en salut de Manhiça (*Centro Investigaçaõ em Saúde da Manhiça*)
CRESIB	Centre de recerca en salut internacional de Barcelona
EV	Enterovirus
Flu	Virus influenza
FR	Freqüència respiratòria
HDM	Hospital Distrital de Manhiça
Hib	Haemophilus influenzae b
HIV	Virus de la immunodeficiència humana (*human immunodeficiency virus*)
hMPV	Metapneumovirus humà
LRTI	Infecció del tracte respiratori inferior (*lower respiratory tract infection*)
OMS	Organització Mundial de la Salut
OR	*Odds rati*
p	p-valor
PCR	Reacció en cadena de la polimerasa (*polymerase chain reaction*)
PIV	Virus parainfluenza
PYAR	Anys a risc per persona (*person year-at-risk*)
RR	Coeficient d'incidències (*rate ratio*)
RSV	Virus respiratori sincitial
RT-PCR	PCR amb transcripció inversa (*reverse transcription-PCR*)
RV	Rinovirus
UNICEF	Fons de Nacions Unides per a la infància (*United Nations Childrens Fund*)
URTI	Infecció del tracte respiratori superior (*upper respiratory tract infections*)
vs.	*versus*
95%CI	Interval de confiança del 95%

INTRODUCCIÓ

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1. Infeccions respiratòries agudes

1.1. Definició i classificació de les infeccions respiratòries agudes

Les infeccions respiratòries agudes (ARI, de l'anglès *acute respiratory infections*) es defineixen com el conjunt d'infeccions de l'aparell respiratori que tenen una durada menor a dues setmanes. Les ARI es classifiquen en ARI del tracte respiratori superior (URTI, de l'anglès *upper respiratory tract infection*), quan la infecció es localitza a les vies altes del tracte respiratori i en ARI del tracte respiratori inferior (LRTI, de l'anglès *lower respiratory tract infection*), quan la infecció afecta les vies baixes del tracte respiratori (figura 1).

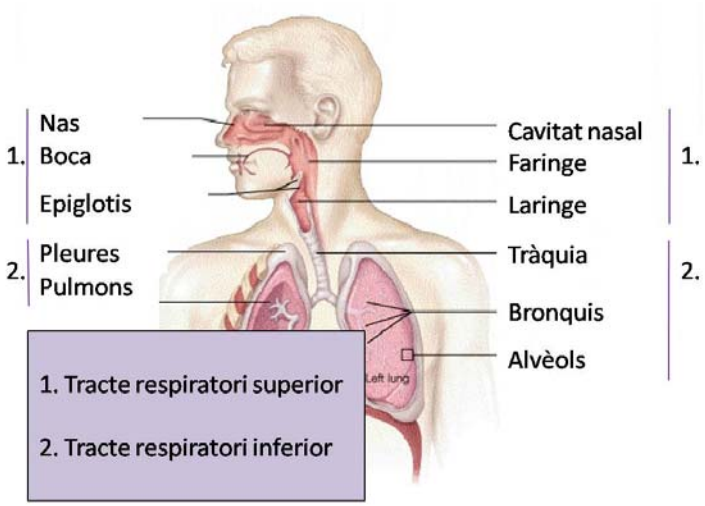

Figura 1. Esquema del tracte respiratori superior i inferior.

1.2. Epidemiologia de les ARI

Les URTI són normalment malalties lleus i autolimitants, generalment d'origen víric, en què es veuen afectades la cavitat nasal, l'oïda, la faringe o la laringe. Malgrat que en la majoria dels casos la prognosi és bona, a causa de la seva elevada freqüència, les URTI comporten una elevada càrrega econòmica i social. S'estima que el 50% de totes les malalties, i el 75% de les malalties en nens, són URTI d'origen víric [1], i aquesta elevada incidència es tradueix en grans despeses econòmiques; en primer lloc, a causa de l'absentisme laboral per malaltia pròpia o per estar cuidant un menor malalt, i en

segon lloc, pel nombre de visites als serveis sanitaris [2, 3]. En determinats casos, i amb més risc en persones amb sistemes immunològics debilitats, les URTI poden evolucionar cap a infeccions del tracte respiratori inferior (LRTI), com la bronquiolitis o la pneumònia, les quals produeixen una presentació clínica més greu.

Les LRTI, i especialment la pneumònia, són la primera causa de mortalitat infantil arreu del món (figura 2) [4, 5]. Segons la darrera estimació de les causes de mortalitat infantil a nivell global, la pneumònia és responsable de 1,6 milions de morts en nens menors de cinc anys [5].

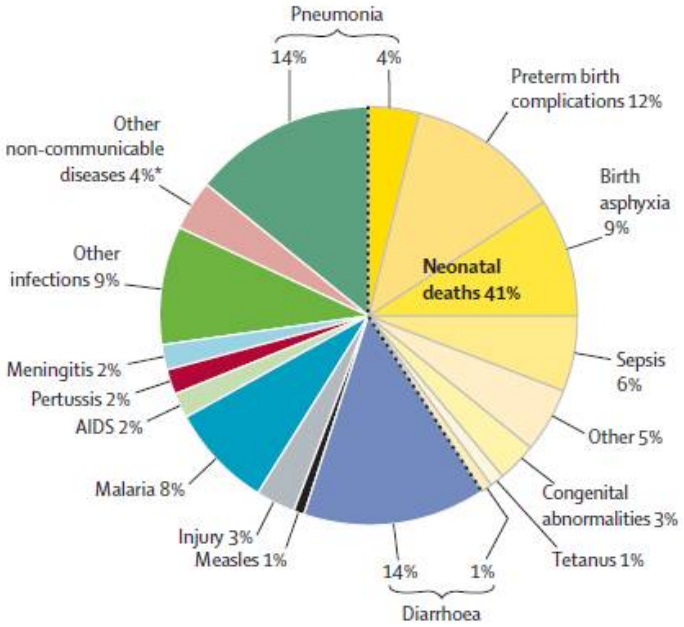

Figura 2. Causes de mortalitat infantil a nivell mundial [5]

Tant la incidència de la pneumònia com la mortalitat associada a aquesta presentació clínica no es reparteixen uniformement (figura 3). Més de 150 milions de nous casos de pneumònia es registren anualment als països en vies de desenvolupament, valor que representa el 95% de tots els casos que es donen a nivell mundial. Pel que fa a la mortalitat associada, s'estima que el 98% de les morts es produeixen als països més pobres i el 50% es produeixen concretament a l'Àfrica subsahariana, representant entre el 19 i el 21% del total de les morts que es produeixen en aquesta zona geogràfica en nens menors de cinc anys [5-8].

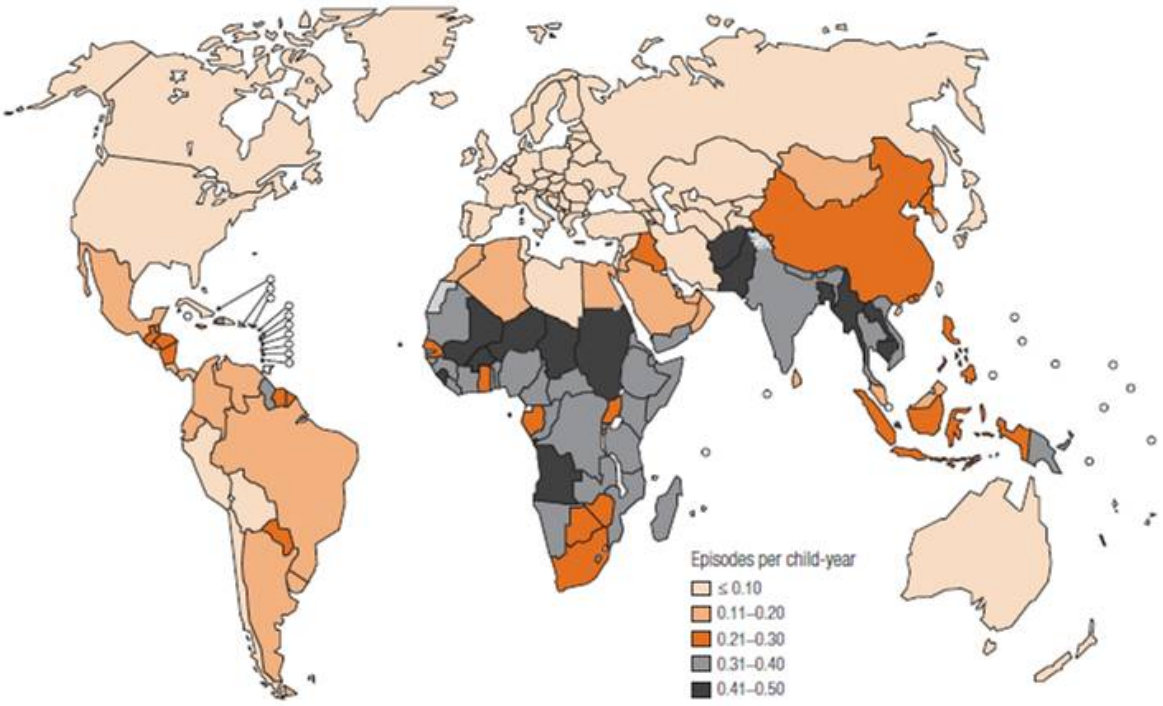

Figura 3. Incidència de pneumònia clínica entre nens menors de cinc anys per països [8].

Aproximadament la meitat d'aquestes morts es produeixen entre nens desnodrits [9]. El baix pes en néixer, la deficiència de zinc, l'exposició al fum de cremar biomassa, la convivència amb molts nens petits, etc., són altres factors lligats a la pobresa que comporten una major incidència i mortalitat de les ARI [9-11]. Molts d'aquests factors de risc són comuns per a altres malalties infeccioses lligades a la pobresa, com la diarrea, el xarampió o la malària (en les zones endèmiques), i la concurrència de dues malalties infeccioses augmenta el risc de mortalitat esperat per cadascuna de les malalties per separat [6].

La pandèmia de l'HIV (de l'anglès *human immunodeficiency virus*) té un paper important en l'epidemiologia de les ARI, especialment a l'Àfrica subsahariana, on es produeixen el 91% de les noves infeccions per l'HIV entre nens. La pneumònia és la principal causa d'hospitalització i mortalitat entre els nens infectats per l'HIV [12-15]. La mortalitat associada a la pneumònia és entre 3 i 6 vegades més elevada en nens infectats per l'HIV que en nens no infectats per l'HIV. La incidència de la pneumònia i la mortalitat associada a aquesta malaltia és també més alta per a nens exposats a l'HIV (nens no infectats fills de mares infectades) que per a nens no exposats [14].

Els factors de risc comentats fins ara són molt importants a l'hora de determinar l'elevada incidència de les ARI en general i, en concret, de les formes més greus com la pneumònia, però la manca d'accés a l'atenció sanitària bàsica és probablement un dels factors que més determina l'elevada mortalitat associada a aquestes infeccions en països de renda baixa [16]. Segons un informe de l'UNICEF (de l'anglès *United Nations Childrens Fund*) i de l'OMS (Organització Mundial de la Salut), a l'Àfrica subsahariana, només 41% dels nens amb pneumònia arriben centres de salut amb capacitat per diagnosticar i tractar la pneumònia [17].

1.3. Etiologia: les ARI víriques

Les ARI poden ser causades per diferents agents etiològics, principalment virus i bacteris, però també per fongs i paràsits. Els virus més importants per freqüència i severitat són el virus respiratori sincitial (RSV), el rinovirus (RV), el virus influenza (Flu), el virus parainflueza (PIV) i el metapneuovirus humà (hMPV). D'altra banda, els principals agents bacterians són *Streptococcus pneumoniae (*pneumococ) i *Haemophilus influenzae* b (Hib).

La majoria de les URTI són d'origen víric [1]. Els virus no formen part de la flora habitual del tracte respiratori, i per tant històricament quan es detecta un virus al tracte respiratori superior es considera que aquest virus és responsable dels símptomes respiratoris observats. Aquest dogma, però, es basa en resultats obtinguts mitjançant tècniques de diagnòstic tradicionals (cultiu de virus i detecció d'antígens) i no és clar si es pot aplicar quan el diagnòstic dels virus es fa utilitzant tècniques basades en la detecció d'àcids nucleics, com ara la reacció en cadena de la polimerasa (PCR, de l'anglès *polymerase chain reaction*) [18]. La PCR té una sensibilitat més elevada que les tècniques de diagnòstic tradicionals i ha permès la detecció de virus, com l'hMPV o diversos genotips d'RV que no s'havien detectat utilitzant altres tècniques [19]. La millora en el diagnòstic dels virus respiratoris ha comportat també un augment en la detecció de virus respiratoris entre pacients asimptomàtics [20-22], fent que actualment es qüestioni si la detecció de virus i la presència de símptomes indica que hi ha, en tots els casos, una relació causal [18].

Les LRTI poden ser d'origen víric o bacterià. Tradicionalment, la bronquiolitis s'ha associat a infeccions víriques i la pneumònia a infeccions bacterianes, però amb l'ús de tècniques basades en la detecció d'àcids nucleics aquesta relació és menys evident. S'han descrit fins a 26 virus respiratoris associats a la pneumònia [23], i els estudis que utilitzen aquestes tècniques detecten almenys un virus respiratori en més del 50% dels casos de pneumònia [24-26]. De la mateixa manera que per a les URTI, la detecció d'un virus durant una LRTI no sempre indica que aquest en sigui l'agent causant. En el cas de les LRTI establir una relació causal entre el virus detectat i els símptomes clínics és encara més arriscat per les següents raons. En primer lloc, la majoria d'estudis utilitzen espècimens del tracte respiratori superior (aspirats o rentats nasofaringis, frotis de la nasofaringe, el coll o el nas, etc.) i és difícil establir una relació causal entre els patògens detectats a les vies altes del tracte respiratori i una infecció que té lloc al tracte respiratori inferior. La detecció d'un virus al tracte respiratori superior pot indicar la coexistència temporal de dues infeccions no relacionades: una URTI causada pel virus detectat i una LRTI causada per un altre patogen [27]. En estudis que utilitzen espècimens del tracte inferior (esput, broncoscòpia, etc.) és difícil d'excloure la possibilitat de contaminació de la mostra amb microorganismes del tracte respiratori superior [28]. En segon lloc, la detecció d'un virus respiratori no exclou la presència d'un altre patogen. La tècnica més utilitzada per detectar pneumònia d'origen bacterià és l'hemocultiu, una tècnica que té una sensibilitat molt baixa i dóna resultats positius només en el 4-18% dels nens amb pneumònia [28]. Per tant, el fet de detectar un virus i tenir hemocultiu negatiu no exclou la possibilitat que la pneumònia estigui associada també a una infecció bacteriana.

Malgrat les limitacions exposades a l'hora d'atribuir causalitat, l'elevat nombre tant de casos d'URTI com d'LRTI en què es detecta almenys un virus indica que, ja sigui de manera directa o pel fet d'augmentar el risc que es produeixi una sobreinfecció bacteriana [29-31], els virus respiratoris tenen un rol important en l'etiologia de les ARI. Estudis que han comparat la detecció de virus en casos amb símptomes d'ARI i controls asimptomàtics han trobat que la prevalença d'infeccions víriques és superior entre els casos [18], especialment per alguns virus com l'RSV [18, 21] i l'hMPV [32, 33] que no es troben normalment entre pacients asimptomàtics. En concordança amb això, segons un estudi

recent, en els controls en que es detecten el Flu A o l'RV, la carrega viral és més baixa que la que es detecta entre pacients simptomàtics [22]. Tot i que calen millores en el diagnòstic de les ARI (incloent-hi obtenció de mostres i interpretació de resultats) per poder-ne atribuir la causa amb certesa, hi ha prou evidència que els virus tenen un rol important tant en les URTI com en les LRTI, inclosa la pneumònia.

2. Virus respiratoris

S'han descrit més de 200 virus antigènicament diferents capaços de causar infeccions respiratòries en nens i adults. Tots ells es classifiquen segons la naturalesa de l'àcid nucleic del virió [ADN (àcid desoxiribonucleic) o ARN (àcid ribonucleic)], la simetria i dimensions de la càpside, i la presència o absència d'embolcall extern (taula 1) [34]. Segons aquests criteris els virus respiratoris s'agrupen en sis famílies diferents: *Paramyxoviridae, Orthomyxoviridae, Picornaviridae, Adenoviridae, Coronaviridae i Herpesviridae,* essent les quatre primeres les més importants.

Família	**Subfamília**	**Gènere**	**Espècie**
Paramyxoviridae	*Paramyxovirinae*	*Respirovirus*	PIV1, PIV3
		Rubulavirus	PIV2, PIV4A, PIV4B
	Pneumovirinae	*Pneumovirus*	RSV
		Metapneumovirus	hMPV
Orthomyxoviridae		*Influenza virus A*	
		Influenza virus B	
		Influenza virus C	
Picornaviridae		*Enterovirus*	
		Rhinovirus	RV A, RV B, RV C
Ademoviridae		*Mastadenovirus*	ADV

Taula 1. Esquema basat en la classificació del virus del tracte respiratoris de Mackie PL [34]. PIV (parainfluenza), RSV (virus respiratori sincitial), hMPV (metapneumovirus humà), RV (rinovirus), ADV (adenovirus).

2.1.Paramyxoviridae

Aquesta família conté alguns dels virus amb major importància com a causants d'infeccions respiratòries: els virus parainfluenza (PIV), el virus respiratori sincitial (RSV) i el metapneumovirus humà (hMPV). Els virions d'aquesta família són normalment esfèrics, d'una mida entre 150 i 300 nm de diàmetre. Tenen una bicapa lipídica, que deriva de la cèl·lula hoste en què el virus ha crescut, on s'insereixen les glicoproteïnes virals (els principals antígens vírics) formant unes punxes que sobresurten de la membrana. A l'interior de la membrana lipídica hi ha la nucleocàpside, una estructura helicoïdal formada per ARN i proteïna de nucleocàpside, a la qual s'uneixen la fosfoproteïna i la polimerasa [35].

2.1.1. Virus parainfluenza

Es coneixen cinc espècies de PIV diferents: 1, 2, 3 i 4A i 4B, tots ells de la subfamília *Paramyxovirinae,* però membres de gèneres diferents. El PIV1 i el PIV3 pertanyen al gènere *Respirovirus*, mentre que el PIV2 i el PIV4 pertanyen al gènere *Rubulavirus* [36]. El primer PIV es va descobrir l'any 1952; inicialment se'l va anomenar Sandai virus i posteriorment PIV1; tres anys més tard (1955), es va aïllar el PIV2 de nens amb laringotraqueobronquitis (en anglès *croup*) i l'any 1985 el PIV3 de nens amb ARI. L'any 1960 es va aïllar el PIV4 (amb dos serotips diferents, PIV4A i PIV4B) de nens amb ARI lleu [37].

Els PIV afecten bàsicament la població pediàtrica i són una de les principals causes d'hospitalització per LRTI en nens petits. Els PIV, especialment el PIV1 i el PIV2, són els principals causants de la laringotraqueobronquitis, tot i que també se'ls troba associats a d'altres URTI. Aproximadament el 15% des les infeccions pels PIV acaben afectant també el tracte respiratori inferior, essent el PIV3 el més associat a patologies més severes [36].

La incidència de les infeccions pels PIV mostren certa estacionalitat, essent el PIV1 el que causa brots més marcats de totes les espècies dels PIV. Segons dades d'Estats Units dels anys 1993-1998, el PIV1 està associats a brots de laringotraqueobronquitis que es produeixen durant la tardor cada dos anys. El PIV2 és menys predictibles, tot i que generalment segueix els brots del PIV1. El PIV3 es detecta cada any durant els

mesos de primavera i estiu, i el PIV4 és l'espècie de PIV detectada de manera menys freqüent i el seu patró estacional no està ben definit [38].

2.1.2. Virus Respiratori Sincitial

L'RSV es classifica dins la subfamília *Pneumovirinae* i gènere *Pneumovirus.* Es va aïllar per primera vegada l'any 1956 de ximpanzés amb símptomes d'ARI. Posteriorment es van detectar anticossos dirigits contra aquest agent en treballadors de laboratori, i es va poder aïllar de les mucoses nasals d'un dels treballadors que estava refredat, cosa que indicava que l'RSV també infectava humans. Poc temps més tard, l'RSV es va aïllar també de nens petits amb ARI [39].

L'RSV es un dels principals causants d'LRTI (i de pneumònia) durant la infància, especialment durant el primer any de vida: aproximadament el 22% dels episodis d'LRTI que es produeixen anualment són causats per aquest patogen. S'estima que l'RSV causa entre 66.000 i 200.000 morts anualment entre nens menors de cinc anys, essent el patogen que causa més morts associades a LRTI després del pneumococ i de Hib [40].

L'RSV té una marcada estacionalitat, causant brots d'uns quatre mesos de durada un cop l'any. En zones temperades de l'hemisferi nord el pic de l'RSV es produeix normalment durant els mesos més freds. En zones tropicals i subtropicals l'estacionalitat de l'RSV està associada a les pluges, i el brot de l'RSV es produeix durant la segona meitat de l'estació plujosa [41-45].

2.1.3. Metapneumovirus humà

L'hMPV forma part de la mateixa subfamília que l'RSV, *Pneumovirinae,* però pertany al gènere *Metapneumovirus.* L'hMPV es va descriure per primera vegada l'any 2001, després de ser aïllat de nens amb infecció respiratòria semblant a la causada per l'RSV amb etiologia desconeguda. Tot i això hi ha proves serològiques que indiquen que l'hMPV portava més de cinc dècades circulant entre la població, i que probablement no s'havia descrit abans a causa del seu lent creixement *in vitro* [46].

L'hMPV causa una clínica similar a l'RSV i produeix tant URTI com LRTI, les darreres especialment en nens menors de cinc anys i persones grans o immunodeprimides. La

incidència de l'hMPV és especialment elevada entre nens menors de 2 anys; en comparació amb l'RSV, l'hMPV en general afecta nens més grans. L'estacionalitat de l'hMPV és menys marcada que la de l'RSV, però també s'observen pics anuals que sovint coincideixen o segueixen el pic d'aquest virus [47].

2.2.Orthomyxoviridae

Aquesta família inclou cinc gèneres: els *influenza virus A, B* i *C* (Flu A, Flu B i Flu C), *Thogotovirus* i *Isavirus*, però només els tres primers causen ARI en humans. Els gèneres *Thogotovirus* i *Isavirus* són molt diferents als *influenza virus* pel que fa als seus hostes (*Thogotovirus* afecta principalment invertebrats i *Isavirus* els salmons), però estudis genètics, moleculars i bioquímics els agrupen a la mateixa família [48].

2.2.1.Influenza virus A, B i C

Els virions dels Flu A, Flu B i Flu C tenen una estructura semblant. Tots ells tenen una única cadena senzilla i polaritat negativa d'ARN segmentada. El Flu A i el Flu B tenen vuit segments, mentre que el Flu C en té set [48]. L'ARN s'associa amb la nucleoproteïna per formar una estructura helicoïdal anomenada nucleocàpside. La nucleoproteïna té tres formes antigèniques diferents que permeten la classificació en els gèneres Flu A, Flu B i Flu C. Al voltant de la nucleocàpside es troben dues proteïnes de membrana envoltades per una bicapa lipídica que constitueix la membrana vírica, on s'insereixen les glicoproteïnes de membrana: l'hemaglutinina i la neuraminidasa, en el cas del Flu A i del Flu B, i l'HEF (de l'anglès *hemagglutinin-esterase-fusion*), en el cas del Flu C. Les funcions de les glicoproteïnes de membrana són clau en el procés infecciós: l'hemaglutinina s'uneix als receptors de la superfície cel·lular en els estadis inicials de la infecció, fent possible la primera interacció amb la cèl·lula hoste, mentre que la neuraminidasa té un paper clau en l'alliberament de nous virions de les cèl·lules infectades. L'HEF combina les funcions d'aquestes dues proteïnes [48-50].

L'hemaglutinina i la neuraminidasa presenten diferents formes antigèniques que s'utilitzen per la classificació en espècies dels diferents gèneres del Flu. Es coneixen quinze subtipus d'hemaglutinina (H1-H15) i nou de neuraminidasa (N1-N9) per al Flu A,

tot i que només s'han aïllat tres subtipus d'hemaglutinina i dos de neuraminidasa en humans. Per al Flu B es coneix només un subtipus de d'hemaglutinina i de neuraminidasa [50].

El comportament epidemiològic del Flu depèn dels dos tipus de variació antigènica que poden tenir les glicoproteïnes de superfície. Anualment hi ha brots de grip causats per noves variants del Flu que apareixen com a resultat de mutacions puntuals a les glicoproteïnes de superfícies, fenomen conegut com a "deriva antigènica" (en anglès *antigenic drift*). Aquestes noves variants estan relacionades amb les variants circulants els anys anteriors però són prou diferents com per escapar al reconeixement del sistema immune i causar brots anuals. Cada cert temps es produeix la recombinació entre virus diferents fent que aparegui un nou tipus del Flu, amb una nova hemaglutinina o una nova neuraminidasa, fet que els fa antigènicament diferents als virus circulats anteriorment i potencialment causants de pandèmies de grip. Aquest fenomen s'anomena "canvi antigènic" (en anglès *antigenic shift*) i només s'observa entre espècies del gènere Flu A [49, 50]. Les infeccions pel Flu poden variar des de formes asimptomàtiques fins a diversos síndromes respiratoris de diferent severitat, que poden ser en molts casos fulminants [51, 52]. El Flu A i el Flu B causen una presentació clínica semblant, però la freqüència d'infeccions severes associades al Flu B és aproximadament quatre cops menor que la de Flu A. El Flu C està principalment associat a URTI [53].

El Flu té una marcada estacionalitat. Causa brots anuals d'una durada d'entre sis i vuit setmanes, normalment durant els mesos de més humitat, coincidint en zones temperades amb els mesos d'hivern i en les zones tropicals amb l'estació humida [3, 45, 49, 54, 55].

El Flu és l'únic virus respiratori pel qual hi ha vacuna disponible actualment. Les vacunes existents són produïdes a partir de virus cultivats en ous fèrtils de gallina i posteriorment inactivats; contenen tot el virus sencer o bé els antígens de superfície (hemaglutinina i neuraminidasa) purificats de tres soques diferents, que varien segons les recomanacions anuals de l'OMS. [49, 50].

2.3. Picornaviridae

Aquesta família inclou un grup molt divers de patògens humans, entre ells els rinovirus (RV) i els enterovirus (EV). El genoma de tots ells consisteix en un únic segment d'ARN de cadena senzilla i polaritat positiva. Els virions d'aquesta família són esfèrics, amb un diàmetre d'aproximadament 30 nm, i no estan embolcallats per cap membrana lipídica: consten únicament d'una càpside icosaèdrica proteica (proteïnes VP1-VP4) i d'una cadena senzilla d'ARN dins d'aquesta [34].

2.3.1.Enterovirus

Els EV són un gènere de família *Picornaviridae,* del qual s'han aïllat en humans més de 80 serotips diferents que es classifiquen en quatre espècies (A-D) [56]. Els EV es repliquen principalment al tracte digestiu, on causen infeccions subclíniques o amb clínica lleu, però en un nombre elevat de casos els virus es poden desplaçar a d'altres òrgans causant infeccions més o menys greus depenent del serotip. Diversos serotips s'associen a ARI [57].

2.3.2. Rinovirus

L'RV es va observar per primera vegada l'any 1953 en cultius cel·lulars, el sobrenedant dels quals era capaç de produir símptomes de refredat comú en voluntaris. Quatre anys més tard es va aïllar per primera vegada en pacients amb refredat comú [58]. El gènere *Rhinovirus* inclou més de 100 genotips diferents, que segons estudis filogenètics es divideixen en diferents espècies: rinovirus A (RV A), rinovirus B (RV B) i rinovirus C (RV C). L'RV C va ser descrit l'any 2007 [59] a partir d'anàlisis filogenètiques que relacionaven diferents genotips del gènere *Rhinovirus* distants d'RV A i d'RV B, aïllats en diferents parts del món de pacients amb ARI a partir de 2006 [60-62].

L'RV és la principal causa del refredat comú, sent responsables de com a mínim el 50% d'aquestes infeccions [63]. Malgrat que el refredat comú és una malaltia lleu i autolimitant amb poques conseqüències a nivell individual, comporta una gran càrrega per a la societat a causa de les elevades pèrdues econòmiques que genera anualment com a causa d'absentisme laboral i saturació dels sistemes sanitaris [2]. En els darrers anys ha augmentat el nombre d'estudis que l'assenyalen com a causant d'asma, bronquiolitis i pneumònia [25, 64-66], i d'acord amb una revisió bibliogràfica recent, hi ha

prou evidències per afirmar que l'RV és també un patogen del tracte respiratori inferior [67]. La clínica associada a la nova espècie d'RV, l'RV C, no està encara ben definida, però diversos estudis han detectat aquest virus de manera freqüent en nens hospitalitzats per LRTI o per asma, fent pensar que aquesta espècie deu ser més patogènica que les altres espècies d'RV [59, 61, 68, 69].

Tot i que les infeccions pels RV es detecten durant tot l'any, a les zones temperades de l'hemisferi nord es detecten dos brots anuals: el primer a finals de primavera i el segon a principis de tardor [70]. Les infeccions per l'RV són freqüents entre tots els grups d'edat, però són especialment habituals entre nens majors d'un any [1].

2.4. Adenoviridae

Aquesta família inclou quatre gèneres, però només n'hi ha un que afecta els humans, el *Mastadenovirus.* Dins d'aquest gènere es classifiquen els adenovirus humans (ADV), dels quals es coneixen sis espècies (A-F) que inclouen 51 serotips diferents [34, 71]. Els virions d'aquesta família formen partícules no embolcallades de 70-90 nm de diàmetre. La càpside dels virus de la família *adenoviridae* està formada per 252 capsòmers que s'ordenen formant un icosàedre. De cadascun dels dotze vèrtexs de l'icosàedre surten estructures proteiques en forma de punxa que faciliten el procés d'unió i entrada a la cèl·lula hoste. A l'interior de la càpside proteica es troba una cadena doble d'ADN no fragmentat[71].

2.4.1. Adenovirus

Els ADV es van aïllar per primer cop el 1953 per investigadors militars, en brots d'ARI entre homes joves i sans que feien el servei militar. Poc temps després es van relacionar també amb infeccions gastrointestinals i oculars, i posteriorment s'han aïllat d'una gran varietat d'òrgans i de pacients de diferents grups d'edat [72].

Els ADV causen infeccions puntuals, endèmiques i epidèmiques a tot el món, amb una presència més elevada als països en vies de desenvolupament o als estrats socioeconòmics més baixos dels països desenvolupats. Es transmeten per diverses vies (contacte directe, aigua, oral-fecal, aerosols, etc.) causant brots ràpids entre grups

amb una convivència estreta, com ara guarderies, centres de dia per a gent gran o casernes militars [71]. La majoria de les infeccions respiratòries associades als ADV són URTI, però en nens, entre el 5-10% de les LRTI s'associen a diversos serotips d'ADV [72].

Tot i que els ADV s'aïllen durant tot l'any, en zones temperades s'observa una major freqüència durant l'hivern i la primavera [71].

OBJECTIUS

OBJECTIUS

1. Objectiu general

Descriure l'epidemiologia de les infeccions respiratòries víriques en pacients pediàtrics a Manhiça.

2. Objectius específics

1. Descriure l'etiologia i l'epidemiologia de les infeccions respiratòries agudes d'origen víric en nens menors d'un any atesos a les consultes externes de l'Hospital Distrital de Manhiça (article 1).

2. Descriure l'etiologia i l'epidemiologia de les infeccions respiratòries agudes d'origen víric en menors de cinc anys ingressats a l'Hospital Distrital de Manhiça amb símptomes de pneumònia severa (article 2).

3. Estudiar en detall els virus més freqüents i amb més rellevància clínica a la zona:
   - 3.1. Calcular la incidència d'infeccions respiratòries associades al Flu en nens menors de cinc anys a Manhiça (article 3).
   - 3.2. Determinar si la pneumònia associada a l'RV durant el primer any de vida és un factor de risc per patir sibilacions durant la infància (manuscrit).

HIPÒTESIS

HIPÒTESIS

1. Un percentatge elevat de les ARI, tant de les URTI lleus com de les pneumònies que requereixen hospitalització, estan associades a virus respiratoris.

2. Les ARI d'origen víric tenen un patró estacional; a Manhiça la màxima incidència s'observa durant l'estació de pluges. Cada virus té un patró estacional característic.

3. La prevalença de cadascun dels virus respiratoris varia en funció del grup d'edat.

4. El nombre de casos amb coinfeccions víriques és elevat.

5. Existeixen algunes diferències clíniques entre les ARI d'origen víric les víric i les ARI d'origen no víric.

6. La malària, les infeccions bacterianes invasives, l'HIV i la malnutrició modifiquen la incidència, l'estacionalitat, la distribució per edat i la clínica de les ARI d'origen víric.

MÈTODES

MÈTODES

1. Àrea d'estudi

Les dades que s'han utilitzat per dur a terme els estudis que s'engloben en aquesta tesi han estat recollides pel Centre de Recerca en Salut de Manhiça (CISM, del portuguès *Centro Investigaçaõ em Saúde da Manhiça*) i pel Centre de Recerca en Salut Internacional de Barcelona (CRESIB) a l'Hospital Districtal de Manhiça (HDM), al districte de Manhiça, una zona rural de la província de Maputo, al sud de Moçambic (figura 4).

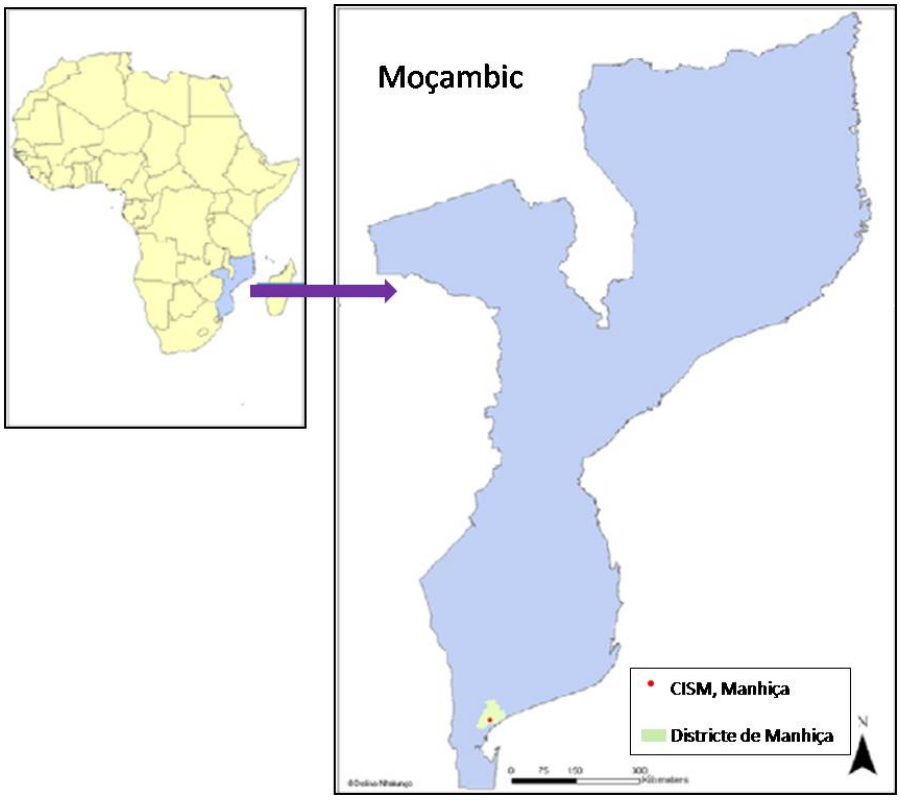

Figura 4. Localització de Moçambic i del districte de Manhiça.

El districte de Manhiça té una població de 140.000 habitants [73]. Els grups ètnics majoritaris són Xangana i Ronga. El districte està dividit en dues zones: la plana inundable del riu Inkomati (figura 5a), poc habitada i d'ús gairebé exclusiu per a la plantació de la canya de sucre i fruita, i un petit altiplà a l'oest del riu, on es concentra la major part de la població del districte. Al districte de Manhiça hi ha dues poblacions principals (Manhiça i Xinavane), però la major part de la població viu en conglomerats

de cases dispersos per tot el districte (figura 5b). La major part de la població es dedica a l'agricultura de subsistència o treballa en alguna de les dues fàbriques de sucre de la zona [42]. Històricament els homes de Manhiça migren cap a Maputo o Sud-àfrica per buscar feina, fent que el 30% de les llars estiguin dirigides per dones [73].

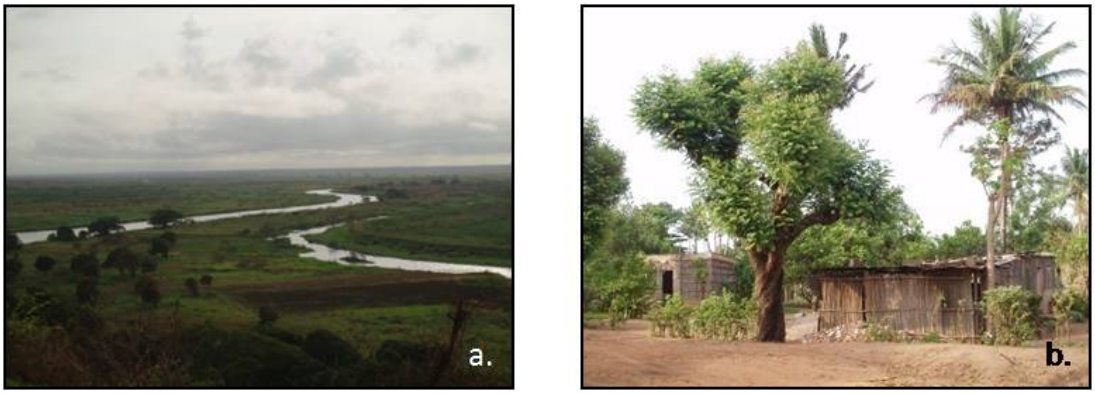

Figura 5. Àrea d'estudi: **a.** Plana del riu Inkomati; **b.** Conglomerat de cases al districte de Manhiça

El clima a la zona és subtropical, amb dues estacions marcades: l'època de pluges amb temperatures més altes entre novembre i abril, i l'època seca amb temperatures més fredes durant la resta de l'any [42]. La precipitació mitjana anual entre el 2001 i el 2006 fou de 1200 mm [74].

La malària, les infeccions invasives bacterianes i l'HIV són infeccions molt freqüents a la zona. La malària és una de les principals causes de morbiditat i mortalitat a Manhiça. Entre el 2003 i el 2005, el 30% de les visites a les consultes externes i el 49% de les hospitalitzacions a l'HDM estaven associades a la malària [75, 76] i aproximadament el 19% de les morts pediàtriques intrahospitalàries registrades durant aquest període foren causades per la malària [76]. La transmissió de la malària a Manhiça és perenne, amb pics durant l'estació de pluges [76]. La majoria de casos són deguts al *Plasmodium falciparum* [77] i transmesos per l'*Anopheles funestus* [78].

Entre el 2001 i el 2006, les infeccions bacterianes invasives van ser responsables del 8% dels ingressos pediàtrics i del 21% de les morts intrahospitalàries [74]. L'any 2006, el 18% dels nens ingressats a l'HDM tenien clínica compatible amb meningitis, i meningitis confirmada en el 7% d'aquests casos [79]. Durant els anys en què van recollir les mostres

per a aquests estudis, les vacunes de Hib i de pneumococ no formaven part del programa ampliat de vacunació a Moçambic.

La prevalença de l'HIV és elevada a la zona i ha crescut del 12% l'any 1999 al 49% l'any 2008 entre dones en edat reproductiva [80]. Fins a l'any 2003 no hi havia cap sistema implantat a l'HDM per prevenir la transmissió vertical de l'HIV i el contagi de mares a fills es produïa en el 25-40% dels casos [81, 82]. Des de l'any 2003, i durant els anys en què es van dur a terme els estudis que formen part d'aquesta tesi, la política de prevenció de la transmissió vertical de l'HIV es basava en l'autoadministració d'una dosi de nevirapina abans del part i una altra dosi administrada al nadó durant les primeres 72 hores de vida, reduint la transmissió al 25% dels casos [83]. El tractament antiretroviral de gran activitat (HAART, de l'anglès *highly active anti retroviral therapy*), està disponible a la Manhiça des de 2004.

La prevalença de malnutrició a la zona és també elevada; segons dades dels anys 2001-2003, el 10% dels nens menors de cinc anys ingressats a l'HDM presentaven malnutrició severa [84].

2. Vigilància demogràfica a Manhiça

L'any 1996 es va fundar el CISM i aquell mateix any va establir un sistema de vigilància demogràfica a l'àrea del voltant del centre que continua funcionant avui dia. A cada persona que viu a la zona sota vigilància demogràfica (o àrea d'estudi) se li atorga un número d'identificació permanent que permet recollir de manera contínua informació demogràfica de tots els residents de la zona. Aquesta informació es recull mitjançant visites a totes les llars de l'àrea d'estudi dos cops l'any, durant les quals s'obté informació sobre migracions, morts, embarassos, avortaments i naixements (figura 6). A més a més hi ha establerta una xarxa d'informadors a la comunitat que setmanalment informen els treballadors de camp del departament de demografia del CISM sobre les morts i els naixements que han tingut lloc a la seva zona [85]. Aquest sistema de vigilància demogràfica permet que es puguin calcular incidències comunitàries tenint en compte el temps a risc de cada individu.

Figura 6. Treballadora del departament de demografia del CISM durant una visita a una casa de l'àrea d'estudi per actualitzar la informació demogràfica.

Tant la informació obtinguda durant les visites a les cases com la proporcionada pels informadors comunitaris, es recull en formularis estandarditzats que es digiten per duplicat al CISM (dues persones diferents digiten cada qüestionari i en cas de discrepància es verifiquen els qüestionaris originals).

L'àrea sota vigilància demogràfica s'ha anat ampliant des de la seva creació, i durant el període en què es van dur a terme els estudis inclosos en aquesta tesi, l'àrea englobava les poblacions de Manhiça, Manciana, Palmeira, Taninga i Ilha Josina, tenia una extensió de 500 km^2 i una població d'aproximadament 80.000 habitants, essent el 18% d'ells nens menors de cinc anys (figura 7).

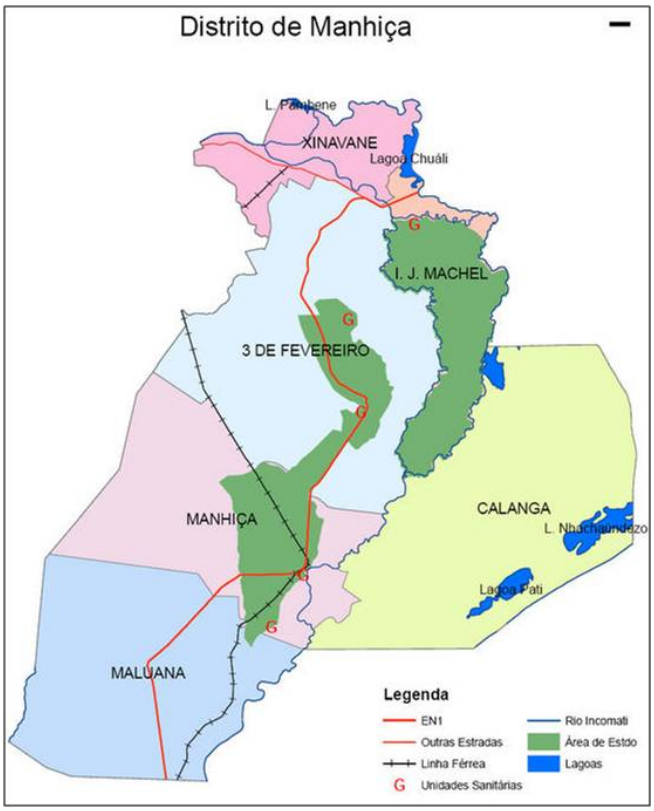

Figura 7. Mapa del districte de Manhiça. La zones marcades en verd correspon a l'àrea sota vigilància demogràfica (http://www.manhica.org).

3. Vigilància Hospitalària

La recollida de mostres utilitzades en els estudis que formen aquesta tesi es va dur a terme a l'HDM, el centre sanitari de referència del districte de Manhiça. L'any 1997 el CISM, en col·laboració amb l'HDM, va iniciar un sistema de vigilància contínua de mobilitat i mortalitat. Actualment hi ha tres ambulatoris a l'àrea d'estudi: Maragra, Taninga i Ilha Josina, que també participen del sistema de vigilància hospitalària. Des de 1997, l'HDM registra totes les visites pediàtriques que tenen lloc a les consultes externes de l'hospital i tots els ingressos hospitalaris. Per a totes les consultes pediàtriques, el metge o agent de salut que visita el nen omple un qüestionari estandarditzat en què es recull informació sobre símptomes i signes clínics, i en el cas que el nen sigui resident a l'àrea d'estudi, registra també el seu número d'identificació permanent. A aquells nens que tenen temperatura axil·lar ≥ 37,5ºC (o la mare reporta que el nen ha tingut febre en les 24 hores anteriors a la visita), se li agafa una mostra de sang del dit per determinar la presència de paràsits *P. falciparum* mitjançant

l'examen microscòpic d'una gota grossa i d'un frotis sanguini tenyits amb colorant Giemsa.

Per als nens ingressats a l'hospital, s'omple un altre qüestionari estandarditzat (en el qual també es registra el número d'identificació permanent, en el cas que es tracti d'un resident de l'àrea d'estudi) que recull símptomes i signes clínics de manera més detallada. A tots els nens menors de dos anys, i a aquells més grans amb temperatura axil·lar ≥ 39ºC o amb signes de severitat, se'ls agafa una mostra de sang (1-3 ml) per fer un hemocultiu. Als nens amb signes neurològics compatibles amb meningitis se'ls aplica efectua una punció lumbar [79].

Tots els qüestionaris, tant els omplerts a la clínica com els que contenen els resultats dels diversos tests de laboratori, es digiten també per duplicat al CISM. Tota la informació d'un mateix nen es pot unir utilitzant els números d'identificació de cada qüestionari. A més a més, per a aquells nens de l'àrea d'estudi, tota la informació recollida a la clínica es pot unir amb la informació demogràfica mitjançant el número d'identificació permanent.

4. Mètodes de laboratori

4.1. Estandardització dels mètodes de diagnòstic de virus respiratoris

El diagnòstic de virus respiratoris es va dur a terme utilitzant diferents PCR prèviament descrites per altres autors [86-88]. Per a la detecció de l'RSV A, l'RSV B, el Flu A i el Flu B vam modificar la tècnica descrita per Bellau-Pujol i col·laboradors [89], substituint la PCR niada (de anglès *nested PCR*) per una digestió amb enzims de restricció (taula 2).

Vam utilitzar els següents controls positius per poder optimitzar les PCR: per als virus Flu A, Flu B, RSV A i RSV B vam utilitzar extractes de cèl·lules infectades amb virus Flu A (H1N1) soca A/PR/8/34, Flu B, RSV A soca long i RSV B soca RSN-2, produïts i comprats a la *National Collection of Pathogenic Viruses* (NCPV, Heath Protection Agency-Centre for Applied Microbiology & Research, Wiltshire, UK), a partir dels quals vam extraure l'ARN víric. Per als altres virus (ADV, PIV1, PIV2, PIV3, PIV4AB, RV, EV i hMPV) vam

utilitzar controls d'ADN víric [producte de RT-PCR (de l'anglès *reverse transcription*-PCR)] clonats en pGEM-T Vector System I (Promega, Madison, WI) i transformats en cèl·lules competents, generats pel laboratori de virus respiratoris, del servei de virologia, del centre nacional de microbiologia del *Instituto de Salut Carlos III* (Madrid) [86-88] i cedits per la Dra. Pérez-Breña. Per determinar la sensibilitat de cadascuna de les PCR vam quantificar la concentració d'ADN del controls positius per espectrofotometria. Primer vam determinar la puresa dels controls positius a partir de la proporció d'absorbàncies a 260 nm i a 280 nm ($A_{260/280}$); vam considerar que els controls eren purs a partir de valors d' $A_{260/280}$ entre 1,2 i 1,8. A partir de l'absorbància a 260 nm, aplicant la llei de Beer Lambert, vam calcular la concentració de molècules d'ADN (o d'ARN)/ml en cadascun dels controls positius. Vam diluir els controls positius fins a arribar a valors exactes de molècules d'ADN/ml i vam fer dilucions successives 1:10 fins a arribar a concentracions de 10^{-4} molècules d'ADN/ml. Vam amplificar (mitjançant les respectives PCR) cadascun dels controls en les diferents concentracions i vam analitzar els productes per electroforesi en gel d'agarosa per tal d'observar el límit de detecció de les diferents reaccions (figura 8). La taula 2 mostra la sensibilitat de les PCR utilitzades en els estudis que formen aquesta tesi.

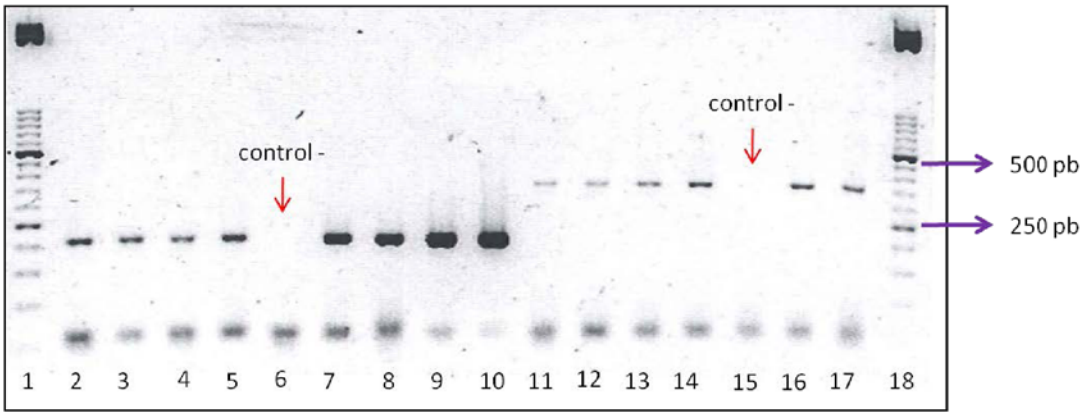

Figura 8. Detecció de diferents concentracions (molècules d'ARN/ml) de Flu A (212 pb) i Flu B (363 pb) en gel d'agarosa. Carril 1 i 18: marcador de pes molecular (XIII Molecular Weight Marker, Roche); carrils 2-5 i 7-9: concentracions de 10^{-3} fins a 10^{4} molècules d'ARN/ml de Flu A; carrils 11-14, 16 i 17: concentracions de 10^{-3} fins a 10^{2} molècules d'ARN/ml de Flu B; carrils 6 i 15: controls negatius.

Vam seguir mesures descrites per Coiras i col·laboradors [86] per evitar la contaminació de les mostres amb productes de l'amplificació de mostres (o controls) analitzades

anteriorment. La preparació de reactius, l'extracció d'ARN/ADN i les primeres reaccions (RT-PCR o PCR), i les segones reaccions (PCR niada, PCR seminiada o digestió amb enzims de restricció) es van dur a terme en cabines diferents, equipades amb material propi (tubs, gradetes, puntes amb filtre i micropipetes) i situades en laboratoris diferents. La detecció dels productes amplificats es va dur a terme en un altre laboratori. A cada laboratori utilitzàvem bates diferents. Després de treballar en qualsevol de les cabines, aquestes les netejàvem amb lleixiu al 10% i etanol, i enceníem la llum ultraviolada durant 15 minuts.

AUTORS	VIRUS	REACCIÓ 1						REACCIÓ 2					SENSIBILITAT
		REACCIÓ 1	PRIMERS	Seqüència (5' → 3')	CONDICIONS			REACCIÓ 2	ENZIMS RESTRICCIÓ/PRIMERS Seqüència (5'→3')	CONDICIONS	MIDA		
Adaptació de Bellau-Pujol et al. (89)	Flu A	Multiplex-RT-PRC	FluA1	CAG AGA CTT GAA GAT GTC TTT GCT GG				Digestió amb enzims de restricció	Nla III	3 hores a 37˚ C	51 + 150 + 11		10^{-3}
			FluA2	GCT CTG TCC ATG TTA TTT G	50˚C	30 min	1 cicle						
	Flu B		FluB1	AAA ATT ACA CTG TTG GTT CGG TG	94˚C	15 min			Nla III		251 + 27 + 29 + 56		10^{-3}
			FluB2	AGC GTT CCT AGT TTT ACT TG	94˚C	30 seg	40 cicles						
	RSV A		RSV1	GGA ACA AGT TGT TGA GGT TTA TGA ATA TGC	55˚C	30 seg			Tsp 509 I		36 + 69 + 174		10^{-3}
			RSV2	TTC TGC TGT CAA GTC TAG TAC ACT GTA GT	72˚C	1 min							
	RSV B		RSV1	GGA ACA AGT TGT TGA GGT TTA TGA ATA TGC	72˚C	10 min	1 cicle		Tsp 509 I	3 hores a 65˚ C	106 + 173		10^{-3}
			RSV2	TTC TGC TGT CAA GTC TAG TAC ACT GTA GT									
López-Huestas et al. (88).	hMPV	RT-PRC	hMPV1	GAG TCC TAC CTA GTA GAC AC				PCR seminiada	hMPV1	GAG TCC TAC CTA GTA GAC AC			
			hMPV2	TTG TYC CTT GRT GRC TCC A	45˚C	45 min	1 cicle		hMPV3	TCT TGC AKA TYY TRC TKA TGC T	95˚C	5:30 min	1 cicle
					95˚C	15 min					95˚C	30 seg	35 cicles
					95˚C	30 seg	45 cicles				55˚C	2 min	
					53˚C	2 min					72˚C	30 seg	
					68˚C	30 seg					72˚C	10 min	1 cicle
					68˚C	5 min	1 cicle						

718 | 10^{0}

Coiras et al. (86).	ADV	PCR	ADV1	CAA CAC CTA YGA STA CAT GAA	48˚C 45 min 1 cicle 95˚C 15 min 94˚C 30 seg 45 cicles 50˚C 2 min 72˚C 1 min 72˚C 5 min 1 cicle	PCR niada	ADV3	CCC ITT YAA CCA CCA CCG	95˚C 5 min 1 cicle 94˚C 30 seg 35 cicles 55˚C 1 min 72˚C 30 seg 72˚C 5 min 1 cicle	181	10^{-1}
			ADV2	KAT GGG GTA RAG CAT GTT			ADV4	ACA TCC TTB CKG AAG TTC CA			
Coiras et al. (87)	PIV1	Multiplex-RT-PRC	PIV13-1	AGG WTG YSM RGA TAT AGG RAA RTC ATA	48˚C 45 min 1 cicle 95˚C 15 min 94˚C 30 seg 45 cicles 55˚C 1:30 min 72˚C 1 min 72˚C 5 min 1 cicle	Multiplex-PCR niada	PIV13-3	ACG ACA AYA GGA ART CAT GYT CT	95˚C 5 min 1 cicle 94˚C 30 seg 35 cicles 55˚C 1 min 72˚C 30 seg 72˚C 5 min 1 cicle	439	10^{1}
			PIV13-2	CTW GTA TAT ATR TAG ATC TTK TTR CCT AGT			PIV1-4	GAC AAC AAT CTT TGG CCT ATC AGA TA			
	PIV2		PIV2-1	TAA TTC CTC TTA AAA TTG ACA GTA TCG A			PIV24-3	CYM AYG GRT GYA YTM GAA TWC CAT CATT		297	10^{1}
			PIV24-2	TRA GRC CMC CAT AYA MRG GAA ATA			PIV2-4	GCT AGA TCA GTT GTG GCA TAA TCT			
	PIV3		PIV13-1	AGG WTG YSM RGA TAT AGG RAA RTC ATA			PIV13-3	ACG ACA AYA GGA ART CAT GYT CT		39	10^{3}
			PIV13-2	CTW GTA TAT ATR TAG ATC TTK TTR CCT AGT			PIV1-4	GAG TTG ACC ATC CTY CTR TCT GAA AAC			
	PIV4AB		PIV4-1	ATC CAG ARR GAC GTC ACA TCA ACT CAT			PIV24-3	CYM AYG GRT GYA YTM GAA TWC CAT CATT		174	10^{4}
			PIV24-2	TRA GRC CMC CAT AYA MRG GAA ATA			PIV4-4	TGA CTA TRC TCG ACY TTR AAA TAA GG			
	RV		RVEV1	CTC CGG CCC CTG AAT RYG GCT AA			RVEV3	ACC RAS TAC TTT GGG TRW CCG TG		110	10^{-1}
			RVEV2	TCI GG IAR YTT CCA SYA CCA ICC			RVEV4	CTG TGT TGA WAC YTG AGC ICC CA			
	EV		RVEV1	CTC CGG CCC CTG AAT RYG GCT AA			RVEV3	ACC RAS TAC TTT GGG TRW CCG TG		226	10^{-1}
			RVEV2	TCI GG IAR YTT CCA SYA CCA ICC			RVEV4	CTG TGT TGA WAC YTG AGC ICC CA			

Taula 2: Protocols per a la detecció de virus respiratoris, mida dels productes de la reacció i sensibilitat (número de còpies d' AND/ml) després de d'optimitzar les PCR als laboratoris del CRESIB

4.2. Diagnòstic dels virus respiratoris

El diagnòstic dels diferents virus respiratoris dels estudis que formen aquesta tesi es va dur a terme a partir d'ANF recollits a l'HDM. Els ANF es recollien durant la consulta/ingrés a l'hospital i immediatament després es duien als laboratoris del CISM, on s'homogeneïtzaven, s'aliquotaven i es congelaven a -70ºC. Els ANF s'enviaven periòdicament als laboratoris del CRESIB a Barcelona, on vam realitzar el diagnòstic dels virus (figura 9).

Figura 9. Recol·lecció i processament de les mostres d'estudi: **a.** Obtenció d'aspirat nasofaringi (ANF) durant l'hospitalització; **b.** Processament inicial de l'ANF als laboratoris del CISM; **c.** Preparació d'un enviament d'ANF des del CISM cap als laboratoris del CRESIB.

L'ARN/ADN es va extraure seguint un protocol manual descrit per Casas i col·laboradors [90]. Tant per l'extracció d'ARN/ADN com per a les PCR, els ANF es van processar en grups de 25, incloent un control negatiu (aigua estèril lliure de RNAses) cada cinc mostres, per evitar contaminació entre mostres i per garantir que els resultats positius no eren fruit d'aquesta. Per a cada PCR incloíem també un control positiu (els mateixos que vam utilitzar per optimitzar les PCR i que he descrit a l'apartat anterior) per assegurar que totes les reaccions funcionaven correctament.

Vam seguir els protocols descrits a l'apartat anterior (taula 2) i vam analitzar els productes finals de les diferents reaccions per electroforesi en gel d'agarosa al 2,5% (2% per hMPV) amb 0,05 µl de SBYR-safe (Invitrogen) per identificar els diferents virus respiratoris, en funció de la mida o patró de bandes observat, utilitzant un marcador de pesos moleculars comercial (XIII Molecular Weight Marker, Roche) (figura 10).

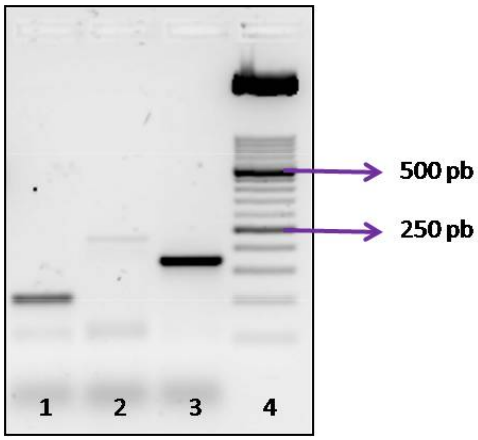

Figura 10. Detecció de diferents virus respiratoris en gel d'agarosa. Carril 1: RV (110 pb); carril 2: EV (226 pb); carril 3: PIV4AB (174pb), carril 4: marcador de pes molecular (XIII Molecular Weight Marker, Roche).

RESULTATS

RESULTATS

1. ARTICLE 1

Viral acute respiratory infections among infants visited in a rural hospital of southern Mozambique

Infeccions respiratòries agudes entre nens menors d'un any atesos en un hospital rural del sud de Moçambic

Cristina O'Callaghan-Gordo, Núria Díez-Padrisa, Fatima Abacassamo, Pilar Pérez-Breña, Inmaculada Casas, Pedro L. Alonso, Anna Roca

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RESUM

1.1. Objectiu

Descriure l'etiologia i l'epidemiologia de les infeccions respiratòries agudes d'origen víric entre nens menors d'un any atesos a les consultes externes de l'HDM.

1.2. Disseny de l'estudi

Aquest estudi es va dur a terme a partir d'una selecció sistemàtica d'ANF collits entre març de 1999 i febrer de 2000 a nens menors d'un any, atesos a les consultes externes de l'HDM amb ARI, en el context d'un estudi sobre l'epidemiologia de l'RSV. Aquesta selecció de 333 ANF [7% (333/4751) dels ANF collits inicialment] va ser testada per a altres virus respiratoris: RV, Flu A i Flu B, ADV, hMPV, PIV1, PIV2, PIV3, PIV4AB i EV mitjançant diferents PCR. Els resultats de l'RSV no estan inclosos en aquest article però es poden trobar en una publicació anterior [42] .

Per a aquest estudi, ARI es va definir com: presència de tos i/o secreció nasal, independentment de la presència d'altres símptomes respiratoris de més severitat.

1.3. Resultats principals

1. En el 56% (185/333) dels ANF testats vam detectar com a mínim un dels virus respiratoris inclosos a l'estudi. En total vam detectar 231 virus: 86 RV [26% (86⁄333)], 51 Flu [15% (51⁄333); 41 Flu A, 10 Flu B], 47 ADV [14% (47⁄333)], 22 hMPV [7% (22⁄333)], 16 PIV [5% (16⁄333); 2 PIV1, 4 PIV2, 9 PIV3, and 1 PIV4AB] i 9 EV [3% (9⁄333)].
2. En l'11% dels ANF testats vam detectar coinfecció vírica: en el 9% (30/333) dels ANF testats vam detectar dos virus i en el 2% (8/333) tres.
3. El 68% (125/185) dels casos amb infecció vírica es van produir durant l'estació de pluges. Vam detectar un patró estacional per Flu, amb dos pics marcats durant els mesos plujosos.
4. Entre els nens amb infecció vírica vam observar supuració de l'orella de manera més freqüent que entre la resta de nens de l'estudi [6% (11/183) *vs*. 1% (2/144); p=0,034].
5. Els nens amb coinfeccions víriques presentaven una clínica més severa, amb una probabilitat més elevada de tenir aleteig nasal [26% (10⁄38) *vs*. 12% (17⁄145); OR = 2,7 (95%CI = 1,1–6,5); p = 0,028] i tiratge [24% (9⁄38) *vs*. 8% (11⁄145); OR = 3,8 (95%CI = 1,4–9,9), p= 0,007].
6. No vam trobar diferències entre nens amb i sense infecció vírica pel que fa a la prevalença de malària [20% (24⁄123) *vs*. 19% (30⁄156); p= 0,953], anèmia [55% (86⁄157) *vs*. 59% (74⁄125); p= 0,456] o malnutrició [13% (24⁄185) *vs*. 14% (20⁄148); p= 0,885].
7. No vam trobar diferències en el risc d'hospitalització entre els nens amb i sense infecció vírica [10% (18⁄185) *vs*. 7% (10⁄148); p= 0,331]. D'altra banda, els nens amb infecció per l'ADV tenien més probabilitat de ser ingressats que els nens no infectats per l'ADV, i els nens amb coinfeccions víriques tenien més probabilitat de ser ingressats que els nens sense coinfeccions víriques [OR=3,35 (95%CI=1,38-8,12); p=0,007 i OR=4,42 (95%CI=1,18-10,76); p=0,001, en l'anàlisi ajustat per edat i sexe, per ADV i coinfeccions víriques respectivament].

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Viral acute respiratory infections among infants visited in a rural hospital of southern Mozambique

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Summary

OBJECTIVES To determine the epidemiology and clinical presentation of virus-associated acute respiratory infections (ARI) in Mozambican infants.

METHODS A systematic selection of nasopharyngeal aspirates (n = 333), collected from infants younger than 12 months who visited Manhiça District Hospital (southern Mozambique) with ARI during a 12 months respiratory syncitial virus surveillance, were tested for other common respiratory viruses. Four different polymerase chain reactions were used to diagnose rhinovirus (RV), influenza (Flu; A and B), adenovirus (ADV), human metapneumovirus (hMPV), parainfluenza (PIV; 1, 2, 3 and 4AB) and enterovirus (EV).

RESULTS At least one study virus was identified in more than half of the samples tested (185/333). Overall, 231 viruses were detected among 185 infants, listed in the order of prevalence: RV (26%), Flu (15%), ADV (14%), hMPV (7%), PIV (5%) and EV (3%). Acute respiratory infections (ARI) cases and viral episodes were seasonal and concentrate during the warm and the rainy season. Clinical features were similar among all study children regardless of the detection of virus, with the exception of ear discharge, which was more frequent among viral cases [6% (11/183) *vs.* 1% (2/144); P = 0.034]. Children with multiple viral infections had higher odds of severity such as nasal flaring and indrawing (OR = 2.7, P = 0.028 and OR = 3.8, P = 0.007, respectively) and higher odds of hospitalisation (OR = 4.42, P = 0.001, adjusted by age and sex).

CONCLUSIONS Viral ARI are frequent among infants visited in MHD. Strategies to prevent mild respiratory infections, and specially their complications, might alleviate health systems of source-limited settings.

keywords acute respiratory infection, respiratory viruses, epidemiology, developing countries

Introduction

Acute respiratory infections (ARI) are the main cause of morbidity and mortality in children under 5 years worldwide. Although lower respiratory infections (LRI) are the most severe forms of infection and cause of approximately 1.9 million annual deaths among children (Rudan *et al.* 2008), upper respiratory infections (URI) are the most frequent presentation of respiratory infections. An estimated 50% of all illnesses (not only respiratory diseases) in all age groups and approximately 75% of illnesses in young children are viral URI (Papadopoulos *et al.* 2006). Limited data available indicate that children have 6–8 and adults 2–4 episodes of URI per year (Heikkinen & Jarvinen 2003).

Although the incidence of ARI could be similar around the world, the prognosis is expected to be worse in rural Africa, where the underlying health status of children is poor and some risk factors such as malnutrition and anaemia, as well as other co-morbidities like malaria and HIV infection, are prevalent (Ezzati *et al.* 2002; Lopez *et al.* 2006).

There are few reports on overall viral ARI (Kusel *et al.* 2006; Fabbiani *et al.* 2009; Niang *et al.* 2010), as most studies concentrate on LRI. This paper presents data on the epidemiology of viral pathogens associated with ARI among infants attending the outpatients department (OPD) of a rural hospital in Mozambique between March 1999 and February 2000. Data on respiratory syncitial virus

(RSV) for the same children were described elsewhere (Loscertales *et al.* 2002) and is not included in the analysis.

Materials and methods

Study area and population

The study was conducted by the *Centro de Investigaçaõ em Saúde da Manhiça* (CISM) at the Manhiça District Hospital (MDH), the referral health facility for Manhiça District, a rural area of Maputo Province in southern Mozambique with approximately 143 000 inhabitants. The climate in the area is subtropical with two distinct seasons: a warm rainy season between November and April and a cool and generally dry season during the rest of the year. The annual rainfall in 1999 was 1600 mm. In February 2000, heavy rains flooded southern Mozambique; in this month alone more rain fell than during the whole previous rainy season (Christie & Hanlon 2001). Other details of the study site and population can be found elsewhere (Loscertales *et al.* 2002; Nhacolo *et al.* 2006).

Malaria transmission in Manhiça is perennial, peaking during the rainy season (Bassat *et al.* 2008). In 1999, the prevalence of HIV infection among pregnant women attending the antenatal clinic was 12% (Perez-Hoyos *et al.* 2011). During the study period, no mother-to-child-transmission (MTCT) control programs existed and MTCT of HIV occurred at an approximate rate of 25–40% (De Cock *et al.* 2000; Kourtis *et al.* 2006), which led to an expected HIV prevalence of approximately 3–5% in the birth cohort.

Clinical surveillance and clinical management

Since 1997, MDH and CISM have jointly operated a round-the-clock morbidity surveillance of all paediatric visits at the OPD and of all admissions to the wards. Hospital surveillance includes records of clinical signs and symptoms, and determination of malaria parasites and invasive bacterial infections.

Study design

This analysis is part of a larger study (Loscertales *et al.* 2002) designed to describe the epidemiology and clinical presentation of RSV among children attending to MDH. Between February 1999 and May 2000, all infants younger than 12 months who visited the OPD of MDH with signs and/or symptoms of ARI (defined as cough and/or nasal secretion) were offered participation in the study. Presence of other concomitant signs/symptoms or severity was not an exclusion criterion. After maternal oral consent, a nasopharyngeal aspirate (NPA) was collected by instilling 2 ml of normal saline into the nasopharynx followed by aspiration. The study was approved by the Institutional Review Board of the Hospital Clínic in Barcelona (Spain). Details of the study can be found elsewhere (Loscertales *et al.* 2002).

For the current analysis, a systematic selection of NPA collected during 12 months (March 1999–February 2000) was tested for common respiratory virus. Viral diagnosis included: rhinovirus (RV; A and B), influenza (Flu; A and B), adenovirus (ADV), human metapneumovirus (hMPV), parainfluenza (PIV; 1, 2, 3 and 4AB) and enterovirus (EV).

Definitions

For the purposes of the study, we defined ARI as cough and/or nasal secretion with or without any other respiratory symptom. 'Reported fever' refers to symptoms described by the mother, whereas 'fever' was considered when the axillary temperature was ≥37.5 °C.

As defined in the previous RSV study (Loscertales *et al.* 2002), anaemia was considered when packed cell volume (PCV) was <33% and classified according to PCV values as mild (PCV 25–33%), moderate (PCV 15–24%) or severe (PCV < 15%).

Mild to moderate malnutrition was diagnosed in children having a weight-for-length of <80%, weight-for-age of <80% or a final diagnosis of 'growth failure' or 'low weight'. Moderate to severe malnutrition was defined as weight-for-length <70%; weight-for-age <60%; or a diagnosis of kwashiorkor, marasmus or marasmic kwashiorkor.

Laboratory methods

Nasopharyngeal aspirate were processed daily: homogenised with 4 ml of Dulbecco's minimal essential medium and kept frozen at −70 °C. Nasopharyngeal aspirate used for the present analysis were transferred frozen to the reference laboratory in Barcelona in March 2006, where two multiplex polymerase chain reactions (PCR) and two individual PCR were performed to detect the study viruses (RV, Flu, ADV, hMPV, PIV and EV). Details on PCR protocols used are described elsewhere (O'Callaghan-Gordo *et al.* 2011).

Data management and analysis

Statistical analysis was performed using STATA/SE 11 (Stata Corp., College Station, TX, USA). Proportions were compared using Chi-square test, and odds ratios and 95% confidence intervals (95% CI) were estimated using logistic regression. Logistic regression (crude and adjusted) was used to describe factors associated with hospital admission.

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Results

Study profile

During the 12 months of surveillance, 5457 infants visited the OPD of MDH; 87% (4768/5457) presented with signs/symptoms of ARI and NPA was available for 99% (4751/4768. A systematic selection of 7% (333/4751) of these NPA were tested and in more than half [56% (185/333)] at least one respiratory virus was identified. Overall, 231 viruses were detected among these 185 samples: 86 RV [26% (86/333)], 51 Flu [15% (51/333); 41 Flu A, 10 Flu B], 47 ADV [14% (47/333)], 22 hMPV [7% (22/333)], 16 PIV [5% (16/333); 2 PIV1, 4 PIV2, 9 PIV3, and 1 PIV4AB], 9 EV [3% (9/333)]. Nine per cent (30/333) of the samples contained two viruses and 2% (8/333) contained three simultaneously. Adenovirus and hMPV had the highest percentages of co-detection [51% (24/47) and 41% (9/22) respectively, Table 1].

Epidemiological features of study children

Median age of children with NPA processed was 6 months (interquartile range: 3–9), whereas for the subgroup of children with at least one virus detected median age was 7 months (interquartile range: 4–9). Fifteen per cent (28/185) of viral cases occurred among infants aged <3 months, 24% (45/185) among infants aged 3–6 months and 61% (112/185) among infants aged 6–12 months. There were no significant differences between the median ages of children infected by the different study viruses.

Most viral episodes [68% (125/185)] occurred during the warmer and rainy months of the follow-up period, from March to May 1999 and from December 1999 to February 2000 (Figure 1A). As shown, Flu detections were seasonal and caused outbreaks during the rainy months (at the start and at the end of the surveillance period). Flu A peaked in March 1999 and was followed by Flu B in April. During the second peak (January–February 2000), only Flu A was detected. During the floods of February 2000, 33% of overall Flu cases were detected. The other study viruses were detected over the study surveillance with no clear pattern of seasonality (Figure 1B). However, ADV peaked at the beginning of the rainy season and hMPV detections increased during the second half of the surveillance period.

Clinical presentation

Signs and symptoms. Clinical features were compared between children with and without viral detection (Table 2). No significant differences were found between groups with the exception of ear discharge, which was more frequent among the viral group [6% (11/183) *vs.* 1% (2/144); $P = 0.034$].

When clinical presentation was stratified by study virus ($n = 185$), again, only few differences were observed between viruses (for these comparisons, we created a group that includes children with viral co-detections). Infants with hMPV detected were more likely to present wheezing than children with other virus detected [15% (2/13) *vs.* 2% (3/170); OR = 10 (95%CI = 1.5–67), $P < 0.016$]. And most importantly, children with multiple viral detection had higher odds of presenting signs of severity such as nasal flaring [26% (10/38) *vs.* 12% (17/145); OR = 2.7 (95%CI = 1.1–6.5), $P = 0.028$] and indrawing [24% (9/38) *vs.* 8% (11/145); OR = 3.8

Table 1 Number and percentages of simple, double and triple viral infections detected among infants with signs and symptoms of acute respiratory infections by combination of virus

	RV, $n = 86$ n (%)*	Flu, $n = 51$ n (%)*	ADV, $n = 47$ n (%)*	hMPV, $n = 22$ n (%)*	PIV, $n = 16$ n (%)*	EV, $n = 9$ n (%)*
RV	58 (67)†	8 (16)	9 (19)	3 (14)	1 (6)	0
Flu	8 (9)	34 (67)†	3 (6)	0	0	0
ADV	9 (10)	3 (6)	23 (49)†	2 (9)	1 (6)	2 (22)
hMPV	3 (3)	0	2 (4)	13 (59) †	1 (6)	0
PIV	1 (1)	0	1 (2)	1 (5)	12 (75)†	0
EV	0	0	2 (4)	0	0	7 (78)†
Triple detections‡	7 (8)	6 (12)	7 (15)	3 (14)	1 (6)	0

ADV, adenovirus; EV, enterovirus; hMPV, human metapneumovirus; PIV, parainfluenza; RV, rhinovirus.
*Percentages calculated by columns.
†Simple detections.
‡Combination of viruses found in triple detections: four cases of Flu A + ADV + RV, two cases of ADV + RV + hMPV, two cases of Flu A + ADV + PIV3 and one case of Flu A + RV + hMPV.

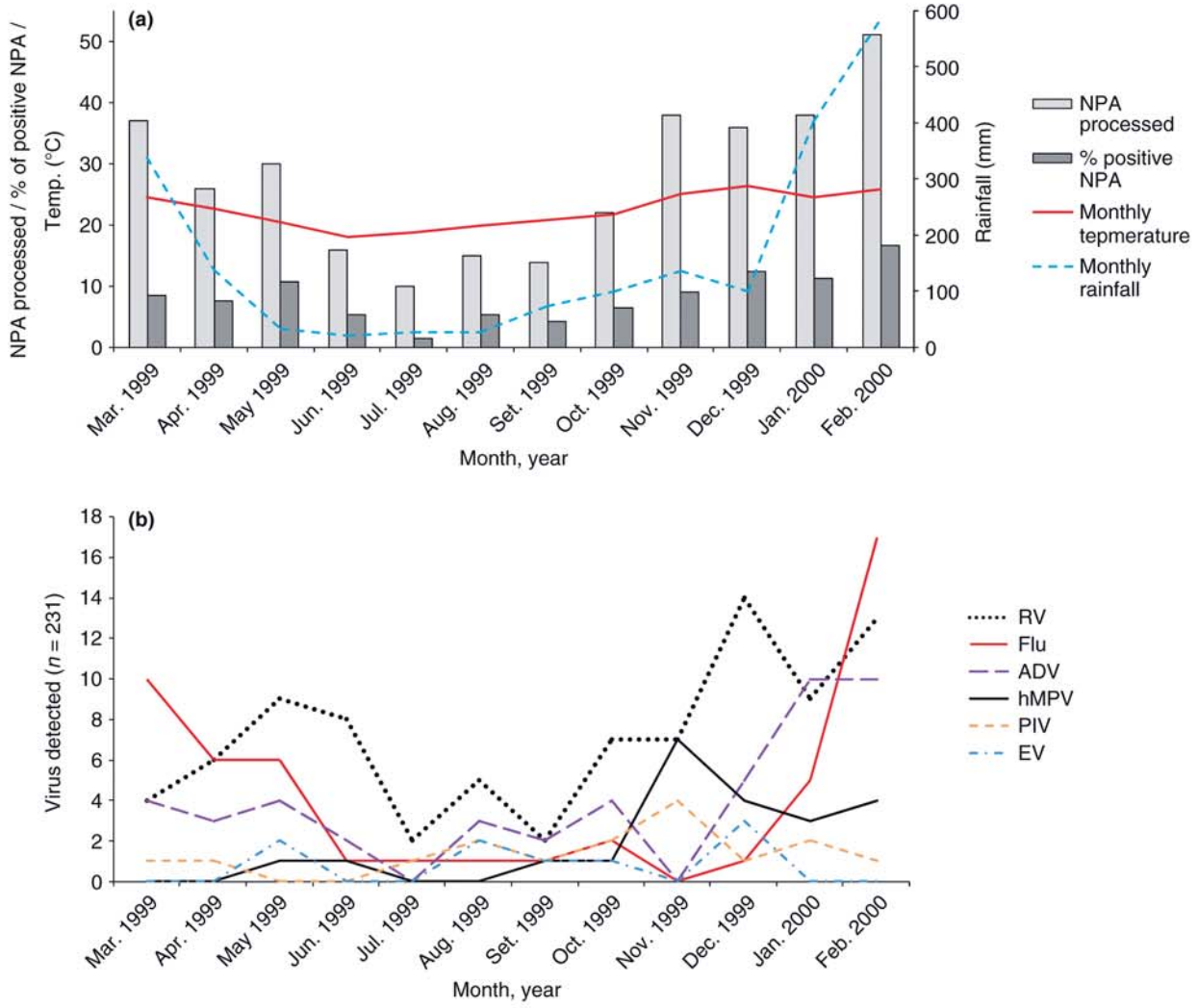

Figure 1 (a) Number of nasopharyngeal aspirate (NPA) tested and percentage of NPA with at leat one study virus detected by month of collection related to monthly temperaturs and rainfall patern. (b) Number of diagnosed virus by month.

(95%CI = 1.4–9.9), $P = 0.007$], as well as a trend for dyspnea [13% (5/38) *vs.* 5% (7 /144); OR = 2.9 (95%CI = 0.88–9.9), $P = 0.075$].

Co-morbidities. Plasmodium falciparum parasitemia, anaemia and malnutrition were common conditions among study children: 19% (54 of 279 children tested for parasitemia) harboured malaria parasites, 57% (160 of 282 with known results) had anaemia and 13% (44/333) presented malnutrition. However, no differences were observed between children with and without virus [20% (24/123) *vs.* 19% (30/156), $P = 0.953$, for malaria parasites; 55% (86/157) *vs.* 59% (74/125), $P = 0.456$, for anaemia and 13% (24/185) *vs.* 14% (20/148), $P = 0.885$, for malnutrition].

Rate of hospitalisation. Overall, 8% (28/333) of study infants were hospitalised. The median age of admitted children was 3 months (interquartile range: 2–3 months); 18% (5/28) presented mild to moderate malnutrition and 23% (6/26 with parasitemia results available) harboured *P. falciparum* parasites. Hospitalisation rates were similar for children with and without viral infection [10% (18/185) *vs.* 7% (10/148), $P = 0.331$].

When looking at each virus (Table 3), we found that infants with ADV and children with multiple viruses had

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Table 2 Clinical features of children <1 year of age seen at Manhiça District Hospital with signs and/or symptoms of acute respiratory infections (cough and/or nasal secretion) and tested for study virus according to the presence of at least one study virus

	All acute respiratory infections cases, n (%)		
	No virus, n (%) n = 148	Virus, n (%) n = 185	P-value
Nasal secretion	148 (100)	185 (100)	1.000
Reported fever	128 (86)	159 (86)	0.887
Cough	137 (93)	167 (90)	0.227
Anxillary temp.≥ 37,5 °C	47 (32)	69 (37)	0.887
Crackles	45 (30)	46 (25)	0.221
IRR*	40 (27)	51 (28)	0.912
Nasal flaring	29 (20)	27 (15)	0.199
Chest indrawing	17 (11)	20 (11)	0.804
Dyspnea	11 (7)	12 (6)	0.714
Vomiting	29 (20)	50 (27)	0.132
Lethargy	7 (5)	16 (9)	0.173
Ear discharge	2 (1)	11 (6)	0.034
Dehydratation	2 (1)	9 (5)	0.079
Weezing	2 (1)	5 (3)	0.405

*Increased respiratory rate (IRR) defined according to age group as 60 or more breaths/min for children <2 months old, 50 or more for children between 2 and 12 months old.

significantly higher odds of hospitalisation than other infants in both crude and adjusted analysis (adjusted by age and sex).

Discussion

According to our results, viral respiratory detections in infants are frequent among outpatient visits in MDH. Most infants visiting the outpatient clinic presented signs/symptoms of ARI and more than half of children with NPA tested had at least one respiratory virus detected. This percentage might underestimate the real frequency of viral detection among infants, as RSV, one of the most prevalent viruses among infants with respiratory signs/symptoms (Kusel *et al.* 2006; Fabbiani *et al.* 2009; Niang *et al.* 2010), was not included in our analysis.

According to the results from the previous study (Loscertales *et al.* 2002), RSV was detected in 9% (30/333) of children (data not shown). We did not include these results in the current analysis for two main reasons. First, the technique used to diagnose RSV was ELISA (TESTBACK RSV; Abbott Laboratories), and the current respiratory viruses were detected by PCR. Is it well known that PCR has a higher sensitivity than ELISA (Magnard *et al.* 1999). On the other hand, RSV diagnostic was carried out with fresh samples shortly after collection, whereas NPA were frozen and stored before being used to diagnose other virus, which might also lead to differences in sensitivity. Although we cannot know how prevalent RSV would be if we had used the same method of diagnostic as for the other respiratory viruses, it is clear that inclusion of RSV diagnosis would have substantially increased the number of viral ARI cases detected among infants seen at the OPD

Table 3 Risk of hospitalization among children <1 years of age seen at Manhiça District Hospital with signs and/or symptoms of acute respiratory infections according to the presence of at least one study virus, according to virus detected and according to the presence of more than one virus at the same time

		Total	No hosptalization n = 305	Hospitalization n = 28	OR*	P-value	95% CI
Any viral infection	No	148	138 (93)	10 (7)	1.53	0.303	0.67–3.46
	Yes	185	167 (90)	18 (10)			
Rhinovirus	No	247	229 (93)	18 (7)	1.67	0.220	0.74–3.78
	Yes	86	76 (88)	10 (12)			
Flu	No	282	258 (91)	24 (9)	0.93	0.92	0.31–2.84
	Yes	51	47 (92)	4 (8)			
Adenovirus	No	286	267 (93)	19 (7)	3.35	0.007	1.38–8.12
	Yes	47	38 (81)	9 (19)			
Human metapneumovirus	No	311	286 (92)	25 (8)	1.79	0.374	0.49–6.52
	Yes	22	19 (86)	3 (14)			
PIV	No	317	291 (92)	26 (8)	1.66	0.519	0.36–7.76
	Yes	16	14 (88)	2 (12)			
Enterovirus	No	324	298 (92)	26 (8)	3.46	0.414	0.66–17.99
	Yes	9	7 (78)	2 (22)			
Multiuple viral infection	No	295	276 (94)	19 (6)	4.42	0.001	1.81–10.76
	Yes	38	29 (76)	9 (24)			

*Adjusted by age group and sex.

of MDH. Seasonality of RSV was similar to Flu, as we have already seen among children admitted with viral pneumonia (O'Callaghan-Gordo *et al.* 2011).

Two out of three hospital consultations at MDH occurred during the rainy season. Previous studies conducted in our setting showed an important increase in the number of outpatients visits during the rainy months of the year associated with different illnesses, such as malaria (Guinovart *et al.* 2008) and LRI (Loscertales *et al.* 2002). The current study showed that mild viral respiratory infections also contribute to the high burden of hospital visits that annually collapse the OPD during these months of the year. According to previous data, the annual RSV outbreak also occurs during these months (Loscertales *et al.* 2002).

During the heavy rains of February 2000, many people from the study area had to flee their flooded homes and take refuge in shelters (Christie & Hanlon 2001). This emergency situation might have favoured the transmission of viral respiratory infections (by overcrowding and poor sanitary conditions in shelters), and indeed up to 15% of all ARI cases were detected during that month, as well as one in three Flu detections. The peak in ARI observed during the rainy month seem to be real and cannot be attributed to any change in case ascertainment of the study.

Whether multiple viral infections are associated with more severe disease remains an unresolved topic. Our results showed that infants with more than one virus presented a higher prevalence of severity signs (nasal flaring, indrawing and dyspnea) and were at higher risk/odds of hospitalisation. These results agree with those of previous studies that describe a worse prognosis among either viral co-infections *per se* (Calvo *et al.* 2008; Richard *et al.* 2008) or certain viral combinations (Aberle *et al.* 2005; Semple *et al.* 2005). However, other investigators found no increased severity of disease in children with viral co-infections (Choi *et al.* 2006; Garcia-Garcia *et al.* 2006; Wolf *et al.* 2006). It is difficult to ascertain whether our results are confounded by other variables not considered in the analysis, as for instance HIV. Prolonged viral shedding has been demonstrated in HIV-infected patients (King 1997) and as long-term shedding increases the possibility of simultaneous detection, viral co-detections might be more frequent among HIV-infected infants.

Similarly to co-infections, we also observed higher odds of hospitalisation among children with ADV. As ADV was the most prevalent virus detected among children with viral co-detections, we cannot know whether it was ADV or the combination with another virus that led to increased disease severity. Either ADV or viral co-infections could have resulted in the higher odds of hospitalisation we observed.

Infants from the viral group had higher risk of acute otitis media [(AOM), defined as ear discharge], a leading cause of care attendance in infants and toddlers all over the world (Rovers 2008). Respiratory viruses have been found in up to 70% of AOM cases, and although colonisation mechanisms are not clear, many studies point at viral infections as an important predisposing factor for development of AOM (Nokso-Koivisto *et al.* 2006).

Evaluating the global burden of mild infections in rural Africa is difficult using a hospital-based study, as it is influenced by health-seeking behaviour and hospital accessibility. Only active case ascertainment would give robust data on the disease burden of these infections. Nonetheless, our study generated valuable data on frequency of viral ARI among infants attending to hospital in rural Mozambique and reinforces the idea that mechanisms to prevent mild respiratory infections might alleviate health systems in poor countries such as Mozambique, as well as reduce the number of more severe respiratory infections frequently produced by complications of ARI.

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2. ARTICLE 2

Etiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique. A malaria endemic area with high prevalence of human immunodeficiency virus

Etiologia i epidemiologia de la pneumònia vírica entre nens hospitalitzats en una zona rural de Moçambic, una àrea on la malària és endèmica i la prevalença del virus de la immunodeficiència humana és elevada

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RESUM

2.1. Objectiu

Descriure l'etiologia i l'epidemiologia de les infeccions respiratòries agudes d'origen víric entre nens menors de cinc anys ingressats a l'HDM amb símptomes de pneumònia severa.

2.2. Disseny de l'estudi

Aquest estudi es va dur a terme entre setembre de 2006 i setembre de 2007 a l'HDM. Durant aquest període, vam recollir ANF a tots els nens menors de cinc anys que ingressaven a l'hospital amb clínica de pneumònia severa per al diagnòstic dels següents virus respiratoris: RSV A, RSV B, RV, Flu A, Flu B, ADV, hMPV, PIV1, PIV2, PIV3, PIV4AB i EV, mitjançant diverses PCR. Durant l'ingrés hospitalari vam recollir també informació clínica detallada sobre diversos signes i símptomes clínics respiratoris utilitzant qüestionaris estandarditzats, i vam realitzar testos per diagnosticar presència de paràsits de la malària (*P.falciparum*), infecció bacteriana invasiva i infecció per l'HIV.

La definició de clínica de pneumònia severa utilitzada en aquest estudi fou la següent: tos i/o freqüència respiratòria (FR) elevada per l'edat segons la definició de l'OMS

(≤2mesos:FR ≥ 60; >2-12mesos:FR ≥ 50; >12-60 mesos: FR ≥ 40) [91] i com a mínim un dels següents signes: tiratge, aleteig nasal, gemecs expiratoris (en anglès *grunting*) o crepitacions (en anglès *crackles*) detectats durant l'examinació del nen pel pediatra de l'estudi.

2.3. Resultats principals

1. Durant els 12 mesos d'estudi van ingressar 835 nens amb clínica de pneumònia severa i van acceptar formar part de l'estudi. Vam recollir i processar els ANF del 97% d'aquests nens (807/835).
2. En el 49% (394/807) dels ANF testats vam detectar com a mínim un virus respiratori. En total vam detectar 475 virus: 196 RV [41% (196/475)], 102 ADV [21% (102/475)], 50 RSV [11% (50/475), tots RSV A], 39 hMPV [8% (39/475)], 39 Flu [8% (39/475); 21 Flu A i 18 Flu B], 31 PIV [7% (31/475); 3 PIV1, 10 PIV2, 7 PIV3 i 11 PIV 4] i 18 EV [4% (18/475)].
3. En el 20% dels casos vam detectar coinfeccions víriques: en el 19% (73/394) dels casos vam detectar dos virus i en l'1% (4/394) tres.
4. La distribució del nombre de casos segons l'edat va variar en funció del virus respiratori detectat. L'ADV i el Flu van ser els virus més freqüents en nens de 12-60 mesos, i l'RSV i l'hMPV els més freqüents en nens menors de tres mesos.
5. El 59% (478/897) dels nens menors de cinc anys amb clínica de pneumònia severa van ingressar durant l'estació de pluges (de novembre a abril), i el 64% (252/394) dels virus respiratoris es van detectar durant el mateix període. El brot anual de l'RSV es va detectar entre gener i juliol, amb un pic marcat entre març i maig, mesos en què es van produir el 78% (39/50) dels casos de l'RSV. El brot del Flu va començar unes setmanes abans que el brot de l'RSV, amb un increment de casos del Flu B seguit d'un increment de casos del Flu A. El brot del Flu va durar fins a mitjans de maig, tot i que el 59% (23/39) de les infeccions pel Flu es van produir durant el mes de març. La resta de virus respiratoris es van detectar durant tot l'any, amb variacions en el nombre de casos segons el mes, però sense un patró estacional gaire clar.
6. L'11% dels nens amb infecció vírica (38 dels 345 amb el resultat de l'hemocultiu no contaminat) tenien infecció bacteriana invasiva, el 15% (57 dels 389 testats) tenien

paràsits de *P.falciparum* i el 25% (67 dels 207 testats) tenien infecció per l'HIV. No vam detectar diferències en la prevalença de cap d'aquestes infeccions en funció del virus respiratori detectat.

7. La incidència d'infecció per qualsevol dels virus respiratoris inclosos a l'estudi va ser entre 5,5 i 16 cops més elevada per a nens infectats per l'HIV. Les incidències d'infecció de tots els virus van ser més elevades per a nens infectats per l'HIV, però amb variacions en funció del virus detectat.
8. La mortalitat intrahospitalària pels nens amb, com a mínim, un virus respiratori detectat fou del 9% (33/359), i del 3% entre aquells nens amb, com a mínim, un virus respiratori però sense cap altra infecció detectada (sense malària, sense infecció bacteriana invasiva i sense HIV). Les probabilitats de morir durant l'ingrés hospitalari van ser més elevades per nens amb coinfecció vírica [OR=2,17 (95%CI=1,00-4,72); p=0,050], amb infecció bacteriana invasiva [OR=7,02 (95%CI=2,88-17,12); p<0,001] o amb infecció per l'HIV [OR=6,75 (95%CI=2,33-19,52); p<0,001].

ORIGINAL STUDIES

Etiology and Epidemiology of Viral Pneumonia Among Hospitalized Children in Rural Mozambique

A Malaria Endemic Area With High Prevalence of Human Immunodeficiency Virus

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Background: The role of viruses in pediatric pneumonia remains poorly studied in sub-Saharan Africa, where pneumonia-associated mortality is high.

Methods: During a 1-year hospital-based surveillance, a nasopharyngeal aspirate (NPA) was collected from children aged <5 years admitted to hospital in rural Mozambique with clinically severe pneumonia. Identification of 12 respiratory viruses was performed by polymerase chain reactions (PCR). Study children were also tested for invasive bacterial infection (IBI), *Plasmodium falciparum* parasitemia, and HIV.

Results: Almost half (394/807) of the children hospitalized with clinically severe pneumonia had at least one respiratory virus detected. A total of 475 viruses were detected among these 394 children, the most prevalent ones were rhinovirus (41%), adenovirus (21%), and respiratory syncytial virus (11%). Eleven percent of viral infected children had concomitant IBI, 15% had malaria parasites, and 25% had HIV coinfection. Viral infection was 5.5 to 16 times more prevalent among HIV-infected children and incidence rate ratios varied according to virus. Inhospital mortality of viral cases was 9%, being highest among cases with IBI coinfection (odds ratio = 7) or HIV infection (odds ratio = 7).

Conclusions: Study results highlight the high prevalence of respiratory viruses among hospitalized pneumonia cases in Mozambique. HIV infection is an important contributor to the high burden of disease and associated mortality of viral pneumonia. IBI also contributes to a worse prognosis of viral cases. Strategies to prevent mother-to-child transmission of HIV as well as introduction of Hib and pneumococcal vaccines could have a substantial impact on reduction of viral pneumonia and associated mortality among children in rural Africa.

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Pneumonia, one of the most important causes of morbidity and mortality in children worldwide,[1–4] causes approximately 900,000 deaths each year in sub-Saharan Africa.[4] Although various respiratory viruses have been described in pneumonia episodes,[5–7] viral data beyond respiratory syncytial virus (RSV) are scarce,[8] especially in developing and rural settings.[9] The relevance of the coexistence and the potential interaction between viruses and bacteria also remains poorly explored[3] and very few data are available from rural Africa.[9] Moreover the HIV pandemic, whose burden is mainly felt in this area of the world,[10] has significantly altered the dynamics of pneumonia.[11,12]

Etiologic differentiation of the most prevalent causes of pneumonia is necessary to provide evidence-based guidance for treatment recommendations and for the design of preventive strategies. This article presents surveillance data on the epidemiology of several respiratory viruses associated with clinical pneumonia in children <5 years admitted to a rural hospital in Mozambique, a malaria endemic area with high HIV prevalence. Viral detection in this study included RSV (A and B), influenza (Flu [A and B]), parainfluenza (PIV [1, 2, 3, and 4]), human metapneumovirus (hMPV), adenovirus (ADV), rhinovirus (RV), and enteroviruses (EV). The role of concomitant bacterial and malaria infections as well as HIV status on viral pneumonia cases was also evaluated.

MATERIALS AND METHODS

Study Area and Population

The study was conducted by the Centro de Investigação em Saúde da Manhiça (CISM) at the Manhiça District Hospital (MDH), the referral health facility for Manhiça District, a rural area of Maputo Province in Southern Mozambique. Details of the study site have been described elsewhere.[13,14] Briefly, the climate of the area is subtropical with 2 distinct seasons: a warm and rainy season between November and April and a cool and dry season during the rest of the year. Malaria transmission in Manhiça is perennial, peaking during the rainy season.[15] In 2004, the prevalence of HIV infection among pregnant women attending the hospital antenatal clinic was 23.6%.[16] During the study period, Hib and pneumococcal conjugated vaccine were not included among routine infant immunizations in Mozambique.

CISM has been running a continuous demographic surveillance system (DSS) in the area since 1996. During the study period, approximately 80,000 inhabitants (18% of them being children <5 years) were under demographic surveillance, covering an area of 500 km^2 surrounding the hospital. Each child living

within the DSS study area is issued a unique permanent identification number that is recorded at each hospital visit. Details of the DSS are described elsewhere.[14] All households in the DSS area are visited every 6 months to record all births, deaths, and changes in residency of the population in addition to active surveillance for vital events.

Hospital Surveillance and Clinical Management

Since January 1997, the Hospital and CISM have jointly operated a round-the-clock morbidity surveillance of all pediatric visits at the outpatient department and of all admissions to the wards.[13] Hospital surveillance includes determination of malaria parasites and invasive bacterial infections (IBIs).[17]

Study Design

This analysis is part of a larger study designed to describe the clinical and epidemiologic characteristics of children admitted with respiratory distress to MDH. As part of the study protocol, children <5 years admitted to MDH during a 1-year period (September 20, 2006–September 19, 2007) with signs or symptoms suggestive of clinical severe pneumonia, defined as cough and/or increased respiratory rate (according to the WHO definition[18]) and at least one of the following: indrawing, nasal flaring, grunting or crackles detected through examination, were considered for the study and carefully investigated and followed up.

Informed written consent was obtained from each child's mother or caregiver before enrolment. Data presented here report the etiology and epidemiology of respiratory viruses detected among the children recruited for the larger study.

Sample Collection Among Study Children

As part of the study protocol, a nasopharyngeal aspirate (NPA) was collected from all study children using NPA kits (M-Pro, Michigan, United States) to diagnose respiratory viruses. A single venous blood specimen was collected for bacterial culture and a lumbar puncture was performed to all children admitted with neurologic signs compatible with meningitis.[19] A finger-prick blood sample was collected for microscopy diagnosis of malaria and packed-cell volume determination from children with fever (axillary temperature, ≥37.5°C). Bacterial surveillance and malaria parasites determination are part of routine clinical practice among admitted patients at the hospital.[13]

Pediatric HIV testing, usually not considered a standard routine determination at MDH, was performed to study children, and required another signed parental consent. HIV testing was limited to study children who resided in the DSS study area, as specific HIV-related treatment could only be guaranteed for people that could be traced back in the community. Children identified as HIV-infected were followed up according to national guidelines for HIV treatment.[20] The study was approved by the Mozambican National Bioethics Committee and the Institutional Review Board of the Hospital Clínic in Barcelona (Spain).

Laboratory Methods

NPA was processed on a daily basis: homogenized with 3 mL of RPMI, aliquoted, frozen at −70°C, and sent periodically to the IDIBAPS laboratory in Barcelona for viral detection. RNA/DNA was extracted using a method described by others.[21] Four different polymerase chain reaction (PCR) were performed for detection of respiratory viruses. RSV A and B, and Flu A and B viruses were detected by a multiplex reverse transcription (RT)-PCR (adapted version of the multiplex RT-heminested-PCR) described by Bellau-Pujol et al.[22] Amplified products obtained in the RT-PCR were digested using specific restriction enzymes (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A572, shows restriction enzymes used and digestion patterns). PIV 1, PIV 2, PIV 3, PIV 4AB, RV, and EV were detected using a multiplex RT-nested-PCR described by Coiras et al.[23] ADV was detected using primers described by Avellon et al[24] with PCR-adapted conditions.[25] hMPV were detected using a heminested-PCR described by López-Huertas et al.[26] Positive controls were included in each PCR assay. A negative control (RNase-free sterile water) was used for every 5 clinical samples processed.

Plasmodium falciparum parasitemia was diagnosed by microscopy and was considered positive when one or more asexual parasites were seen after observation (by 2 different microscopists) of 200 leukocytes in a thick and thin Giemsa-stained blood film.

Blood and cerebrospinal fluid (CSF) were processed following conventional microbiologic methods.[19] IBI was diagnosed when bacterial growth was observed in either blood or CSF culture. Coagulase-negative *staphylococci*, *Bacillus* species, or micrococci are considered blood and CSF contaminants, whereas *Streptococcus viridans* is considered contamination only in blood cultures.

HIV-1 seropositive children were identified using a sequential testing algorithm with 2 rapid HIV-1 antibody tests (Determine [Abbott Laboratories, North Chicago, IL] and Unigold [Trinity Biotech, Bray, Ireland]) according to national guidelines. Filter paper blood samples were collected for subsequent confirmatory testing that was performed monthly using the Amplicor HIV-1 DNA assay (version 1.5, Roche Molecular Systems, Branchburg, NJ) with dried blood spots. Children older than 18 months were assumed to be HIV infected if both rapid tests were positive. For seropositive children younger than 18 months and older children with discordant serologic tests, HIV infection was confirmed by a positive Amplicor test.

Data Management and Analysis

Proportions were compared using χ^2 test. Odds ratio and 95% confidence intervals (95% CI) were estimated using logistic regression. Unless otherwise indicated, comparisons used "children" as unit of analysis, rather than "viruses detected." Inhospital mortality excluded children with unknown outcome because of transfer to another hospital or withdrawal from hospital against medical advice.

Incidence rates were calculated for each of the study viruses and stratified by HIV status (only episodes from children with known HIV status were included). For the stratified incidence rate, calculation time at risk for both HIV-infected and HIV-uninfected children was estimated (as HIV prevalence among the birth cohort in the DSS study area was unknown) considering the following data: (i) Children <5 years of age during the study surveillance (21,767, according to DSS data); (ii) data on HIV prevalence among pregnant women attending the antenatal clinic in the study setting (23.6%[16]); and (iii) Mother-to-child transmission (MTCT) of HIV. A sensitivity analysis of incidence rates was performed considering 2 different values of MTCT of HIV as it varies depending on measures available to decrease vertical transmission. According to data from the study setting, when nevirapine is given following clinical protocol at the onset of labor, MTCT of HIV is 12.4%,[27] and this prevalence was considered the minimum value in our analysis of sensitivity. When no measures are available, MTCT of HIV can be as high as 34% according to data from the neighboring South Africa,[28] and this percentage was considered the maximum value in our analysis of sensitivity. Using these 2 values of MTCT of HIV, estimated prevalence of HIV in the birth cohort was between 2.9% and 8.0%. Mortality rate among HIV-uninfected children was considered 10% (data on the overall mortality rate among children from the DSS study area during the surveillance period), and mortality rate among HIV-infected children was considered 62%, (according to data from Rwanda[29]).

Although there is no data on <5 mortality among HIV-infected children in the study area, infant mortality is the same in both the study in Rwanda and our study area.[27] All deaths were considered at midpoint of the study. Incidences were expressed as episodes per 1000 person-years at risk.

All analyses were performed using STATA/SE 11 (StataCorp 2009, Stata: Release 11, Statistical Software, College Station, TX).

RESULTS

Study Profile

During the 12 months of surveillance 2951 children <5 years were admitted to the MDH. Twenty-eight percent of these children (835/2951) met study criteria, provided consent and were recruited. NPA was available from 97% (807/835) of them. Almost half of the nasopharyngeal samples (49%, 394/807) were positive for at least one of the 12 viruses tested. Four hundred seventy-five viruses were detected from 394 children, being in decreasing order of prevalence: RV (41% [196/475]), ADV (21% [102/475]), RSV (11% [50/475]; all being RSV-A), hMPV (8% [39/475]), Flu (8% [39/475]; 21 Flu A and 18 Flu B), PIV (7% [31/475]; 3 PIV 1, 10 PIV 2, 7 PIV 3 and 11 PIV 4), and EV (4% [18/475]). Nineteen percent (73/394) of the cases had double viral detection, ADV and EV had the highest percentage of codetection (41% and 42% respectively), and the most frequent viral combination found was RV-ADV (44% [32/73] of cases with double viral detection). One percent (4/394) of the viral cases had triple viral detection (1 for each of the following combinations: PIV, EV, and RV; RSV, ADV, and PIV; ADV, RV, and hMPV; and Flu, ADV, and RV).

Epidemiologic Features of Children With Viral Infection

Approximately 60% (478/807) of the NPA were collected during the rainy season, and the number of viral detected cases was also higher during this period (64% [252/394], $P = 0.008$). Figure 1 illustrates the number of viruses detected per month during the overall surveillance (n = 475). RSV, Flu, and PIV infections were seasonal. RSV cases were found from January to July, peaking between March and May (78% [39/50]). Flu main outbreak overlapped with RSV outbreak but was shorter; 59% (23/39) of Flu cases occurred in March only. Flu B peaked in early March and was followed by Flu A extending throughout April. The rest of viruses were detected throughout the year (Fig. 1).

Median age of study children with at least one virus detected was 11 months (interquartile range 4–21), and as shown in Table 1 age distribution varied according to virus detected ($P < 0.001$).

Viral Distribution and Concomitant Infections

Almost all children with virus detected were tested for IBI and malaria parasites on admission. Concomitant IBI was found in 11% (38 of 345 children tested for IBI and with a valid result [contaminations excluded]) of children with virus detected. All IBI cases were bacteremias, and 6 of them also had positive results from the CSF culture. *Streptococcus pneumoniae* (29% [11/38]), *Haemophilus influenza* b (29% [11/38]), and *Staphylococcus aureus* (11% [4/38]) were the most common bacterial pathogens. Malaria parasites were found in 15% (57 of 389 children tested for parasitemia) of children with virus detected. HIV infection was detected in 25% (67 of 270 children with available HIV results) of viral episodes. Table 1 shows prevalence of these coinfections stratified by study virus.

Most of the viral cases (74% [254/342]) tested for both IBI and malaria parasites had no concomitant infection, and 78% of them were HIV-uninfected (138 of 178 with HIV results).

Incidence Rates of Viral Infections

Table 2 presents the estimated incidence rates of each respiratory virus stratified by HIV status. Because episodes from children within the DSS study area not tested for HIV were not considered in the analysis, both HIV-uninfected and HIV-infected rates might be slightly diluted. According to the sensitivity analysis performed, incidence of viral infection was between 5.5 and 16 times higher among HIV-infected children. This trend was observed for all tested viruses, but varied according to virus (details in Table 2). Data for EV were not presented due to the low number of cases detected.

Mortality

Inhospital mortality among children with virus detected was 9% (33/359 children with known outcome). One-third of these deaths occurred among children with concomitant IBI (34%, 10/29 children with bacterial results available). Viruses found among the other 19 fatalities were listed as follows: 8 RV, 2 ADV, 2 RSV, 2

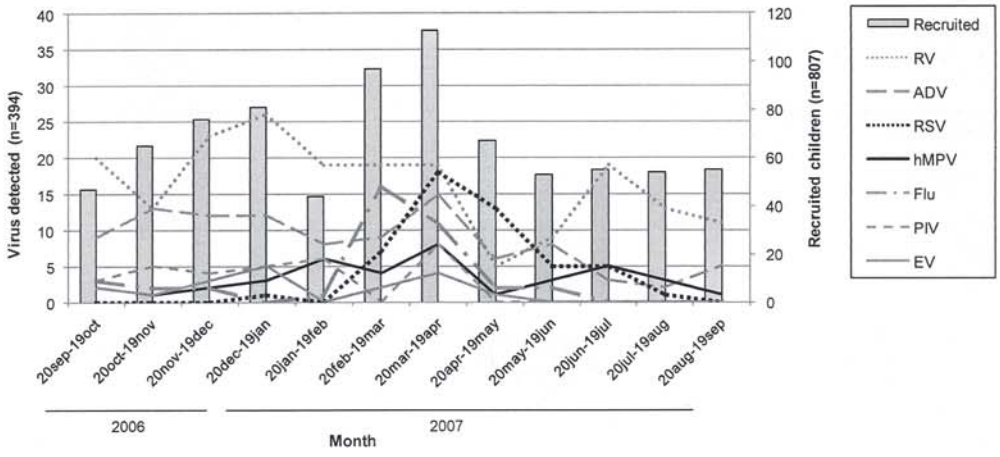

FIGURE 1. Number of children enrolled in the study (right y-axis) and number of diagnosed virus (left y-axis) by month (months go from the 20th of each month until the 19th of the following).

TABLE 1. Epidemiologic Characteristics of Different Isolated Viruses and Viral Coinfections Detected Among Children <5 Years of Age Admitted to MDH With Viral Pneumonia

	Total n	RV n (%)	ADV n (%)	RSV n (%)	hMPV n (%)	Flu n (%)	PIV n (%)	EV n (%)	Coinfection* n (%)	*P*
Total	394	135 (34)†	57 (14)†	38 (10)†	29 (7)†	28 (7)†	20 (5)†	10 (3)†	77 (20)†	
Age (mo)										
<3	50 (13)	14 (10)	1 (2)	11 (29)	6 (21)	4 (14)	4 (20)	3 (30)	7 (9)	
3–12	149 (38)	67 (50)	12 (21)	16 (42)	16 (55)	7 (25)	7 (35)	3 (30)	21 (27)	
12–<60	195 (49)	54 (40)	44 (77)	11 (29)	7 (24)	17 (61)	9 (45)	4 (40)	49 (64)	<0.001
Sex (male)	250 (63)	85 (63)	35 (61)	22 (58)	20 (69)	18 (64)	12 (60)	6 (60)	52 (68)	0.974
Rainy season	252 (64)	76 (56)	34 (60)	24 (63)	15 (52)	24 (86)	15 (75)	9 (90)	55 (71)	0.015
IBI‡	38 (11)	16 (13)	6 (12)	1 (3)	3 (11)	1 (4)	1 (5)	0	10 (16)	0.408
Parasitemia§	57 (15)	19 (14)	11 (19)	2 (5)	3 (11)	2 (7)	4 (20)	0	16 (21)	0.181
HIV infected¶	67 (25)	26 (30)	9 (24)	3 (10)	7 (33)	4 (25)	3 (19)	1 (14)	14 (25)	0.475
IH mortality‖	33 (9)	10 (8)	4 (8)	2 (6)	3 (11)	1 (4)	1 (6)	1 (11)	11 (15)	0.627

*One hundred fifty-eight viruses were isolated among this group: 61 RV, 45 ADV, 12 RSV, 10 hMPV, 11 Flu, 11 PIV, and 8 EV.

†Percentages among the number of virus infected children (n = 394).

‡Percentages among children tested for IBI and with valid results (n = 345).

§Percentages among children tested for parasitemia (n = 389).

¶Percentages among children with available HIV results (n = 270).

‖Percentages among children with known outcome (n = 359).

RV indicates rhinovirus; ADV, adenovirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; Flu, influenza virus; PIV, parainfluenza virus; EV, enteroviruses; IBI, invasive bacterial infection; HIV, human immunodeficiency virus; IH, inhospital.

TABLE 2. Burden of Viral Pneumonia Associated to Each Respiratory Virus in HIV-infected and HIV-uninfected Children

Definition	HIV-infected			HIV-uninfected			IRR†	
	Episodes	Rate* (1000 PYAR)		Episodes	Rate* (1000 PYAR)			
		Analysis 1 (PYAR‡ = 440)	Analysis 2 (PYAR‡ = 1205)		Analysis 1 (PYAR‡ = 20,073)	Analysis 2 (PYAR‡ = 19,020)	Analysis 1	Analysis 2
RV	30	68.2	24.9	71	3.5	3.7	19.5	6.7
ADV	14	31.8	11.6	43	2.1	2.3	15.1	5.0
RSV	4	9.1	3.3	28	1.4	1.5	6.5	2.2
hMPV	8	18.2	6.6	17	0.8	0.9	22.8	7.3
Flu	5	11.4	4.1	11	0.5	0.6	13.0	6.8
PIV	4	9.1	3.3	15	0.7	0.8	5.8	4.1
Viral infection	55	125.0	45.6	157	7.8	8.3	16.0	5.5

The table shows 2 values for Rates and IRR because they were estimated from a sensitivity analysis considering a minimum and a maximum value of mother-to-child-transmission of HIV infection.

*Rates were calculated by dividing episodes by the 2 values of PYAR (sensitivity analysis).

†Ratios were calculated by dividing rates obtained under the same estimated HIV prevalence.

‡PYAR (person-years at risk) were calculated based on the population <5 years of age living in the DSS study area considering the 2 estimated values of HIV prevalence: 2.9% (analysis 1) and 8% (analysis 2). See details in text.

HIV indicates human immunodeficiency virus; RV, rhinovirus; ADV, adenovirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; Flu, influenza virus; PIV, parainfluenza virus; DSS, demographic surveillance system; IRR, incidence rate ratio.

hMPV, 1 Flu, and 4 dual detections (3 RV and ADV; 1 RV and hMPV). In addition, 2 cases (of 18 children tested for malaria parasites) of these nonbacteremic children had parasitemia and 4 (of 8 with HIV results) were HIV-infected. When excluding cases with other coinfections (IBI, malaria parasites, or HIV), inhospital mortality for children with virus detected was 3% (4/127). Table 3 shows relationship between certain characteristics of viral cases and odds of mortality. Of all the variables explored dual viral detection, IBI and HIV infection were the only ones associated with mortality in the univariate analysis ($P = 0.05$, $P < 0.001$, and $P < 0.001$, respectively) and their odds of an associated fatal outcome were 2, 7, and 7 times higher, respectively.

DISCUSSION

We present here unique data on incidence rates of viral associated hospitalized pneumonia in an African malaria endemic country with high prevalence of HIV infection. Results emphasize the role played by respiratory viruses in the etiology of hospitalized pneumonia and associated mortality in rural Mozambique. At least one respiratory virus was detected from almost half of the children admitted at MDH with clinical presentation compatible with severe pneumonia. No other concomitant etiology (IBI or malaria) was found in 74% of these viral cases. As expected,[11,12,30] HIV infection entailed an increased risk of viral detection in pneumonia episodes. Prognosis was worsened for both HIV-infected and bacterial coinfected viral cases.

The high prevalence of respiratory viruses among hospitalized children with severe pneumonia and the occurrence of dual viral detection that we report here have previously been described in other areas of the world, such as Europe, the United States,[6,7,31] and to a lesser extent in Africa.[9,11] In contrast with our results, most hospital-based studies in which viral diagnosis was included showed that RSV was the most prevalent viral pathogen.[9,31] In our results RSV was the third viral etiology in frequency after RV and

TABLE 3. Risk Factors for Death Among Children Admitted to MDH With Viral Pneumonia

Variable	Inhospital Mortality*		Univariate Analysis		*P*
	No (%)†	Yes (%)†	OR	95% CI	
Age (n = 359)	n = 326	n = 33			
<3 mo (n = 44)	40 (91)	4 (9)	1	—	—
3–<12 mo (n = 138)	123 (89)	15 (11)	1.22	0.38–3.89	0.737
1–5 yr (n = 177)	163 (92)	14 (8)	0.86	0.27–2.75	0.798
Sex (n = 359)	n = 326	n = 33			
Male (n = 226)	208 (92)	18 (8)	1	—	—
Female (n = 133)	118 (89)	15 (11)	0.68	0.33–1.40	0.296
Season (n = 359)	n = 326	n = 33			
Dry (n = 132)	121 (92)	11 (8)	1	—	—
Rainy (n = 227)	205 (90)	22 (10)	1.18	0.55–2.52	0.668
Viral coinfection (n = 359)	n = 326	n = 33			
Yes (n = 72)	61 (85)	11 (15)	1	—	—
No (n = 287)	265 (92)	22 (8)	2.17	1.00–4.72	0.050
IBI (n = 316)	n = 287	n = 29			
Yes (n = 30)	20 (67)	10 (33)	1	—	—
No (n = 286)	267 (93)	19 (7)	7.02	2.88–17.12	<0.001
Parasitemia (n = 354)	n = 323	n = 31			
Yes (n = 54)	49 (91)	5 (9)	1	—	—
No (n = 300)	274 (91)	26 (9)	1.08	0.39–2.94	0.887
HIV infection (n = 243)	n = 227	n = s16			
Yes (n = 55)	45 (82)	10 (18)	1	—	—
No (n = 188)	182 (97)	6 (3)	6.75	2.33–19.52	<0.001

*Children transferred and absconded from the hospital were excluded from this analysis.
†Percentages are calculated by row.
MDH indicates Manhiça district hospital; OR, odds ratio; IBI, invasive bacterial infection; HIV, human immunodeficiency virus.

ADV, representing only 11% of overall viral cases. The comparatively lower RSV prevalence in this series might be explained by yearly variations in incidence and severity of RSV and other viral epidemics. In addition, 2 other factors might have also reduced the number of RSV cases detected in our study. On the one hand, RSV infection among admitted children was probably under-represented because wheezing per se was not an inclusion criteria and many RSV episodes, often presenting exclusively with wheezing,[32,33] may have not been included. Similar findings were previously reported by Cevey-Macherel et al in a pneumonia study where wheezing was an exclusion criteria.[7] On the other hand, RSV incidence was lesser increased among HIV-infected children compared with other study viruses; this observation is in concordance with a previous report of Madhi et al.[11]

The rate of IBI among viral cases was high. Because of the low sensitivity of blood culture to diagnose bacterial pneumonia,[34] real viral-bacterial coinfection was probably even higher. Some reports suggest that bacterial coinfections are often an essential part of the pathogenesis of most severe viral infections progressing to pneumonia.[35] Conversely, infection with respiratory viruses might predispose to bacterial superinfection.[11,36] To ascertain whether viruses detected among the study children caused pneumonia symptoms by themselves, contributed to the severity of the episode, and/or contributed to a bacterial superinfection, which is challenging and other approaches, such as a case-control design, would appear necessary. As well as IBI, detection of malaria parasites among study children was common for all the study viruses.

Calculated incidence rates of pneumonia with viral detection were very high for both HIV-infected and HIV-uninfected. However, real incidences might be even higher since case ascertainment was passive (hospital based) and episodes from children residents of the DSS study area with no HIV results were not included. The overall risk of viral detection among study patients was higher among HIV-infected children. As previously described by Madhi et al,[11] the increased risk among HIV-infected varied according to respiratory virus and was lowest for RSV. Increased risk of viral infection among HIV-infected children is also reflected in the high HIV prevalence among children with virus detected (25%), contrasting with the estimated prevalence of HIV infection among the birth cohort (2.9%–8.0%).

Inhospital mortality associated with viral pneumonia reported here (9%) is higher than in other studies.[37,38] High prevalence of IBI as well as HIV infection partly explains the high inhospital mortality detected. As previously reported,[11,38,39] these 2 coinfections were associated with higher risk of fatal outcome (odds ratio = 7 in both cases) among study children. On the contrary, detection of malaria parasites does not increase the risk of mortality among viral cases. Still, 4 deaths occurred among study children with no other coinfection detected.

This study has other limitations beyond those intrinsic of a surveillance design exposed earlier. Although the isolation of respiratory viruses in the nasopharynx has been associated with infection from the lower tract, isolation of certain study viruses, such as ADV, Flu, or PIV, has also been detected in healthy children.[40] In addition, results presented here correspond to only 12 months of surveillance. As previously reported, wide interannual variations on viral disease burden, viral distribution, and viral seasonality often occurs.[41] However, current findings of seasonality and disease burden of RSV are in accordance with previous data generated during 2 years of RSV surveillance in the same setting.[13,42]

Notwithstanding its limitations, this study describes for the first time the burden of disease associated with viral detection among hospitalized children with clinical presentation compatible with severe pneumonia in a malaria endemic African setting with high HIV prevalence. Results highlight the high prevalence of viruses, high rates of other coinfections, and high rates of mortality among study children. The HIV epidemic is an important contributor to the high burden of disease and associated mortality of viral pneumonia; therefore, strengthening strategies to prevent MTCT of HIV infection would also have an impact on reducing viral

pneumonia among children in rural Africa. Similarly, bacterial coinfection increases mortality associated to viral pneumonia, hence prevention of IBI by introduction of already available pneumococcal and Hib vaccines may also reduce mortality associated to viral pneumonia in places where these vaccines are not routinely administrated. In addition, mechanisms to improve early diagnostic and appropriate case management of pneumonia in these rural communities are necessary to reduce child mortality in rural Africa.

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3. ARTICLE 3

The global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis

Càrrega global de malaltia produïda per les infeccions respiratòries causades pel virus estacional de la grip en nens: metaanalisis i revisió bibliogràfica sistemàtica

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RESUM

3.1. Objectiu

Estimar la càrrega global de malaltia atribuïble a infeccions respiratòries associades al Flu entre nens menors de cinc anys.

3.2. Disseny de l'estudi

Aquest estudi es va dur a terme pel Grup d'Estudi d'Infuenza. Es va realitzar una cerca bibliogràfica sistemàtica per trobar tots els articles publicats entre l'1 de gener de 1995 i el 31 de desembre de 2010 sobre la incidència del Flu i la mortalitat associada a aquest virus en nens menors de cinc anys. A més a més, es van incloure dades similars encara no publicades d'estudis que tenien el rigor científic necessari.

Pel fet que cada estudi utilitzava definicions de casos lleugerament diferents, es van establir unes definicions comunes per a aquest estudi per definir els casos de síndrome gripal (en anglès *influenza-like illness*), d'LRTI i d'LRTI greu.

A partir dels estudis detectats i les definicions establertes es va estimar la incidència del Flu i aquestes estimacions es van aplicar a la població mundial de l'any 2008.

3.2. Resultats principals

1. Es van detectar i incloure al metaanàlisis 27 estudis publicats i 16 estudis no publicats amb dades d'incidència del Flu.
2. L'any 2008 es van produir aproximadament 90 milions (95%CI=49-162 milions) d'infeccions noves pel Flu i 20 milions (95%CI=13-32 milions) de casos d'LRTI (tan greu com no greu) associats al Flu, en nens menors de cinc anys.
3. L'any 2008 es van produir aproximadament 1 milió (95%CI=1-2 milions) de casos nous d'LRTI greu associats al Flu entre nens menors de cinc anys. La incidència d'LRTI greu, però, varia àmpliament segons l'any, depenent del tipus de Flu que circuli.
4. Entre 28.000 i 115.000 de les morts que es van produir l'any 2008 en nens menors de cinc anys es poden atribuir a LRTI greu associades al Flu. El 99% d'aquestes morts es van produir a països en vies de desenvolupament.

Articles

Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis

Harish Nair, W Abdullah Brooks, Mark Katz, Anna Roca, James A Berkley, Shabir A Madhi, James Mark Simmerman, Aubree Gordon, Masatoki Sato, Stephen Howie, Anand Krishnan, Maurice Ope, Kim A Lindblade, Phyllis Carosone-Link, Marilla Lucero, Walter Ochieng, Laurie Kamimoto, Erica Dueger, Niranjan Bhat, Sirenda Vong, Evropi Theodoratou, Malinee Chittaganpitch, Osaretin Chimah, Angel Balmaseda, Philippe Buchy, Eva Harris, Valerie Evans, Masahiko Katayose, Bharti Gaur, Cristina O'Callaghan-Gordo, Doli Goswami, Wences Arvelo, Marietjie Venter, Thomas Briese, Rafal Tokarz, Marc-Alain Widdowson, Anthony W Mounts, Robert F Breiman, Daniel R Feikin, Keith P Klugman, Sonja J Olsen, Bradford D Gessner, Peter F Wright, Igor Rudan, Shobha Broor, Eric A F Simões, Harry Campbell**

Summary

Background The global burden of disease attributable to seasonal influenza virus in children is unknown. We aimed to estimate the global incidence of and mortality from lower respiratory infections associated with influenza in children younger than 5 years.

Methods We estimated the incidence of influenza episodes, influenza-associated acute lower respiratory infections (ALRI), and influenza-associated severe ALRI in children younger than 5 years, stratified by age, with data from a systematic review of studies published between Jan 1, 1995, and Oct 31, 2010, and 16 unpublished population-based studies. We applied these incidence estimates to global population estimates for 2008 to calculate estimates for that year. We estimated possible bounds for influenza-associated ALRI mortality by combining incidence estimates with case fatality ratios from hospital-based reports and identifying studies with population-based data for influenza seasonality and monthly ALRI mortality.

Findings We identified 43 suitable studies, with data for around 8 million children. We estimated that, in 2008, 90 million (95% CI 49–162 million) new cases of influenza (data from nine studies), 20 million (13–32 million) cases of influenza-associated ALRI (13% of all cases of paediatric ALRI; data from six studies), and 1 million (1–2 million) cases of influenza-associated severe ALRI (7% of cases of all severe paediatric ALRI; data from 39 studies) occurred worldwide in children younger than 5 years. We estimated there were 28 000–111 500 deaths in children younger than 5 years attributable to influenza-associated ALRI in 2008, with 99% of these deaths occurring in developing countries. Incidence and mortality varied substantially from year to year in any one setting.

Interpretation Influenza is a common pathogen identified in children with ALRI and results in a substantial burden on health services worldwide. Sufficient data to precisely estimate the role of influenza in childhood mortality from ALRI are not available.

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Introduction

Acute lower respiratory infections (ALRI) such as pneumonia and bronchiolitis are a leading cause of morbidity and mortality in young children.[1] Around 156 million new episodes of ALRI occur worldwide every year and about 1·56 million young children died as a result of such infections in 2008.[2,3] Respiratory viruses are commonly associated with ALRI episodes in young children.[4–10] We previously estimated that respiratory syncytial virus (RSV) is present in 22% of such episodes, making it the most prevalent pathogen in children with ALRI.[11] Influenza has long been regarded as an important disease in the elderly because of its high incidence and concomitant high rate of hospital admissions and mortality in individuals older than 65 years.[12] However, studies in the past decade suggested that the burden of disease due to hospital admissions for influenza-associated ALRI in young and very young children is also substantial.[13–16]

Previously, no estimates of the global burden of disease from seasonal influenza virus-associated ALRI in young children have been made. We identified only two systematic reviews of the incidence of influenza-associated pneumonia,[17,18] neither of which provided summary incidence rates. Recent estimates of global ALRI incidence and mortality associated with *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and RSV[11,19,20] do not fully explain the paediatric ALRI burden, and so the role of other pathogens needs to be explored. Influenza is associated with a large but unknown number of hospital admissions in young children globally and is vaccine preventable. Globally, there is an increasing capacity for laboratory-confirmed diagnosis of influenza infection which led to increased recognition (especially) of severe

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influenza-related illness in children and adults in developing countries during the influenza A H1N1 pandemic in 2009. Additionally, studies from developing countries have provided population-based estimates of burden of influenza in children that have added to the evidence of the health effects of the disease worldwide. Moreover, the influenza A H1N1 (2009) pandemic raised questions about the baseline incidence and mortality from seasonal influenza in young children so as to better assess the need for and structure of vaccination programmes.

Many data for incidence and mortality from influenza-associated ALRI in developing countries remain unpublished. Therefore, we formed an international Influenza Study Group to supplement our systematic literature review with unpublished data. We aimed to estimate the burden of disease due to influenza-associated ALRI in children younger than 5 years for 2008 globally and for six WHO regions.

Methods

Search strategy and selection criteria

We undertook a systematic literature review with various search terms (webappendix pp 3–4) and hand searched online journals and scanned reference lists of identified citations. We restricted the search to Medline (Ovid), Embase, CINAHL, Global Health, Web of Science, WHOLIS, LILACS, IndMed, grey literature (SIGLE), and Chinese language databases and to studies published between Jan 1, 1995, and Oct 31, 2010. Panel 1 shows study eligibility criteria. No language or publication restrictions were applied. Two authors (HN and VE) independently did the literature search and extracted data. Any disagreements were resolved after discussion. The Influenza Study Group agreed on a common approach to data analysis and formulated common case definitions. We invited participation of other researchers and contacted authors of published studies who had done similar population-based studies of paediatric influenza (webappendix pp 6–7).

Definitions

Most investigators used modified versions of the case definitions for clinical pneumonia, severe pneumonia, and influenza surveillance that were established by WHO and the US Centers for Disease Control and Prevention (CDC; webappendix pp 8–29).[21,22] We chose to use the terms ALRI and severe ALRI because a proportion of children with lower respiratory complications of influenza might not only present with pneumonia but also with bronchiolitis. We defined influenza-associated ALRI as cough or difficulty in breathing (with fast breathing for age) in a child with influenza virus identified with valid diagnostic tests. We defined influenza-associated severe ALRI as identification of influenza virus with valid diagnostic tests in a child with either cough or difficulty in breathing with indrawing of the lower chest wall (with or without fast breathing for age) or hospitalisation for a respiratory ailment. We also included a category of influenza episodes that included the entire spectrum of respiratory burden from influenza-positive influenza-like illness (webappendix p 56), influenza-associated ALRI, and influenza-associated severe ALRI.

We used a modification of the definition for influenza season that was provided by Izurieta and colleagues.[23] The influenza season included any month in which at least 10 samples were analysed and the virus was detected in more than 5% of specimens. We designated countries as developed or developing on the basis of the Global Burden of Disease Study regions[24] as previously described[11] and child population estimates for every region for 2008 as in *The State of the World's Children Special Edition*.[25]

Data imputation

For studies that did not report disease incidence in children aged 0–4 years, we used imputation to calculate missing data by use of the median incidence rate ratio (for details see webappendix p 5).[11,26] If the duration of the study was not in exact multiples of 1 year, we calculated and reported a yearly incidence. We also decided that, if only a proportion of eligible cases were sampled (with a systematic method) and data for all eligible cases were available, the incidence could be adjusted by scaling for the proportion sampled. Figure 1 summarises our overall approach and associated rationale for decisions adopted.

Panel 1: Study eligibility criteria

Inclusion criteria

- Studies with data for laboratory-confirmed influenza (eg, mild influenza or influenza like illnesses, acute respiratory infections, acute lower respiratory infections, or severe acute lower respiratory infections)
- Studies of children younger than 5 years, or data reported separately for this age group
- Studies published between Jan 1, 1995, and Oct 31, 2010
- Study should have been carried out for at least 1 year (apart from in temperate regions where influenza seasonality is more clearly defined and for studies reporting case fatality ratio); this criterion is important since influenza is a seasonal disease
- Studies reporting influenza incidence or mortality for at least the first year of life

Exclusion criteria

- Studies in which influenza was studied as co-infection rather than primary outcome
- Case definition not clearly defined or not applied consistently
- Case ascertainment done only during the epidemic period
- Incidence and mortality estimated with modelling techniques

Statistical analysis

We did a meta-analysis of data for disease incidence and case fatality and report pooled estimates and 95% CIs. We used the random-effects model (DerSimonian-Laird method) if there was significant heterogeneity in the data ($p<0·05$).[27] Investigators who use a passive case ascertainment usually report substantially lower incidence of influenza-associated ALRI than do those who use an active approach, which is expected in developing countries in which access to health services is restricted. Therefore, we based our incidence estimates of influenza episodes and influenza-associated ALRI for developing countries on a selection of data from developing country studies that did active case ascertainment only (webappendix pp 35–46), consistent with the approach adopted in our previous global estimate of ALRI associated with RSV.[11] We estimated incidence for developed and developing countries and then applied these incidence estimates to the population of children younger than 5 years in 2008 to yield the number of new episodes of all three categories in 2008.[25] We also calculated the incidence of influenza-associated severe ALRI for WHO regions on the basis of incidence meta-estimates for the individual regions.

Because data were scarce, we did not attempt to model a point estimate for influenza-associated ALRI mortality. Instead, we used two approaches to assess the probable upper and lower bound of mortality that could be plausibly attributed to influenza. First, we applied the meta-estimate of influenza-associated case fatality ratio from hospital-based reports to incidence data for influenza-associated severe ALRI reporting to hospitals or clinics (calculated separately for developing and developed countries). Because access to hospital care in most developing countries is poor, we defined this result as the lower bound for mortality.

Our second approach was much the same as the method previously used[11] to estimate mortality from ALRI associated with RSV in children. We assumed that all excess mortality from ALRI in children younger than 5 years during the influenza season was caused by seasonal influenza virus, and that non-influenza mortality is equal within and between periods of influenza epidemics. Because this approach is an extreme case scenario, we assumed that this method yielded an upper bound for influenza-associated ALRI mortality. We defined the duration in months of the influenza season for every

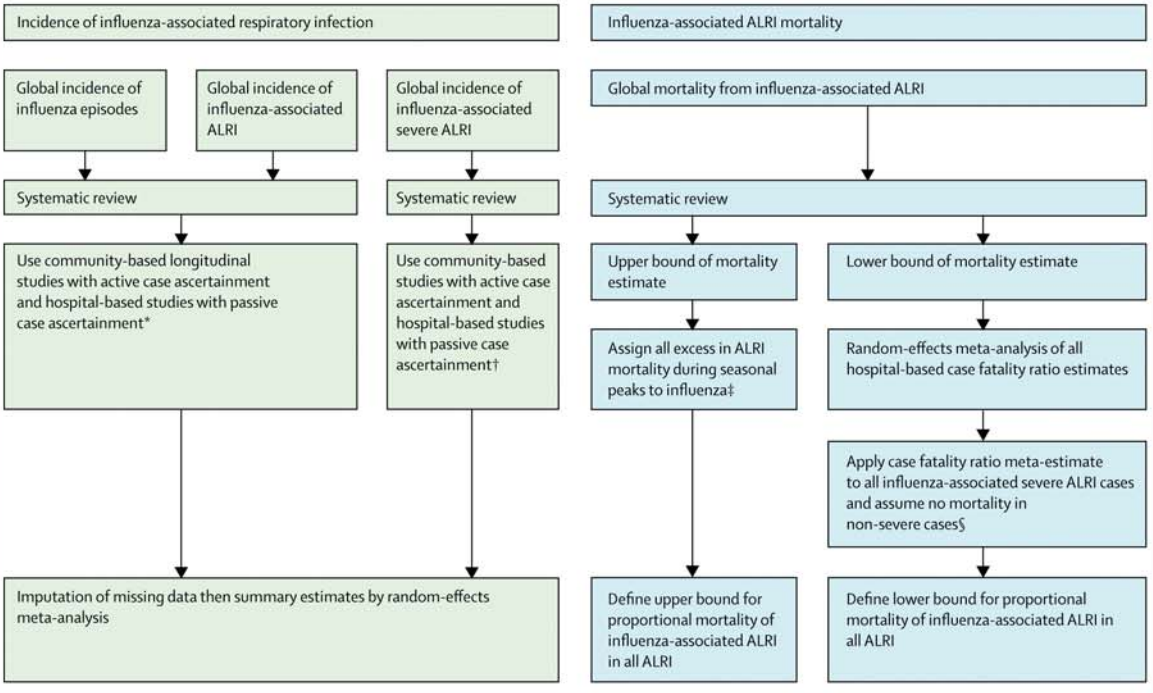

Figure 1: **Approaches for estimation of global influenza incidence and mortality in children aged 0–4 years**

*Approach justified by large difference in reported incidence between studies using active and passive case ascertainment in the case of developing countries; studies with passive case ascertainment reported much lower estimates than did those with active ascertainment. †Approach justified by the decision that hospital-based data would be most useful for population-based projections, since all severe episodes are likely to need hospital treatment; also, we noted no difference in reported incidence of influenza-associated severe ALRI between studies with active and passive case ascertainment. ‡Approach based on the assumptions that baseline proportional mortality of influenza-associated ALRI in all ALRI would be similar to proportional incidence of influenza-associated severe ALRI in all severe ALRI, and that there is no overall effect from all other respiratory pathogens; then if all excess ALRI mortality during influenza seasonal peaks is assigned to influenza as the only cause in a setting (with many seasonal peaks) and this mortality is added to baseline mortality estimates, this approach is likely to overestimate the contribution of influenza to mortality from all ALRI. §Approach deemed to yield a lower bound for influenza-associated ALRI mortality because an unknown proportion of influenza-associated ALRI mortality occurs outside the hospital. ALRI=acute lower respiratory infections.

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See Online for webappendix

	Case ascertainment	Study population (n)	Specimen and diagnostic tests	Incidence of influenza episodes (per 1000 children per year)*			Incidence of influenza-associated ALRI (per 1000 children per year)*			Incidence of influenza-associated severe ALRI (per 1000 children per year)*		
				Aged <1 year	Aged <2 years	Aged <5 years	Aged <1 year	Aged <2 years	Aged <5 years	Aged <1 year	Aged <2 years	Aged <5 years
Western Europe												
Kiel, Germany; urban; 1996–2000[42]	Passive, hospital (inpatient)	Census-derived estimate	NPA; RT-PCR	NA	NA	NA	NA	NA	NA	2	(1)	1
Madrid, Spain; urban; 1997–2003[43]	Passive, hospital (inpatient)	Census-derived estimate (n=149 602)	Nasal or throat aspirate; viral culture and subsequent fluorescent staining with monoclonal antibodies	NA	NA	NA	NA	NA	NA	(1)	(1)	(1)
Multicentre, Germany; mixed urban-rural; 1999–2001[44]†	Passive, hospital (inpatient)	Census-derived estimate	NPA; PCR	NA	NA	NA	11	12	(11)	2	2	(1)
Berne, Switzerland; urban; 1999–2004[45]	Active, community based	Defined population base (n=187)	Nasal swab; RT-PCR	21	(24)	(22)	NA	NA	NA	NA	NA	NA
Turku, Finland; urban; 2000–02[30]†	Passive, clinic (outpatient)	Defined population base (n=1270)	Nasal swab; viral culture and subsequent immunoperoxidase staining with monoclonal antibodies	188	186	186	NA	NA	NA	NA	NA	NA
Leicester, UK; mixed urban-rural; 2001–02[31]	Passive, hospital (inpatient)	NHS database (n=56 395)	Nasal and throat swabs; PCR	NA	NA	NA	NA	NA	NA	2	2	2
Gipuzoka, Spain; mixed urban-rural; 2001–04[32]	Passive, hospital (inpatient)	Census-derived estimate	NPA; viral culture and RT-PCR	NA	NA	NA	NA	NA	NA	3	2	1
East London, UK; urban; 2002–04[46]	Passive, hospital (inpatient)	Census-derived estimate (n=15 177)	NPA; IF and PCR	(18)	19	16	NA	NA	NA	(3)	3	2
East sub-Saharan Africa												
Manhiça district, Mozambique; rural; 2006–07 (Roca and colleagues)	Passive, hospital (inpatient)	Defined population base (n=13 291 cyo)	NPA; multiplex RT-PCR	NA	NA	NA	NA	NA	NA	4	3	2
Kilifi district, Kenya; rural; 2007 (Berkley and colleagues)	Passive, hospital (inpatient)	Defined population base (n=44 544)	Nasal wash; multiplex real-time PCR	NA	NA	NA	NA	NA	NA	3	2	1
Bondo district, Kenya; rural; 2007–09 (Ope and colleagues)	Passive, hospital (inpatient)	Census-derived estimate (n=55 117)	Nasopharyngeal and/or oropharyngeal wash, real-time RT-PCR	NA	NA	NA	NA	NA	NA	1	2	1
Kibera, Nairobi, Kenya; urban; 2008 (Katz and colleagues)	Passive, hospital (outpatient)	Census-derived estimate (n=3434 cyo)	Nasopharyngeal and oropharyngeal swabs; real-time RT-PCR	NA	NA	NA	NA	NA	NA	5	6	9
Lwak, Kisumu, Kenya; rural; 2008 (Katz and colleagues)‡	Passive, clinic (outpatient)	Census-derived estimate (n=3825 pyo)	Nasopharyngeal and oropharyngeal swabs; real-time RT-PCR	NA	NA	NA	NA	NA	NA	1	1	1
West sub-Saharan Africa												
The Greater Banjul area and Upper River Region, The Gambia; periurban and rural; 2007–08 (Howie and colleagues)§	Passive, hospital (inpatient and outpatient)	Defined population base (n=24 378)	NPA; mass-tag PCR	NA	NA	NA	14	6	3	0	1	0
Southern sub-Saharan Africa												
Soweto, South Africa; urban; 1998–2004 (Madhi and colleagues)¶	Passive, hospital (inpatient)	Defined population base (n=39 876)	NPA; DFA	NA	NA	NA	NA	NA	NA	2	2	1
South Asia												
Mirzapur, Bangladesh; rural; 1993–1996[47]	Active, community based	Defined population base (n=252)	NPA; ELISA	NA	NA	NA	NA	NA	NA	(2)	2	(1)
Ballabgarh, India; rural; 2001–05 (Broor and colleagues)	Active, community based	Defined population base (n=281)	Nasopharyngeal wash; DFA	180	178	(184)	33	44	(34)	NA	NA	NA
Kamalapur, Bangladesh; urban; 2004–07[41]	Active, community based	Defined population base (n=5000)	Nasopharyngeal wash; viral culture	132	117	99	11	31	27	1	1	1

(Continues on next page)

	Case ascertainment	Study population (n)	Specimen and diagnostic tests	Incidence of influenza episodes (per 1000 children per year)*			Incidence of influenza-associated ALRI (per 1000 children per year)*			Incidence of influenza-associated severe ALRI (per 1000 children per year)*		
				Aged <1 year	Aged <2 years	Aged <5 years	Aged <1 year	Aged <2 years	Aged <5 years	Aged <1 year	Aged <2 years	Aged <5 years
(Continued from previous page)												
Kamalapur, Bangladesh; urban; 2008 (Brooks and colleagues)‡	Active, community based	Defined population base (n=5710)	Nasopharyngeal wash; RT-PCR and tissue culture	75	188	204	35	61	46	2	2	1
Southeast Asia												
Bohol, Philippines; mixed urban-rural; 2000–04 (Lucero and colleagues)‡	Passive, hospital (inpatient and outpatient)	Defined population base (n=20 516 pyo)	NPA; viral culture and PCR	NA	NA	NA	5	4	(5)	2	2	(1)
Sa Kaeo and Nakhon Phanom, Thailand; rural; 2005–08 (Simmerman and colleagues)‡	Passive, hospital (inpatient)	Census-derived estimate (n=83 200)	Nasopharyngeal swabs; RT-PCR and viral culture	NA	NA	NA	NA	NA	NA	6	7	5
Nha Trang, Vietnam; urban; 2007–08[33]	Passive, hospital based (inpatient)	Census-derived estimate (n=13 952)	NPA; PCR	NA	NA	NA	NA	NA	NA	17	18	9
East Asia												
Hong Kong, China; urban; 1997–99[34]‖	Passive, hospital (inpatient)	Census-derived estimate (n=324 538)	NPA; IF followed by viral culture and serology	NA	NA	NA	NA	NA	NA	5	5	3
Hong Kong, China; urban; 2003–06[35]	Passive, hospital (inpatient)	Census-derived estimate	NPA; DFA and viral culture	NA	NA	NA	NA	NA	NA	7	7	7
Suzhou district, China; mixed urban-rural; 2007–08[36]**	Passive, hospital (inpatient)	Census-derived estimate (n=481 470)	NPA; DFA	NA	NA	NA	NA	NA	NA	1	0	0
High-income Asia-Pacific												
Soma, Japan; urban; 2002–08 (Sato and colleagues)	Passive, hospital (inpatient and outpatient)	Defined population base (n=5692)	Nasal swab; immunochromatography	39	45	45	NA	NA	NA	6	6	5
Australasia												
South Australia, Australia; mixed urban-rural; 1996–2006[48]**	Passive, hospital (inpatient)	Census-derived estimate	Details of specimen not available; viral culture, PCR	NA	NA	NA	NA	NA	NA	2	(1)	1
High-income North America												
Nashville, TN, USA; urban; 1974–99[15]	Passive, clinic (outpatient) and hospital (inpatient)	Defined population base (n=3041 cyo)	Nasal wash; viral culture	93	102	95	11	11	8	3	3	2
Boston, MA, USA; urban; 1993–2004[49]	Passive, hospital (ED)	Census-derived estimate (n=40 640)	NPA; DFA and viral cultures	NA	NA	NA	(15)	21	(15)	NA	NA	NA
Milwaukee, WI, USA; mixed urban-rural; 1996–98[50]	Passive hospital (inpatient)	Census-derived estimate	Nasopharyngeal swabs, bronchoalveolar lavage, throat swabs, endotracheal aspirates; MPCR, tissue culture, EIA	NA	NA	NA	NA	NA	NA	(3)	(3)	1
Monroe County, (NY) and Davidson County (TN), USA; urban; 2000–01[37]	Passive, hospital (inpatient)	Defined population base	Nasal swab and throat swab; viral culture and RT-PCR	NA	NA	NA	NA	NA	NA	2	1	1
Nashville, Rochester (NY) and Cincinnati (OH) USA; urban; 2000–04[16]	Passive, clinic (outpatient); hospital (ED and inpatient)	Defined population base	Nasal swab and throat swab; viral culture and RT-PCR	(71)	(73)	73	NA	NA	NA	(2)	(2)	1
Philadelphia, PA, USA; urban; 2000–04[51]**	Passive, hospital (inpatient)	Census-derived estimate (n=87 216)	Nasal aspirate; solid-phase immunoassay, DFA and viral culture	NA	NA	NA	NA	NA	NA	(4)	4	2
Colorado, USA; mixed urban-rural; 2000–08 (Simões and colleagues)**	Passive, hospital (inpatient)	Census-derived estimate (n=334 810)	Nasal wash; viral culture, ELISA, RT-PCR	NA	NA	NA	NA	NA	NA	3	3	1

(Continues on next page)

	Case ascertainment	Study population (n)	Specimen and diagnostic tests	Incidence of influenza episodes (per 1000 children per year)*			Incidence of influenza-associated ALRI (per 1000 children per year)*			Incidence of influenza-associated severe ALRI (per 1000 children per year)*		
				Aged <1 year	Aged <2 years	Aged <5 years	Aged <1 year	Aged <2 years	Aged <5 years	Aged <1 year	Aged <2 years	Aged <5 years
(Continued from previous page)												
Salt Lake County, UT, USA; mixed urban-rural; 2001–04[38]**	Passive, hospital (inpatient)	Census-derived estimate (n=71784)	NPA; DFA	NA	NA	NA	NA	NA	NA	2	2	1
Davidson County, TN, USA; mixed urban-rural; 2003–04[52]	Passive, hospital (inpatient)	Census-derived estimate (n=37813)	Nasal and throat swabs; viral culture, RT-PCR, rapid tests, IFA, serology	NA	NA	NA	NA	NA	NA	(5)	5	2
Multistate, USA; mixed urban-rural; 2003–04[39]**†	Passive, hospital (inpatient)	Census-derived estimate (n=1164869)	Viral culture, DFA, IFA, rapid antigen test, RT-PCR	NA	NA	NA	NA	NA	NA	2	2	1
Navajo and WMA reservations, USA; rural; 2003–05 (Bhat and colleagues)‡	Passive, hospital (inpatient)	Defined population base (n= 857)	NPA; viral culture and serology	NA	NA	NA	NA	NA	NA	(3)	(3)	(2)
Davidson County (TN), Monroe County (NY) and Hamilton County (OH), USA; mixed urban-rural; 2004–05[53]	Passive hospital (inpatient)	Census-derived estimate (n=141338)	Nasal and throat swabs; viral culture, RT-PCR, rapid tests, IFA, serology	NA	NA	NA	NA	NA	NA	(4)	3	2
Multistate, USA; mixed urban-rural; 2004–08[40]†	Passive, hospital (inpatient)	Census-derived estimate (n=5633069)	Nasopharyngeal and oropharyngeal swabs; viral culture, DFA, IFA, rapid antigen test, RT-PCR	NA	NA	NA	NA	NA	NA	1	1	0
Central Latin America												
Santa Rosa, Guatemala; mixed rural and small towns; 2008 (Lindblade and colleagues)	Passive, hospital and clinics (inpatient and outpatient)	Census-derived estimate (n=34465)	Nasopharyngeal and oropharyngeal swabs; real-time RT-PCR	(91)	(93)	93	NA	NA	NA	(1)	(1)	1
Tropical Latin America												
Rio de Janeiro, Brazil; urban; 1987–89[54]	Passive, hospital (inpatient)	Defined population base (n=262)	NPA; IFA, viral culture	NA	NA	NA	NA	NA	NA	(5)	(5)	3
Managua, Nicaragua; urban; 2007–08 (Gordon and colleagues)**	Passive, hospital and clinics (outpatient)	Defined population base (n=1024)	Nasal and throat swabs; RT-PCR	(203)	(205)	(205)	(18)	(17)	(13)	(4)	(4)	(3)

For more details of the unpublished studies see webappendix pp 6–7. ALRI=acute lower respiratory infection. NPA=nasopharyngeal aspirate. NA=not available. IF=immunofluorescence assay. cyo=child-years observed. pyo=person-years observed. DFA=direct immunofluorescence. MPCR=multiplex PCR. EIA=enzyme immunoassay. IFA=indirect immunofluorescence assay. ED=emergency department admission. WMA=White Mountain Apache. *Data in parentheses are computed incidence estimates from data imputation. †Detailed age-specific incidence estimates obtained directly from authors. ‡Some included patients were hospitalised. §Included children aged 2 months to 4 years. ¶Included children aged 6 weeks to 4 years. ||Incidence estimated with hospital discharge records and laboratory data. **Included children aged 2–4 years.

Table 1: **Incidence estimates of influenza episodes, influenza-associated-ALRI, and influenza-associated severe ALRI in children younger than 5 years from published and unpublished studies by Global Burden of Diseases, Injuries and Risk Factors regions**

calendar year of the study (MonFLU). For every year, we calculated the average number of total ALRI deaths in the community that occurred per month during (AvgFLU) and outside (AvgOTHER) the influenza season, and the total number of deaths (TOTAL) during the year. The proportion of yearly deaths due to influenza was then calculated as:

$$\frac{(\text{AvgFLU} - \text{AvgOTHER}) \times \text{MonFLU}}{\text{TOTAL}}$$

Population-based data to define influenza season and monthly death records (with reported causes of death based on verbal autopsy data) from the same populations for 3 years were available from Ballabgarh, Haryana in India and Nairobi in Kenya.[28,29] However, the Kenyan data were not suitable for our analytical approach because influenza virus was circulating throughout 2003–05, making an influenza season impossible to define (webappendix p 53). Application of the second approach to the estimated mortality of children younger than 5 years from ALRI in India in 2008[3] provided an estimate of all ALRI deaths attributable to influenza if community-based case ascertainment was used. We then applied the ratio between influenza-associated ALRI deaths (determined with this approach) and influenza-associated ALRI deaths in hospitalised cases in India (determined with the first approach) to the lower bound of influenza-associated ALRI mortality in developing countries to estimate an upper bound of

global ALRI mortality attributable to influenza in children younger than 5 years.

We did all data analyses with Stata version 11.1.

Role of the funding source

The funding sources supported a meeting of the Influenza Study Group in Edinburgh, UK (Feb 3–4, 2010). The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. HN had full access to all the data in the study and HN and HC had final responsibility for the decision to submit for publication.

Results

We identified 43 studies[15,16,30–54] with suitable data (table 1, figure 2): 18 were population-based studies reporting incidence of influenza-associated severe or non-severe ALRI in populations under surveillance; 10 were studies estimating incidence on the basis of hospital-discharge records or laboratory reports and a census-based denominator of children at risk; and 15 were population-based studies with unpublished data, reporting a clear denominator of children at risk (figure 3). Only 24 studies (13 published[15,30–41] and 11 unpublished; webappendix p 5) reported disease incidence for children aged 0–4 years and data were imputed for 19 studies. Most studies were passive hospital-based (inpatient), but five used active community-based case ascertainment and ten used a passive hospital or clinic-based (outpatient) approach.

Most studies reported the highest incidence of influenza episodes, influenza-associated ALRI, and influenza-associated severe ALRI in the first year of life (table 1). Data from studies included in the meta-analyses were heterogeneous ($p<0·0001$).

Three studies from developing countries estimating incidence of influenza-associated ALRI used active community-based case ascertainment[41] in which children with ALRI or severe ALRI identified by field workers during weekly home visits were referred to an on-site clinic where the child was examined by a doctor.[41,55] Incidence of influenza episodes and influenza-associated ALRI was highest in children after the first year of life. We estimated that about 90 million (95% CI 49–162 million) new cases of influenza and 20 million (13–32 million) episodes of influenza-associated ALRI (both severe and non-severe) occurred worldwide in children aged 0–4 years in 2008 (table 2).

We based the estimate of influenza-associated severe ALRI incidence on studies with either active or passive case-ascertainment as the incidence estimates for influenza-associated severe ALRI were much the same (table 2, webappendix pp 47–52). Thus, we estimated that 1 million (95% CI 1–2 million) new episodes of influenza-associated severe disease occurred worldwide in children younger than 5 years in 2008 (table 2). The incidence of influenza-associated severe ALRI varied widely from year to year, dependent on the (sub)type of circulating influenza virus (webappendix pp 31–32). Table 3 shows the estimated incidence of influenza-associated severe ALRI and the number of new episodes of severe disease in 2008 by WHO region (excluding the eastern Mediterranean region, from which data were not available).

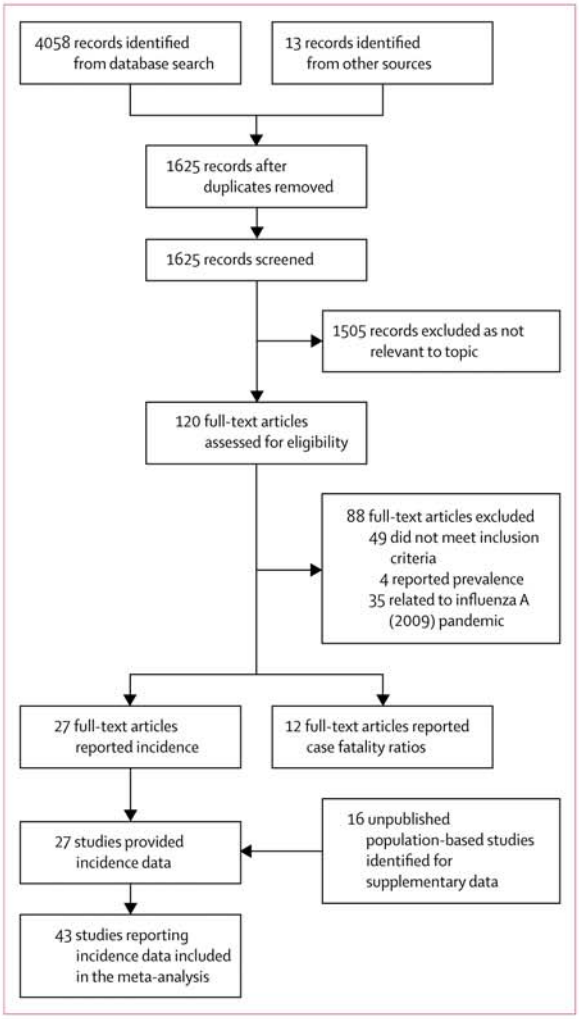

Figure 2: **Flow diagram for selection of studies**

We identified 12 published and eight unpublished studies providing data for case fatality ratios for deaths in children who were admitted to hospital with influenza-associated severe ALRI (table 4).[31,32,34,38,40,48,51,56–60] We estimated the case fatality ratio meta-estimate from these studies and found that the meta-estimate for developing countries was roughly more than 17 times those for developed countries.

Approach 1 was based on the estimated number of new cases of influenza-associated severe ALRI from hospital-based or clinic-based studies in the year 2008 (table 2) and the case fatality ratios from children admitted with severe disease reported in hospital-based studies calculated separately for developing and developed countries

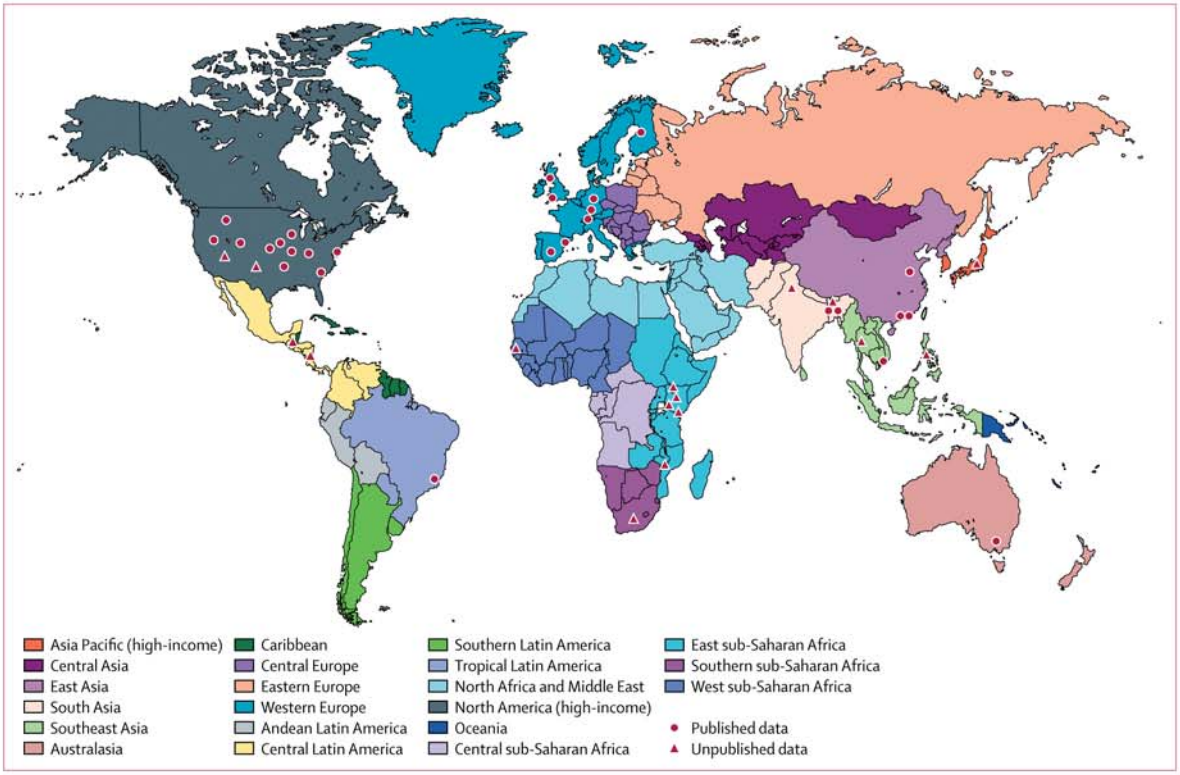

Figure 3: **Location of the 43 studies by Global Burden of Diseases, Injuries and Risk Factors region**

(table 4). With the first approach, we estimated that 27 800 (95% CI 7400–48 000) children younger than 5 years died because of influenza-associated severe ALRI in 2008 (panel 2). We did not have sufficient data to calculate case fatality ratio estimates in younger age categories. Because this estimate includes only children admitted to hospital we judged it to represent a plausible lower bound of influenza-associated severe ALRI mortality.

Approach 2 used cause of death data in children not admitted to hospital, assigned by verbal autopsy, and concurrent influenza virus isolations in the same population. Such data were available only from Ballabgarh in India for 2006–08.[40] Influenza isolation data from a sample of the same population accessing outpatient services for influenza-like illnesses were available from the referral hospital at Ballabgarh (figure 4).

We estimated that the number of deaths calculated with approach 2 was about four times higher than the number estimated with approach 1 (table 5, panel 2). Available data suggest that RSV circulated entirely outside the influenza season with no overlap. Furthermore, the site has low malaria activity.[61] If we assume that these data are broadly representative of India, then 6·5% of all paediatric ALRI deaths in India were associated with influenza in 2006–08. If extrapolated to other developing countries, this approach yields a crude estimate (for developing countries) of 111 500 (range 21 000–245 000) deaths attributable to influenza-associated ALRI in young children in 2008 (panel 2). However, this method probably overestimates deaths because it assumes that all excess ALRI mortality during the influenza season is because of influenza. This assumption is probably untrue because of the shared seasonality of other respiratory pathogens and the likelihood that influenza deaths occur outside the defined influenza season in tropical and subtropical regions.[35,59]

Our rough data-derived estimate of the plausible lower and upper bounds for influenza-associated ALRI mortality in young children are consistent with influenza being associated with 2–7% of deaths from ALRI in children. Data from India (table 5 and figure 4) and Kenya (webappendix p 53), show substantial yearly variation in magnitude of influenza epidemic activity and associated ALRI deaths, suggesting that national, regional, and global influenza mortality could also vary widely from year to year.

Discussion

Our study is the first to estimate global incidence of influenza-associated ALRI and resultant mortality in children younger than 5 years. We estimated that, in 2008, there were about 90 million (95% CI 49–162 million) new cases of influenza episodes, 20 million (13–32 million) cases of influenza-associated ALRI, and 1 million (1–2 million) cases of influenza-associated severe ALRI in this group, causing 28 000–111 500 deaths. Estimates are very variable within countries or regions and between

	Influenza episodes			Influenza-associated ALRI			Influenza-associated severe ALRI		
	Aged <1 years	Aged <2 years	Aged <5 years	Aged <1 years	Aged <2 years	Aged <5 years	Aged <1 years	Aged <2 years	Aged <5 years
Developing countries									
Active									
Studies*	3 (0)	3 (0)	3 (1)	3 (0)	3 (0)	3 (1)	3 (1)	3 (0)	3 (1)
Incidence (95% CI)†	119 (77–186)	156 (108–227)	154 (84–275)	23 (9–57)	44 (26–74)	35 (22–55)	1 (1–2)	2 (1–2)	1 (1–1)
Passive									
Studies*	2 (2)	2 (2)	2 (1)	3 (1)	3 (1)	3 (2)	16 (3)	16 (3)	16 (2)
Incidence (95% CI)†	140 (64–306)	142 (66–307)	142 (66–307)	10 (4–25)	7 (4–12)	5 (3–9)	3 (2–5)	3 (2–4)	2 (1–3)
Active and passive									
Studies*	5 (2)	5 (2)	5 (2)	6 (1)	6 (1)	6 (3)	19 (4)	19 (3)	19 (3)
Incidence (95% CI)†	128 (90–183)	153 (115–205)	150 (98–229)	15 (7–31)	18 (7–44)	14 (6–3)	3 (2–4)	3 (2–4)	2 (1–3)
Developed countries									
Active									
Studies*	1 (0)	1 (1)	1 (1)	0	0	0	0	0	0
Incidence (95% CI)†	NA	NA	NA	NA	NA	NA	NA	NA	NA
Passive									
Studies*	5 (2)	5 (1)	5 (0)	3 (1)	3 (0)	3 (2)	20 (6)	20 (6)	20 (3)
Incidence (95% CI)†	60 (30–117)	65 (34–124)	62 (31–127)	15 (14–16)‡	15 (9–23)	12 (7–18)	2 (2–3)	2 (2–3)	1 (1–2)
Active and passive									
Studies*	6 (2)	6 (2)	6 (1)	3 (1)	3 (0)	3 (2)	20 (6)	20 (6)	20 (3)
Incidence (95% CI)†	52 (28–99)	57 (31–105)	56 (28–106)	15 (14–16)‡	15 (9–23)	12 (7–18)	2 (2–3)	2 (2–3)	1 (1–2)
Global									
Developing countries									
Incidence (95% CI)§	119 (77–186)	156 (108–227)	154 (84–275)	23 (9–57)	44 (26–74)	35 (22–55)	3 (2–4)	3 (2–4)	2 (1–2)
Number of new cases (thousands)	14 634	..	87 198	2763	..	19 807	341	..	934
Developed countries									
Incidence (95% CI)	52 (28–98)	57 (31–105)	55 (28–106)	15 (14–16)‡	15 (9–23)	12 (7–18)	2 (2–3)	2 (2–3)	1 (1–2)
Number of new cases (thousands)	588	..	3056	165	..	650	26	..	66
Total									
Number of new cases (thousands)¶	15 222 (9684–23 883)	..	90 254 (49 257–161 694)	2927 (1244–7176)	..	20 457 (13 009–32 174)	368 (254–532)	..	1000 (665–1503)

ALRI=acute lower respiratory infection. *Data are number of studies and number of studies with imputed data in parentheses. †Data are incidence meta-estimates from random effects model; incidence estimates are per 1000 children per year ‡Incidence estimates are based on fixed effects model as data were not significantly heterogeneous (p=0·25). §Incidence estimates for influenza episodes and influenza-associated ALRI based on meta-estimate for studies with active case ascertainment only and for severe ALRI based on the meta-estimate for studies with both active and passive case ascertainment. ¶Number of new cases globally in the year 2008 is the sum of new cases in children residing in developing and developed countries in 2008; data in parentheses are 95% CIs.

Table 2: **Estimates of incidence (per 1000 children per year) and number of new cases of influenza episodes, influenza-associated ALRI, and influenza-associated severe ALRI in children younger than 5 years from studies with active and passive case ascertainment, by Global Burden of Diseases region**

regions (table 1, table 4), partly due to methodological differences and partly due to variation in influenza epidemiology between study populations and yearly variations in influenza severity. The real uncertainty in estimates is wider than that expressed in a standard 95% CI. There were insufficient data to provide global incidence estimates by type or subtype of influenza virus although incidence of influenza A was generally higher than was that for influenza B. Influenza A (particularly H3N2 subtype) results in higher morbidity and mortality than does influenza B.[62,63] Several factors affect estimates, including the method of case ascertainment, precise case definitions for severe or non-severe ALRI, the proportion of eligible patients tested for influenza virus, and differences in sensitivity and specificity of influenza assays. Hospital-based passive case ascertainment probably yields substantial underestimates of influenza-associated ALRI incidence, especially in developing countries, partly due to poor access to health care.[64,65] Studies in Kenya and The Gambia have shown two-fold to 10-fold decreases in hospital pneumonia admissions in areas farthest from hospital.[64,66,67] In one study,[68] investigators attempted to reduce this effect through provision of reimbursements for travel costs; nonetheless, about 25% of referred children did not attend the hospital. This finding supports our decision to base estimates of influenza episodes and influenza-associated ALRI incidence in developing countries on active case-ascertainment studies.

The studies that we included used various standard case definitions, nasal sampling methods, and diagnostic assays and some only sampled a random proportion of eligible cases or did not include children in the full 0–4 year age

	Countries	Incidence* (95% CI)	Children younger than 5 years in 2008 (thousands)	New episodes in children younger than 5 years in 2008 (thousands)†
Americas	15	1 (1–2)	76 903	94 (63–140)
Western Pacific	7	2 (1–5)	121 005	255 (105–620)
Europe	6	1 (1–2)	51 875	55 (37–82)
Southeast Asia	4	1 (0–6)	180 892	256 (65–1020)
Africa	7	1 (1–3)	131 307	180 (97–332)
Summed regional estimate‡	..	..	..	841 (367–2194)
Developing countries	..	2 (1–2)	566 411	935 (617–1410)
Developed countries	..	1 (1–2)	56 038	66 (48–92)
Global estimate	..	..	622 449	1001 (665–1503)

ALRI=acute lower respiratory infection. *Per 1000 children younger than 5 years per year. †Data in parentheses are 95% CIs. ‡No regional estimate exists for the Eastern Mediterranean region as there are no data from this region; this absence contributes to the difference in summed regional estimates and global estimates.

***Table 3:* Incidence and number of new episodes of influenza-associated severe ALRI in children younger than 5 years, by WHO region**

	Study dates	Case fatality for influenza-associated severe ALRI
Developed countries		
South Australia, Australia[48]	1996–2006	4/626 (0·64%)
Hong Kong[34]*	1997–99	7/5471 (0·13%)
Philadelphia, PA, USA[51]	2000–04	5/573 (0·87%)
Leicester, UK[31]	2001–02	0/33
Gipuzoka, Spain[32]	2001–04	0/70
Salt Lake County, UT, USA[38]	2001–04	1/325 (0·31%)
Sydney, Australia[60]	2003	1/16 (6·25%)
Canada[56]	2003–04	1/424 (0·23%)
Multicentre, USA[40]	2003–08	7/2998 (0·23%)
Hong Kong, China[57]*	2005	1/86 (1·16%)
Developing countries		
Paraná State, Brazil[58]	1996–2001	3/45 (6·67%)
Soweto, South Africa (Madhi and colleagues)	1998–2004	10/178 (5·61%)
Bohol, Philippines (Lucero and colleagues)†‡	2000–04	3/40 (7·50%)
Kuala Lumpur, Malaysia[59]	2002–07	3/116 (2·59%)
Sa Kaeo and Nakhon Phanom, Thailand (Simmerman and colleagues)	2005–08	1/430 (0·20%)
Kilifi, Kenya (Berkley and colleagues)	2007	1/41 (2·43%)
Bondo district, Kenya (Ope and colleagues)	2007–09	3/67 (4·48%)
SARI Sentinel sites, Jordan, Oman, and Egypt (Dueger and colleagues)	2008	2/80 (2·50%)
Santa-Rosa, Guatemala (Lindblade and colleagues)‡§	2008	2/7 (28·57%)
Takeo town, Cambodia (Vong and colleagues)	2008	1/20 (5·00%)

For more details of the unpublished studies see webappendix pp 6–7 and 33. For developed countries, the case fatality ratio (CFR) meta-estimate was 0·17% (95% CI 0·08–0·26; p for heterogeneity=0·76). For developing countries, the CFR meta-estimate was 2·96% (0·79–5·13; p for heterogeneity=0·06). ALRI=acute lower respiratory infection. *Although China is classed as a developing country, Hong Kong was regarded as a developed country as socioeconomic and demographic indicators are much the same as those in developed countries. †Children in this study were aged 0–<2 years; the CFR meta-estimates if this study were excluded was 2·75%. ‡The CFR meta-estimate if Philippines and Guatemala studies were excluded was 2.71%. §The CFR meta-estimate if this study were excluded was 2·92%

***Table 4:* Case fatality because of influenza-associated severe ALRI in children younger than 5 years who were admitted to hospital**

***Panel 2:* Estimated mortality caused by influenza-associated acute lower respiratory infections (ARLI) in children younger than 5 years**

Approach 1: Case fatality ratio and incidence rate

- *a* Estimated new cases per year of influenza-associated severe ALRI in children younger than 5 years in developed countries: 66 000
- *b* Estimated case fatality ratio for children younger than 5 years caused by influenza-associated severe ALRI yearly in developed countries: 0·17%
- *c* Estimated mortality from influenza-associated severe ALRI in children younger than 5 years in developed countries: $a \times b = 112$
- *d* Estimated new cases per year of influenza-associated severe ALRI in children younger than 5 years in developing countries: 934 600
- *e* Estimated case fatality ratio for children younger than 5 years caused by influenza-associated severe ALRI yearly in developing countries: 2·96%
- *f* Estimated mortality from influenza-associated severe ALRI in children younger than 5 years in developing countries: $d \times e = 27\,664$
- *g* Estimated global mortality caused by influenza-associated severe ALRI in children younger than 5 years: $c + f = 27\,776$

Approach 2: ALRI mortality during influenza season based on data from Ballabgarh, India

- *h* Average proportion of ALRI mortality attributable to influenza during 3 years: 0·06
- *i* Estimated mortality caused by ALRI in Indian children younger than 5 years: 371 605
- *j* Estimated mortality due to influenza-associated ALRI in children younger than 5 years: 24 179 (mean of the three yearly estimates)
- *k* Estimated mortality due to influenza-associated ALRI in Indian children (from Approach 1, with incidence rates from table 2 and case fatality ratio for developing countries): 5998
- *l* Proportion of mortality from this approach compared to approach 1: $j \div k = 4{\cdot}03$
- *m* Estimated global mortality due to influenza-associated ALRI (by extrapolating Indian model): $4{\cdot}03 \times f = 111\,486$

range. These factors would also have contributed to some residual variation in reported incidence estimates (webappendix pp 8–29 and p 34). There are several reasons why we might have overestimated true influenza incidence. First, estimates of influenza-associated ALRI are based on only three studies with active community-based case ascertainment from south Asia. Second, influenza virus has been previously isolated from asymptomatic children,[69] although this proportion is probably very low.[9,70] Third, incidence estimation depends on the relative sensitivity and specificity of the WHO respiratory rate cutoffs for true ALRI. This definition was developed for community case

management of paediatric ALRI in developing countries and is thus highly sensitive but has comparatively low specificity (86% for infants and 93% for children aged 1–4 years).[71]

There are several reasons why we might have underestimated true influenza incidence. First, seven studies identified infection either by rapid tests such as ELISA or immunofluoresence alone and 12 used them in combination with either PCR or viral culture. Immunofluorescence assays have variable and lower sensitivity (40–100%) and specificity (86–99%) than does PCR.[72–76] However, the overall effect of this discrepancy depends on relative sensitivity and specificity of the assays, which were unknown for most studies. Second, although we based our estimates of influenza-associated ALRI on data from community-based studies with active case ascertainment, which encouraged referral of patients to hospital, they could have still missed an unknown proportion of cases. Finally, access to hospital care is typically poor in most low-income settings and thus studies using passive hospital-based case ascertainment would have underestimated the true burden of severe disease.

Substantial uncertainty surrounds case fatality estimates from developing countries. First, many studies only tested a random sample of eligible patients. Some sites reported that some eligible children were not sampled because they were critically ill, refused participation, or were discharged or died before sampling (webappendix p 33). Thus, we might have obtained falsely low estimates because mortality tends to be highest in these groups. Second, the degree to which studies are representative of wider population groups is unknown. Finally, infection with influenza virus has been shown to predispose to bacterial infection, particularly pneumococcal pneumonia.[77–81] Results from a study[77] of a nine-valent pneumococcal vaccine probe in South Africa suggest that at least 45% of influenza-associated severe ALRI have co-infection with *S pneumoniae*. Although bacterial infections have higher case fatality ratios in developing countries, the sensitivity of bacterial diagnostic tests is low.[65,82,83] To fairly interpret childhood pneumonia deaths, mortality should be coattributed to influenza and bacterial pneumonia in cases of co-infection.

We show a more than 15-fold difference in meta-estimates of influenza-associated case fatality ratio between developing and developed regions. This difference could be attributable to epidemiological factors such as population immunity, circulation of *S pneumoniae*, or circulating type or subtype of influenza virus; clinical factors such as availability of oxygen, mechanical ventilation, antivirals, and trained nursing staff; and access to care. We based our estimate of lower bound on reported incidence of influenza-associated severe ALRI and on reported case fatality ratio in patients in hospital. The incidence estimates for developing countries are probably underestimated. Furthermore, hospital-based case fatality ratios cannot be regarded as representative of whole population groups and in most resource-poor settings might be higher than are these reported estimates. However, our estimates for children aged 0–4 years in the USA were consistent with estimates reported elsewhere[84] in 1990–99.

Our estimate of the upper bound was made on the basis of only one study and so replication in other settings is needed. Moreover, although we attributed all excess ALRI mortality during influenza season to influenza (strengthened by the lack of co-circulation with RSV), several other viral pathogens (eg, parainfluenza virus and human metapneumovirus) causing ALRI have unknown seasonal patterns and could account for as much as a third of the ALRI admissions with an equivalent case fatality ratio.[85–89] Conversely, our assumption that no influenza mortality in young children occurred outside the influenza season is unlikely to be true in developing countries in tropical and subtropical areas, leading to an underestimate.[35,59] Furthermore, a substantial proportion

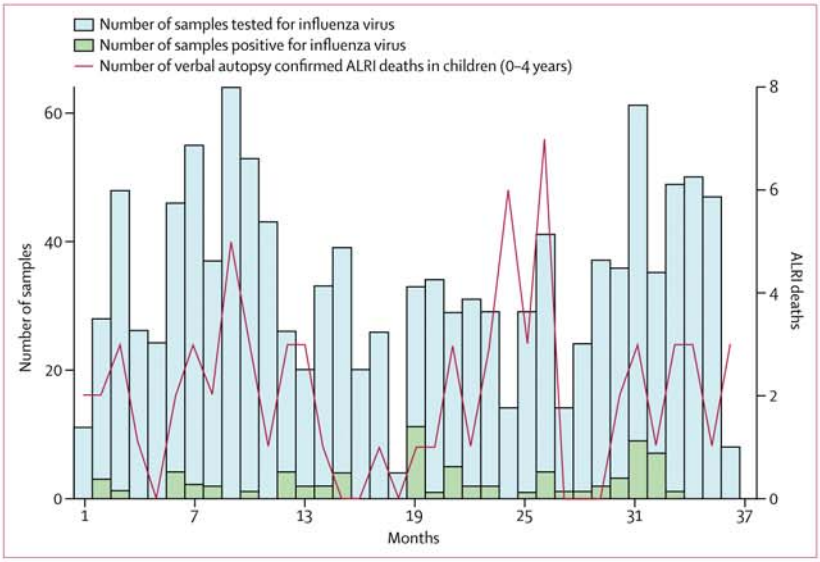

Figure 4: **Pattern of verbal autopsy confirmed ALRI deaths in children younger than 5 years by circulation of influenza virus in the community in Ballabgarh, India (2006–08)**
Month 1 is January, 2006, and month 36 is December, 2008. ALRI=acute lower respiratory infections.

	Duration of influenza season (months)	Mean number of deaths per month during influenza season	Mean number of deaths per month outside influenza season	Overall ALRI deaths per year	Proportion of ALRI deaths caused by influenza	Influenza-associated ALRI deaths in India
2006	3	2·33 (0·58)	2·22 (1·48)	27	0·01	4588
2007	7	1·70 (1·25)	1·60 (2·51)	20	0·04	14 864
2008	5	2·60 (2·70)	1·86 (1·46)	26	0·14	53 086
Mean per year	..	..	..	..	..	24 179 (25 556)

Data are n or mean (SD). ALRI=acute lower respiratory infection.

Table 5: **Estimated influenza-associated ALRI deaths in India based on verbal autopsy confirmed ALRI deaths occurring in the community in children younger than 5 years in Ballabgarh, India**

of the upper bound of influenza mortality that has been attributed to influenza might be the result of co-infection or subsequent infection with a bacterial pathogen (although influenza could have predisposed the child to bacterial infection).[80,81]

The burden on health services of hospitalisation is substantial in influenza, with 1 million episodes of influenza-associated severe ALRI (accounting for 7% of all paediatric severe ALRI episodes) in 2008. Nonetheless, the evidence to support valid and precise estimates of global influenza-associated ALRI mortality is sparse and of low quality. Some sites might have started to improve data collection after the emergence of pandemic influenza A H1N1 (2009). However, this improvement needs to be sustained and expanded to other areas, especially where no data are presently available. Development and consistent application of standardised case definitions and study protocols (at least regionally) would make an important contribution towards addressing gaps in the data and substantially improving these estimates. Further large-scale unselected case series reporting age-specific case fatality ratios from many well described clinical settings in developing countries and large-scale post-mortem studies of ALRI cases that include investigation of influenza virus as a possible cause would also substantially improve the evidence base for this estimate. Influenza is the second most common pathogen identified in children with ALRI and contributes substantially to the burden of hospitalisation and mortality in young children. Our estimates should inform public health policy and vaccine strategy, especially in developing countries. Our report should also help inform donor agencies in assigning funding priorities for novel vaccine development and implementation or other influenza prevention strategies. Until the widespread implementation of an effective influenza vaccine is achievable, reliable provision of effective case management (including oxygen therapy for hypoxaemia and antibiotic treatment of secondary bacterial infections) will substantially reduce sequelae and mortality associated with this disease.

Contributors

HN led the literature search, data analysis, data interpretation, and report writing and contributed to study design and data collection. WAB, MK, AR, SAM, JMS, SH, KAL, OC, DG, WA, RFB, SJO, PFW, SB, and EAFS contributed to study design, data collection, data analysis, data interpretation, and review of manuscript. JAB, AG, AK, MO, ML, WO, ED, SV, MC, PB, EH, MV, TB, RT, DF, and KPK contributed to data collection, data analysis, data interpretation, and review of manuscript. PC-L, LK, NB, and AB contributed to data analysis, data interpretation, and review of manuscript. ET contributed to data interpretation and review of manuscript. MS, MK, and COG-C contributed to data collection, data analysis, and review of manuscript. VE contributed to literature search, data collection, and data analysis. BG did experimental work in the laboratory and contributed to data analysis. M-AW contributed to study design, data analysis, data interpretation, and review of manuscript. AWM, BDG, and IR contributed to study design, data interpretation, and review of manuscript. HC conceptualised the study, provided oversight to literature review, data collection, data analysis, and data interpretation and contributed to report writing and critical review of manuscript.

Conflicts of interest

SAM has received research funding from Wyeth for the Soweto study that contributed to the data. He has received consultancy from Pfizer, GSK, and Novartis and speaker fees from Pfizer and GSK. However, no honoraria were received for work included in this study. BDG has received consultancy from WHO and a travel grant from Sanofi Pasteur to attend a conference on influenza in 2010. He is employed by Agence de Médecine Préventive, which has received funding from WHO, Pfizer, GlaxoSmithKline, and Merck. In 2010, the Agence de Médecine Préventive was hired by Sanofi Pasteur to organize a conference on influenza. However, no grants or honoraria were received for work included in this study. EAFS has received speaker fees and consultancy from MedImmune and research grants from Roche and MedImmune. However, no grants or honoraria were received for work included in this study. NB is employed by Johns Hopkins University which has received funding from Aventis Pasteur and Evans-Powderject. All other authors declare that they have no conflicts of interest.

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4. MANUSCRIT

Human rhinovirus and wheezing: Do lower respiratory tract infections associated with rhinovirus during infancy increase rates of wheezing during childhood?

Rinovirus humà i sibilacions: les infeccions del tracte respiratori inferior associades al rinovirus durant el primer any de vida augmenten la incidència de sibilacions durant la infància?

Cristina O'Callaghan-Gordo, Quique Bassat, Núria Díez-Padrisa, Luis Morais, Sónia Machevo, Tacilta Nhampossa, Llorenç Quintó, Pedro L. Alonso, Anna Roca

Resultats no publicats

RESUM

4.1. Objectiu

Determinar si les infeccions del tracte respiratori inferior associades a l'RV durant el primer any de vida són un factor de risc de tenir sibilacions durant la infància.

4.2. Disseny de l'estudi

Aquí presentem un estudi derivat d'un estudi dut a terme a l'HDM entre setembre de 2006 i setembre de 2007, durant el qual vam agafar ANF en tots aquells nens menors de cinc anys que ingressaven a l'hospital amb tos i/o freqüència respiratòria (FR) elevada per l'edat (≤2mesos:FR ≥ 60; >2-12mesos:FR ≥ 50; >12-60 mesos: FR ≥ 40) i com a mínim um dels següents signes: tiratge, aleteig nasal, gemecs expiratoris o crepitacions detectats durant l'examinació del nen pel pediatra de l'estudi, per diagnosticar diversos virus respiratoris, incloent-hi l'RV. Per a aquesta anàlisi vam incloure tots els nens menors d'un any en el moment de l'ingrés. Aquests nens es van seguir per vigilància passiva (de l'anglès *passive case detection*) a l'HDM durant un màxim de 4 anys i 9 mesos (fins al 20 de juny de 2011) per tal d'enregistrar les visites hospitalàries amb presència de sibilacions durant l'examen mèdic.

Les visites hospitalàries amb sibilacions es van considerar com una aproximació (de l'anglès *proxy measure*) de la presència d'asma.

4.3. Resultats principals

1. Entre el 20 de setembre de 2006 i el 19 de setembre de 2007, 220 nens menors d'un any ingressats a l'HDM amb clínica de pneumònia severa van acceptar formar part de l'estudi. En el 25% dels casos (54/220), vam detectar infecció per l'RV.
2. La probabilitat de tenir infecció per l'RV en el moment d'ingrés fou més elevada per a nens amb infecció per l'HIV [OR=2,66 (95%CI=1,23-5,78); p=0,009].
3. Vam poder seguir fins al 20 de juny de 2011 el 59% (130/220) dels nens que havien acceptat formar part de l'estudi inicialment. L'altre 41% (90/220) dels nens van migrar fora de l'àrea d'estudi o van morir abans d'aquesta data.
4. La incidència de visites a l'HDM amb sibilacions entre els nens d'estudi fou de 296,33 visites (95%CI=250,71-331,25) per 1000 anys a risc per persona (PYAR, de l'anglès *person year-at-risk*)
5. Entre els nens amb RV detectat en el moment d'ingrés per LRTI, la incidència de visites a l'HDM va ser un 75% més elevada que la incidència observada entre nens sense RV detectat inicialment [RR (de l'anglès *rate ratio*)=1,75 (95%CI=1,07-2,86); p=0,026].
6. Després d'ajustar pel sexe, l'edat al moment d'hospitalització i la presència d'infecció per l'HIV, la incidència de visites a l'HDM amb sibilacions fou un 67% [RR=1,67 (95%CI=1,02-2,73); p=0,041] més elevada pels nens amb RV detectat en el moment de l'admissió que pels nens en què no vam detectar RV. Després d'estratificar pel temps des de l'hospitalització per pneumònia, vam veure que aquesta associació desapareixia un cop transcorregut un any de l'ingrés per pneumònia.

Human rhinovirus and wheezing: Do lower respiratory tract infections associated with rhinovirus during infancy increase rates of wheezing during childhood?

INTRODUCTION

Asthma is the commonest chronic disease in children and according to the International Study of Asthma and Allergies in Childhood (ISAAC) phase three, its prevalence is increasing globally (1). Risk factors for asthma are diverse and include, amongst others, air pollution, passive smoking and genetic factors (2, 3). Moreover, several studies have shown that viral lower respiratory tract infections (LRTI) during infancy also predispose to asthma later in life (4-7).

Viral respiratory infections produce wheezing in all age groups, but especially in children (8). Most wheezing episodes during infancy will disappear at school age. However, in some children wheezing is the first clinical manifestation of subsequent asthma (9). Therefore, viral infections in infancy may have a role in development of asthma later in life. Direct association of former viral infection and asthma has been observed for respiratory syncytial virus (RSV) (10, 11), the most common cause of LRTI in children (5, 12), but little is known about the role of other respiratory viruses.

Until recently, RV was considered a mild pathogen of the upper respiratory tract, mainly associated with the common cold (13). Current evidence suggests that RV can infect the lower respiratory tract (14) and become highly prevalent among children with LRTI(15-20) in both developed and developing countries. The association between RV infection and subsequent risk of asthma has also been shown in infants hospitalized for wheezing or at increased risk of developing allergies and asthma, or atopy according to parental history (21-26).

Data on RV from sub-Saharan Africa is scarce and the association between RV and asthma has not been studied in developing countries. The aim of this article in to estimate the incidence of wheezing, as a proxy measure of asthma, in a cohort of infants hospitalized with LRTI in a rural area of southern Mozambique, and to evaluate if LRTI associated with RV during infancy is a risk factor for wheezing during childhood.

METHODS

Study area and population

The study was conducted by the *Centro de InvestigaçaõemSaúde da Manhiça* (CISM) and Barcelona Centre for International Health (CRESIB) at the Manhiça District Hospital (MDH), the referral health facility for Manhiça District, a rural area of Maputo Province in Southern Mozambique. A complete description of the area can be found elsewhere (27, 28). Briefly, Manhiça District has an estimated population of 140.000 inhabitants. The climate of the area is

subtropical with 2 distinct seasons: a warm and rainy season between November and April and a cool and dry season during the rest of the year. Malaria transmission is perennial, peaking during the rainy season (29). In 2004, the HIV prevalence among pregnant women attending the antenatal clinic was 23.6% (30), and was estimated to be between 2.9 and 8% among newborns(20). During the study period, *Haemophilusinfluenzae b* (Hib) and pneumococcal conjugated vaccine were not included among routine infant immunizations in Mozambique.

Demographic surveillance in the area

A demographic surveillance system (DSS) was established in the area surrounding the centre in 1996. Each person living within the DSS area is issued a with unique permanent identification number. Information on vital events is collected by six-monthly household visits (27).

Clinical surveillance and clinical management

Since 1997, MDH and CISM have jointly operated a round-the-clock morbidity surveillance of all paediatric visits at the outpatients department and of all admissions to the wards. Hospital surveillance includes records of clinical signs and symptoms, and determination of malaria parasites and invasive bacterial infections (IBI). For children living in the DSS area the unique permanent identification number is also recorded.

Study design

This analysis is part of a larger study conducted in Manhiça District Hospital (MDH) between September 2006 and September 2007. During that period, participation in the study was offered to all children <5 years old hospitalized at MDH with symptoms of LRTI, defined as cough and/or increased respiratory rate (according to WHO definition(31) and at least one of the following: indrawing, nasal flaring, grunting or crackles detected trough examination, to diagnose RV and other common respiratory viruses. A nasopharyngeal aspirate (NPA) was collected to all study children. Details on the study design can be found elsewhere (20).The study was approved by the Mozambican National Bioethics Committee and the Institutional Review Board of the Hospital Clinic of Barcelona (Barcelona, Spain).

For the current analysis all children residents in the DSS area, that were <1 year old at the time of hospitalization and survive the LRTI episode, were followed-up from study enrolment until the 20th of June 2011to detect visits to MDH outpatients department with wheezing. Detection of visits was done using the morbidity surveillance system established at the MDH. For children with more than one admission during the study period only one register was kept: data from the first admission with RV detected or data from the first visit at all if all of them were RV negative.

Data management and analysis

The main exposure under study was LRTI associated with RV, defined as detection of RV in NPA collected during LRTI hospitalization.

The baseline characteristics of the children enrolled in the cohort were compared according to presence of RV at study enrolment using Chi-square test. Odds ratio (OR) and 95% confidence intervals (95%CI) were estimated using logistic regression.
Crude incidence rates and rate ratios (RR) of visits with wheezing in exposed and non-exposed children were calculated using a Poisson regression model. Time at risk was defined as the time from study enrolment to one of the following events: migration, death, or end of the follow-up period. After one visit with wheezing children were treated as not being at risk during an arbitrarylag period of 15 days to avoid multiple visits during the same episode of wheezing. Multiple events in the same child were taken into account by fitting Random Effects model to the Poisson regression model. Data was set using date of admission at MDH as origin and date of LRTI admission at MDH or end of each lag period as dates of entry into each risk period. Lexis expansion was used to split individual's follow-up time in years since LRTI hospitalization to study the effect of time since RV-detection and incidence of wheezing.

A Random Effects Poisson regression model was used to calculate adjusted RR for hospital visits with wheezing in children with and without RV detected during LRTI hospitalization. The model included the following confounders: sex, age at admission and HIV status. Data were stratified by time since LRTI hospitalization.

All analyses were performed using STATA/SE 11 (Stata-Corp 2009, Stata: Release 11, Statistical Software, College Station, TX).

RESULTS

Study profile

Between the 20th of September 2006 to the 19th of September 2007, 566 children <1 year were hospitalized in MDH with LRTI; 220 of them [39% (220/566)] fulfil the study criteria and entered the cohort. Fifty-four children of the cohort [25% (54/220)] had RV detected during LRTI hospitalization (exposed children) and 166 children of the cohort [75% (166/220)] did not have it (non-exposed children). Baseline characteristics of children in the cohort according to RV detection are presented in table 1. The only difference found between children with and without RV detected during LRTI hospitalization was prevalence of HIV infection as children with RV detected were more likely to be HIV infected [OR=2.66 (95%CI=1.23-5.78); p-value=0.009].
Fifty-nine per cent (130/220) of children were followed-up until the 20th of June 2011 and the remaining children [41% (90/220)] either migrated out of the DSS area or died before this date. Median time-at-risk per individual was 4.01 years, with no differences between exposed and non-exposed children (4.05 years and 3.61 years, respectively; p-value=0.117).

Crude analysis

For the 220 study children, 193 visits with wheezing were recorded during the follow-up period. Fifty-four per cent (119/220) of participants had no episode of wheezing, 25% (56/220) had one episode and 20% (45/220) had more than one episode. The Random Effects model provided strong evidence of within-child clustering of visit with wheezing (LRT for clustering p-value<0.001).

The crude rate of visits with wheezing in the study cohort was 296.33/1000 95%CI=257.34-341.23) person year-at-risk (pyar). Children who had RV detected at admission had 75% higher incidence rate of visits with wheezing during the follow-up period [rate ratio (RR)=1.75 (95%CI=1.07-2.86); Wald test p-value=0.026].

Adjusted analysis

RR of hospital visits with wheezing according to RV detection during LRTI hospitalization adjusted for sex, age at time of hospitalization and HIV infection was 1.67 (95%CI=1.02-2.73; Wald test p-value=0.041). Adjusted RRs stratified by time since LRTI hospitalization are presented in table 2. Results show that LRTI associated with RV during the first year of life increases the incidence rate of visits with wheezing one year after the episode by 68% [RR=1.68, (95%CI=1.03-2.75); Wald test p-value=0.039]. This association seems to disappear after one year of LRTI hospitalization; no evidence of increased incidence rate of visits with wheezing was observed during the remaining follow-up period.

DISCUSSION

To the best of our knowledge, this is the first study assessing the association between LRTI associated with RV in infancy and the presence of wheezing during childhood in Africa. Data shows evidence of an increased risk of wheezing after an initial episode of LRTI with RV.

Our results are in agreement with previous studies that evaluated the same association in developed countries (21-26). Those studies were conducted among children already at increased risk of asthma, since they either had presence of wheezing during LRTI (21-23), or parental history of asthma (24-26). On the contrary, the entry criterion for infants in the present study was hospitalization for LRTI regardless the presence of other risk factors for asthma.

Results from the current study show that infants hospitalized for LRTI with RV have a higher risk of wheezing during the first year following hospitalization. This association, however, is lost after one year. Dilution of the association after the first year could be attributed to a real decrease of the effect of RV on wheezing when children get older, as a consequence of the maturation of their respiratory system. A high percentage of children who wheeze during early childhood stop wheezing after their third year of life (32). Alternative explanations of our results could be

reduction of power to detect differences in an outcome that decreases with age (32) or changes in health seeking behaviour of mothers as children grow older.

As described by Gern (33) there are currently two main hypotheses relating to the association under study. On the one hand, RV might damage the airways of children not previously predisposed to asthma, causing remodelling of their airways and leading to subsequent wheezing episodes and asthma. On the other hand, RV might cause LRTI in infants with underlying lung impairment, who already have a tendency for asthma. The design of the present study does not allow us to support either of the two hypotheses, since children entered the cohort when they had LRTI associated with RV. Therefore it is not possible to ascertain whether study children were already at risk of LRTI by a viral pathogen such as RV or whether subsequent increased risk of wheezing was the result of exposure to RV in infancy.

A major limitation of the current study is the use of children also hospitalized with LRTI, but without RV detected, as a comparison group. Some of the children in the non-exposed group had LRTI due to organisms known to increase risk of subsequent wheezing (20), such as RSV (5, 10, 11, 34). Therefore, the effect observed for RV on the subsequent risk of wheezing, might have been higher if the non-exposed group had been taken from healthy children in the community. The main reason for selecting children similarly ill as the non-exposed group is that in rural areas of sud-Saharan Africa health seeking behaviour widely differs among individuals. Children in our non-exposed group have a similar heath seeking behaviour than exposed children and also attend to hospital when they have a respiratory related infection. The strength of the study could have been improved by collecting information on important risk factors of asthma, some of them highly prevalent in sub-Saharan Africa such as exposure to biomass smoke (i.e. burning of biomass fuels for cooking).

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TABLES

Table 1. Baseline characteristics of infants in the study cohort and crude analysis of risk factors according to RV-detection.

Variable		Total (n=220) n (%)	RV - (n=166) n (%)	RV + (n=54) n (%)	p-value**	OR	95% CI	p-value***
Sex	Boy	130 (59)	93 (56)	37 (69)		1		
	Girl	90 (41)	73 (44)	17 (31)	0.105	0.58	0.30 - 1.13	0.106
Age (months)	≤ 3	82 (37)	67 (40)	15 (28)		1		****
	4-≤6	56 (25)	41 (25)	15 (28)		1.63	0.72 - 3.72	0.237
	7-≤12	82 (37)	58 (35)	24 (44)	0.238	1.85	0.88 - 3.89	0.100
Season of birth	Dry	102 (46)	79 (48)	23 (43)		1		
	Rainy	118 (54)	87 (52)	31 (57)	0.522	1.22	0.66 - 2.28	0.523
Coinfections								
Other viruses*	No	151 (69)	110 (66)	41 (76)		1		
	Yes	69 (31)	56 (34)	13 (24)	0.184	1.76	0.31 - 1.26	0.185
IBI	No	173 (79)	130 (78)	43 (80)		1		
	Yes	14 (6)	10 (6)	4 (7)		1.21	0.36 - 4.07	0.758
	No data	33 (15)	26 (16)	7 (13)	0.848	0.81	0.33 - 2.01	0.655
Parasitemia	No	197 (90)	148 (89)	49 (91)		1		
	Yes	20 (9)	16 (10)	4 (7)		0.75	0.24 - 2.37	0.630
	No data	3 (1)	2 (1)	1 (2)	0.835	1.51	0.13 - 17.13	0.738
HIV infected	No	162 (74)	129 (78)	33 (61)		1		
	Yes	37 (17)	22 (13)	15 (28)		2.66	1.23 - 5.78	0.009
	No data	21 (9)	15 (9)	6 (11)	0.033	1.56	0.56 - 4.36	0.389
Otherconditions								
Malnutrition	No	212 (55)	93 (56)	28 (52)		1		*****
	Moderate	78 (35)	58 (35)	20 (37)		1.14	0.59 - 2.22	0.688
	Severe	21 (10)	15 (9)	6 (11)	0.834	1.33	0.47 - 3.76	0.592
Wheezing	No	162 (74)	126 (76)	36 (67)		1		
	Yes	58 (26)	40 (24)	18 (33)	0.181	1.78	0.80 - 3.09	0.182

* Viruses included: RSV, influenza, adenovirus, human metapneumovirus, parainfluenza virus 1, 2, 3 and 4AB, and enterovirus. ** Chi-square test; *** Wald test; **** Test for trend p-value=0.103 ; ***** Test for trend p-value= 0.548

Table 2. Adjusted incidence rate and RR of recurrent wheezing according to RV-detection during infancy by time since LRTI hospitalization.

Time since LRTI hospitalization			Num. of visitis with wheezing (n=193)	pyar	Crude RR	95% CI	p-value*	Adjusted RR**	95% CI	p-value*
Overall	**RV**	No	139	514.93	1			1		
		Yes	54	136.36	1.75	1.07 - 2.86	0.026	1.67	1.02 - 2.73	0.041
1 year	**RV**	No	81	142.63	1			1		
		Yes	39	42.11	1.67	1.03 - 2.72	0.037	1.68	1.03 - 2.75	0.039
2 years	**RV**	No	32	125.98	1			1		
		Yes	11	31.33	1.51	0.70 - 3.25	0.291	1.53	0.71 - 3.32	0.278
3 years	**RV**	No	17	113.13	1			1		
		Yes	3	28.37	0.76	0.21 - 2.74	0.677	0.77	0.21 - 2.78	0.691
≥4 years	**RV**	No	9	113.19	1			1		
		Yes	1	34.55	0.46	0.06 - 3.77	0.471	0.47	0.06 - 3.84	0.481

* Wald Test; ** Adjusted for age, sex and HIV infection

DISCUSSIÓ

DISCUSSIÓ

En el moment de la publicació d'aquesta tesi, la informació sobre el rol dels virus respiratoris en les ARI entre nens menors de cinc anys d'edat a l'Àfrica subsahariana és limitada. Hem trobat estudis de Gàmbia [92-95], Kènia [21, 96, 97], Sud-àfrica [98-103], Nigèria [104] i Senegal [105]. Molts d'aquests treballs se centren en el paper d'un únic virus (principalment l'RSV) i tots ells, amb l'excepció de l'article de Senegal [105], estudien només casos d'LRTI. En aquesta tesi presentem dades de dotze virus respiratoris associats a les ARI, tant entre nens amb URTI atesos a les consultes externes com entre nens ingressats amb pneumònia, en una zona rural de Moçambic.

Els resultats dels dos primers articles que formen aquesta tesi mostren que la càrrega de malaltia associada a les infeccions respiratòries víriques és molt elevada, fins i tot en una zona on la malària és endèmica. Segons els resultats del primer article, que se centra en la descripció d'URTI, gairebé el 90% dels nens menors d'un any atesos a les consultes externes de l'HDM entre febrer de 1999 i març de 2000, presentaven símptomes d'ARI i en més de la meitat de les mostres analitzades vam detectar com a mínim un virus respiratori. A més, el percentatge de virus respiratoris en aquest grup és, probablement, més elevat que el que nosaltres vam detectar ja que els nostres resultats no inclouen els casos amb infecció per l'RSV, un dels virus més freqüents durant el primer any de vida [105-107]. D'acord amb els resultats de l'estudi previ sobre l'RSV [42], en el 9% dels nens inclosos en el nostre estudi es va detectar l'RSV. Segons dades del segon article, que se centra en la descripció de casos de pneumònia, el 28% dels nens ingressats a l'HDM entre setembre de 2006 i setembre de 2007 presentaven símptomes de pneumònia severa. També en aproximadament la meitat dels casos vam detectar almenys un virus respiratori. L'elevada prevalença de virus en casos de pneumònia concorda amb dades dels darrers anys d'altres zones de l'Àfrica subsahariana [98, 104] i d'altres regions geogràfiques [24-26].

Els resultats dels dos primers estudis, duts a terme durant diferents anys, indiquen que la incidència de les ARI a Manhiça és més elevada durant l'estació de pluges, entre novembre i abril. Els virus amb una estacionalitat més marcada són el Flu i l'RSV, que

com ja s'ha descrit en altres regions [108], provoquen pics que se solapen durant aproximadament les mateixes setmanes (a Manhiça entre gener i maig).

En ambdós estudis vam trobar que en el 20% dels casos amb infecció vírica es detectava més d'un virus simultàniament, tant entre els nens amb URTI com entre els nens amb pneumònia, i d'acord amb els nostres resultats, les coinfeccions víriques s'associaven a una major severitat. El significat i la importància clínica de les infeccions víriques múltiples, però, és un tema de debat; hi ha estudis que recolzen els nostres resultats [109-112] i d'altres que no detecten una simptomatologia més severa entre els casos amb coinfeccions víriques [113, 114 115].

Vam estimar que la prevalença de l'HIV entre nens a la comunitat els anys 1999 i 2006 era d'entre el 3-5% i el 3-8% respectivament. La infecció per l'HIV té un paper important en l'epidemiologia, l'etiologia i en la clínica de les ARI [12, 14, 15]. Segons dades de Sud-àfrica, tant la càrrega de malaltia com la mortalitat associada a les LRTI amb virus és més elevada en nens infectats per l'HIV que en nens HIV negatius [98, 102]. Els resultats del segon estudi que formen aquesta tesi troben resultats similars i indiquen que la incidència amb què detectem virus respiratoris entre nens ingressats amb pneumònia severa és de 6 a 16 vegades superior per a nens HIV positius que per a nens HIV negatius. La mortalitat per pneumònia amb almenys un virus detectat és 7 vegades superior entre nens infectats per l'HIV que entre nens no infectats.

El tercer article i el manuscrit inclosos a la tesi se centren en el paper de dos dels virus actualment més rellevants en l'etiologia de les ARI: el Flu, a causa de l'elevada càrrega de malaltia associada i al seu potencial per causar pandèmies, i l'RV, per la seva associació, cada cop descrita de manera més freqüent, amb LRTI i asma.

El tercer article engloba totes les dades d'incidència i mortalitat associada al Flu en nens menors de cinc anys que hi ha actualment a tot el món. Entre aquestes dades hi ha les generades durant el segon estudi presentat en aquesta tesi. Utilitzant tota la informació disponible sobre el Flu, s'ha estimat que el 13% dels casos d'LRTI i el 7% dels casos d'LRTI severa estan associats al Flu, i es posa de manifest que el Flu és el segon patogen més comú en nens amb LRTI, després de l'RSV.

En el quart manuscrit que forma aquesta tesi hem mirat els efectes a llarg termini de les infeccions severes associades a l'RV. Els resultats del seguiment dels nens ingressats amb clínica de pneumònia severa durant el primer any de vida, mostren una elevada incidència de visites a l'HDM amb sibilacions (288/1000 PYAR). Les sibilacions són una simptomatologia molt associada a les infeccions respiratòries víriques [116-120]; per tant, l'elevada incidència detectada entre els nens de l'àrea d'estudi concorda amb els resultats dels altres estudis que formen la tesi, que indiquen una elevada càrrega de malaltia associada a infeccions respiratòries víriques. Els resultats d'aquest darrer treball indiquen que els nens ingressats amb pneumònia associada a l'RV tenen una incidència més elevada de visites amb sibilacions que els nens ingressats amb pneumònia en els quals no es va detectar l'RV, durant el primer any de vida després de l'episodi de pneumònia amb l'RV [RR ajustada=1,59 (95%CI=0,98-2,58)] però no durant els anys posteriors.

Els estudis inclosos en aquesta tesi comparteixen, com a mínim, una limitació comuna: per a tots ells hem realitzat la detecció de casos a l'hospital i per tant no hem tingut en compte tots aquells episodis que es produeixen a la comunitat però no es veuen a l'hospital. D'acord amb dades de Manhiça, més del 54% de les morts es produeixen a casa, i només el 55% de les persones que moren a casa són ateses en un centre de salut durant el transcurs de la malaltia terminal [121]. Dades de Gàmbia [94] i de Kènia [122] indiquen que la incidència d'hospitalitzacions per LRTI o per pneumònia disminueix a mesura que augmenta la distància a l'hospital. Aquestes dades indiquen que en utilitzar una detecció passiva de casos probablement hem subestimat la prevalença de les ARI i que per tant les dades presentades aquí corresponen a la incidència mínima de les ARI a la zona. Creiem que aquesta limitació deu ser més marcada per a malalties lleus, la qual cosa concorda amb dades de l'estudi de Gàmbia [94], i per tant és probable que hagi afectat de manera més marcada la detecció de casos en el primer estudi (nens amb URTI) i en el quart (detecció de nens amb sibilacions) d'aquesta tesi.

Malgrat aquesta limitació i les exposades de manera individual a la discussió de cadascun dels quatre estudis, aquesta tesi recull dades sobre l'epidemiologia i la freqüència de les ARI víriques, en una zona on les ARI causen una elevada morbiditat i

mortalitat i on no hi havia dades prèvies sobre l'epidemiologia de la majoria de virus respiratoris.

CONCLUSIONS GENERALS

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1. La càrrega de malaltia associada als virus respiratoris al districte de Manhiça és elevada: en aproximadament la meitat dels pacients pediàtrics atesos a l'HDM amb símptomes d'ARI, tant d'URTI com de pneumònia, detectem, com a mínim, un virus respiratori.
2. Els virus detectats de manera més freqüent en nens menors d'un any amb URTI, atesos a l'HDM entre febrer de 1999 i març del 2000 -sense haver inclòs l'RSV en el diagnòstic de les URTI- van ser l'RV, el Flu i l'ADV. En nens menors de cinc anys ingressats amb pneumònia al mateix hospital entre setembre de 2006 i setembre de 2007, els virus més freqüent foren l'RV, l'ADV i l'RSV.
3. La detecció de virus respiratoris augmenta durant l'estació de pluges, entre els mesos de novembre i abril. L'RSV i el Flu són els virus amb una estacionalitat més marcada; ambdós causen un brot anual durant la segona meitat de l'estació de pluges (entre febrer i abril).
4. En els nens atesos a l'HDM, les coinfeccions víriques s'associen a una presentació clínica més severa (en els casos d'URTI) i a una major mortalitat (en els casos de pneumònia).
5. La infecció per l'HIV incrementa la incidència de les infeccions víriques en nens amb pneumònia i n'empitjora la prognosi. La millora en les mesures de prevenció de la transmissió vertical de l'HIV reduirien tant la incidència com la mortalitat associada de les infeccions greus del tracte respiratori inferior associades a virus respiratoris.
6. La mortalitat associada a les ARI d'origen víric és més elevada en els casos amb coinfecció bacteriana invasiva; per tant, la introducció de les vacunes dirigides contra patògens bacterians (com ara les vacunes conjugades de pneumococ o Hib) podria reduir també la mortalitat associada a les pneumònies associades a virus respiratoris.
7. A nivell mundial, el Flu estacional comporta una elevada càrrega de malaltia en nens menors de cinc anys. Actualment no hi ha prou dades per poder estimar de manera acurada la mortalitat associada al Flu.

8. Les infeccions respiratòries greus associades a l'RV -el virus detectat de manera més freqüent en nens hospitalitzats amb pneumònia a l'HDM- poden incrementar el risc de tenir sibilacions durant l'any següent a l'hospitalització.

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