UNIVERSIDADE FEDERAL DA BAHIA
FACULDADE DE MEDICINA DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO
EM CIÊNCIAS DA SAÚDE

PRIMEIRO EPISÓDIO DE SIBILÂNCIA, BACTÉRIAS E VÍRUS RESPIRATÓRIOS E COMPLICAÇÕES ASSOCIADAS EM LACTENTE COM INFECÇÃO RESPIRATÓRIA AGUDA

Juliana Rebouças de Oliveira

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Salvador (Bahia), 2017

COMISSÃO EXAMINADORA

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- ✓ Eduardo Martins Netto – Doutor, médico epidemiologista e professor do Programa de Pós-Graduação em Medicina e Saúde e em Ciências da Saúde da Universidade Federal da Bahia (UFBA);
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- ✓ Cristiana Maria Costa Nascimento de Carvalho – Doutora, livre-docente professora associada do Departamento de Pediatria da Faculdade de Medicina da Bahia da UFBA;

“ Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas graças a Deus, não sou o que era antes”.

(Marthin Luther King)

À minha filha Victória de Oliveira Malvar, minha eterna inspiração, que me deu força e me fez seguir em frente mesmo quando as forças já haviam se esgotado. Aos meus pais, Valdeci Ferreira de Oliveira e Dilmar Rebouças de Oliveira que me deram a vida e que me ensinaram desde tenra idade que a educação é o caminho da dignidade e que nada conseguimos sem esforços.

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SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

ANF	Aspirado nasofaríngeo
FR	Frequência respiratória
hMPV	Metapneumovírus humano
IRA	Infecção respiratória aguda
VSR	Vírus sincicial respiratório
OMA	Otite média aguda
PCV10	Vacina pneumocócica 10 valente
HRV	rinovírus
EV	enterovírus
AdV	adenovírus
PIV	vírus Parainfluenza
FLU	vírus influenza
RSV	vírus sincicial respiratório
HCoV	coronavírus
hBoV	bocavírus

I. RESUMO

TÍTULO: Primeiro episódio de sibilância, bactérias e vírus respiratórios e complicações associadas em lactente com infecção respiratória aguda

A infecção respiratória aguda (IRA) é a doença mais comum entre crianças pequenas; como tal, é uma fonte de morbidade significativa. Sibilância em lactentes, na vigência de infecção respiratória aguda (IRA), está frequentemente associada à infecção respiratória viral. **Objetivos**: Avaliar a associação entre fatores ambientais, bactérias e vírus respiratórios detectados em aspirado nasofaríngeo (NPA), primeiro episódio de sibilância detectado no exame físico e as complicações associadas em crianças de 6 e 23 meses com IRA. **Métodos**: Estudo corte transversal prospectivo seguido de uma coorte, realizado em emergência pediátrica. Crianças com idade entre 6-23 meses com IRA foram avaliadas entre Setembro/2009 e Outubro/2013. Os critérios de exclusão foram ter sido transferido de outro hospital ou relatar episódio anterior de sibilância. Foram coletados dados demográficos e clínicos juntamente com o aspirado nasofaríngeo (NPA). Todas as crianças elegíveis foram contatadas para reavaliação no 14º dia após a avaliação inicial; complicação foi definida como hospitalização, pneumonia ou otite média aguda (OMA). **Resultados**: Foram avaliadas um total de 1154 crianças; das 576 que preenchiam critérios de inclusão, 16 (2,8%) não coletaram NPA e 1(0,2%)

teve quantidade insuficiente de NPA. Foram então inscritas 559 crianças, e destas 92 (16,5%) apresentaram sibilância, 120 (21,5%) e 88 (15,7%) relataram cães e pássaro em casa, respectivamente. Rinovírus (48,1%) e vírus parainfluenza 1 (32,0%) foram os vírus mais frequentemente encontrados. *Staphylococcus aureus* (98,0%) e *Haemophilus influenzae* (97,1%) foram as bactérias mais comuns. Por regressão logística multivariada, *Haemophilus influenzae* (AdjOR=0,319; IC 95%: 0,110-0,927), cão (AdjOR=0,476; IC 95%: 0,246-0,920) e pássaro (AdjOR=1,828; IC 95%: 1,021-3,272) em casa foram independentemente associados com sibilância. Pássaro em casa (OR [IC 95%]: 5,80 [1,73-19,38]) e roncos (OR [IC 95%]: 6,39[1,96-20,85]) foram associados com OMA e PCV10 foi inversamente associada (OR [IC 95%]: 0,16 [0,05-0,52]) **Conclusão:** *Haemophilus influenzae* em NPA e cão em casa protegem de forma independente contra o primeiro episódio de sibilância em crianças com IRA, enquanto pássaro em casa é um fator de risco para tal. A PCV10 foi associada com menor probabilidade de OMA, enquanto pássaro em casa e roncos são fatores de risco.

Palavras-chave: infecção respiratória aguda; sibilância; vírus respiratório; fator de risco; Vacina Pneumocócica Conjugada; lactente.

II. OBJETIVOS

II.1. GERAL

Avaliar a associação entre fatores ambientais, bactérias e vírus respiratórios detectados em aspirado nasofaríngeo (NPA) e sibilos detectados no exame físico em crianças de 6 e 23 meses com infecção respiratória aguda.

II.2. ESPECÍFICOS

II.2.1. Estimar a frequência de complicações em crianças de 6 a 23 meses durante o episódio de IRA.

II.2.2. Avaliar os fatores de risco presentes no recrutamento de lactentes com IRA, associados às complicações após a implantação universal da vacina pneumocócica 10 valente (PCV10) em nossa região.

III. INTRODUÇÃO

As infecções respiratórias agudas (IRA) em crianças com menos de cinco anos de idade são as principais causas de mortalidade infantil em todo o mundo (Nair et al., 2013). Foi relatada uma frequência ajustada de 6,2 episódios de IRA por criança-ano entre crianças com menos de três anos de idade (Budge et al., 2014). IRA têm sido reconhecidas como a principal razão para as famílias que procuram assistência pediátrica (Nair et al., 2010). Segundo o Ministério da Saúde do Brasil, as doenças respiratórias são responsáveis por 35,7% de todas as hospitalizações de crianças < 1ano de idade no estado da Bahia (DATASUS, 2011).

As IRA são classificadas como infecção de vias aéreas superiores (IVAS) e infecção de vias aéreas inferiores (IVAI) segundo sua localização anatômica. A IVAS compreende a rinofaringite, faringite, amigdalite, otite media, sinusite e laringite; e IVAI a bronquiolite e as pneumonias. Geralmente as IRA são autolimitadas, porém podem evoluir para complicações, principalmente em lactentes (Budge et al., 2014; Marcone et al., 2013). As principais complicações são a otite média aguda (OMA), pneumonia e hospitalização (Chonmaitree et al., 2008; Antonova et al., 2012; Neuzil et al., 2002). Em geral, a desnutrição, a amamentação inadequada e a imunização precária são os principais fatores de risco envolvidos na ocorrência de complicações por IRA (Ujunwa et al., 2014).

Os vírus são os principais agentes causadores das IRA (Griffin et al., 2004); além disso as IRA virais são as principais causas de sibilância aguda em lactentes (Turunen et al., 2015). Em relação ao primeiro episódio de sibilância, ocorrendo principalmente no primeiro ano de vida, está associado à sibilância recorrente e asma na infância (Cunha et al., 2010); como tal, é uma fonte significativa de morbidade infantil (Jartti et al., 2008; Caliskan et al., 2013). Temos demonstrado em estudo anterior que fatores ambientais tais como a frequência à creche e a presença de pássaro em casa, são fatores associados à sibilância detectada no exame físico (Bouzas et al., 2014). Porém não existem grandes estudos associando, de forma ampla, fatores ambientais, agentes virais e bacterianos em NPA e primeiro episódio de sibilância, como também complicações associadas a IRA em latentes.

IV. REVISÃO DA LITERATURA

IV.1. Infecção Respiratória Aguda

As IRA são uma das principais causas de morbidade e mortalidade em crianças, principalmente nos primeiros anos de vida (WHO, 2009). A cada ano, cerca de 156 milhões de novos episódios de IRA ocorrem em todo o mundo e cerca de 1,57 milhões de crianças morreram como resultado de tais infecções (Rudan et al., 2008; Black et al., 2010). Em 2010, 1,4 milhões de crianças morreram em decorrência de infecções respiratórias, sobrecarregando globalmente o sistema de saúde (Liu et al., 2012). Estima-se 25 milhões de atendimentos médicos por IRA em criança anualmente nos Estados Unidos (Gonzales et al., 2001; Heikkinen et al., 2003), 22 milhões de dias perdidos no ano letivo, 20 milhões de faltas ao trabalho pelos pais ou responsáveis, além dos custos financeiros com medicamentos, gerando um alto ônus social (Bramley et al., 2002; Heikkinen et al., 2003). Outro estudo na Alemanha mostrou um custo anual estimado de U$ 213 milhões decorrente de IRA em crianças (Tregoning & Schwarze, 2010).

As infecções respiratórias, na grande maioria de etiologia viral, são muito comuns no início da vida. A exposição às infecções respiratórias, que ocorrem neste período, pode aumentar ou diminuir o risco de desenvolver sibilância recorrente e asma na infância, dependendo de fatores do

hospedeiro, fatores ambientais e o tipo de microrganismo. (Ramsey et al., 2007)

O quadro clínico das IRA seja de causa viral ou bacteriana é semelhante, portanto, o diagnóstico etiológico preciso depende inteiramente de investigações laboratoriais. A detecção precoce dos agentes causais relacionados é crucial para proporcionar um regime de tratamento adequado, diminuindo o uso de antibióticos desnecessários, limitando a disseminação da infecção, evitando as complicações e encurtando a duração da hospitalização (Javadi et al.,2015; Mahony et al.,2008).

IV.2. Agentes etiológicos

Cerca de 90% de todas as IRA nas crianças são causadas por vírus e apenas 10% são causadas por bactérias (Pavia et al., 2011). Os vírus mais frequentemente associados com IRA são: rinovírus (RV), enterovírus (EV), adenovírus (AdV), vírus parainfluenza (PIV), vírus influenza (FLU), vírus sincicial respiratório (RSV) e coronavírus (HCoV). Nos últimos anos, com novas técnicas de diagnóstico molecular, seis novos vírus respiratórios foram identificados, incluindo metapneumovírus humano (hMPV) e bocavírus (hBoV) (Berry et al., 2015). Dentre as bactérias as mais frequentemente identificadas em crianças são: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, e *Staphylococcus aureus*. (Goktas et al., 2016)

As novas técnicas de diagnóstico molecular para múltiplos agentes etiológicos estão definindo com mais precisão a epidemiologia da doença e o papel potencial das coinfecções (Griffin et al., 2004). Em estudos recentes as coinfecções vêm se destacando, principalmente entre as crianças, sendo demonstrado em 27,2% dos casos. Dentre os vírus aqueles mais identificados nas coinfecções são os AdV, HCoV, HBoV, hMPV, RV/EV e PIV; dentre as bactérias o *Mycoplasma pneumoniae* e *Chlamydia pneumoniae*. (Goktas et al., 2016)

IV. 3 Microbioma respiratório

O trato respiratório superior é colonizado por uma grande variedade de bactérias que constituem a microbiota respiratória. Entre as bactérias, as mais conhecidas são: *Streptococcus pneumoniae*, *Haemophilus influenzae* e *Staphylococcus aureus* (Schenck et al., 2016). A microbiota respiratória parece influenciar no desenvolvimento da resposta imune local e sistêmica (Hasegawa e Camargo Jr, 2015), podendo com isto aumentar ou diminuir a gravidade das IRA em crianças (Lynch SV, 2014).

Vários estudos demonstraram que podem ocorrer interação entre vírus e bactérias, levando a atividade sinérgica ou efeito de supressão, capazes de impactar significativamente a etiologia, patogênese e frequência das infecções respiratórias. Como exemplo, observou-se que a colonização das vias aéreas por *Moraxella catarrhalis* e *Haemophilus influenzae* está

associado a uma resposta inflamatória mista Th1/ Th2/Th17 da mucosa das vias aéreas modulando a resposta inflamatória de forma sinérgica (Tam TT et al., 2007), enquanto a presença de *Streptococcus pneumoniae* reduz o risco de colonização por *Staphylococcus aureus e* do *Haemophilus influenzae* (Bogaert D et al., 2004; Cundell DR et al., 1995). Outros estudos relataram que alguns vírus podem favorecer significativamente o desenvolvimento de infecções bacterianas, sendo um dos principais exemplos o sinergismo observado entre o Vírus Influenzae e *Streptococcus pneumoniae* (McCullers, 2006), podendo ocorrer também um efeito protetor em alguns casos (Bosch et al., 2013; Lenoir-Wijnnkoop et al., 2015).

IV. 3. Diagnóstico laboratorial

Estão disponíveis diferentes métodos de diagnóstico laboratorial para determinar os patógenos causais relacionados com as IRA. Os métodos convencionais, tais como cultura viral e detecção de antígenos (teste de imunoensaio enzimático e teste de anticorpos fluorescentes) são eficazes e frequentemente complementares, mas têm algumas limitações. Embora a técnica de cultura celular seja considerada como o padrão-ouro para a detecção de vírus, o processo é laborioso e demorado, e é quase impossível obter resultados durante a fase aguda da doença. Os testes de detecção de antígenos podem fornecer resultados mais rápidos, mas são menos

sensíveis e/ou específicos. Os métodos de diagnóstico molecular permitem a identificação de uma ampla gama de agentes virais e bacterianos, com excelente sensibilidade e especificidade. Os ensaios de reação em cadeia de polimerase em tempo real (PCR) permitem a amplificação simultânea de vários vírus ou bactérias em uma única reação e facilitam a detecção de agentes causadores das IRA (Salez et al., 2015; Pillet et al.,2013).

Atualmente novas técnicas usando o sistema de expressão gênica que testam simultaneamente vírus e bactérias como NanoString nCounter, que captura e faz contagens de transcritos de mRNA individuais, vêm sendo usadas (Geiss et al, 2008). Este método permite a identificação de múltiplos patógenos em amostras NPA de pacientes com IRA numa única reação, que apresentam baixo rendimento de RNA (10-50 ng). (Fukutani et al, 2015)

IV 3. Infecção respiratória aguda e sibilância

Infecção respiratória aguda por vírus respiratório pode causar sibilância em crianças pequenas, e com isto sendo causa frequente de morbidade e hospitalização (Turunen et al, 2015). Estudos mostram que mais de 88% dos casos de crianças com sibilância aguda estão associados com infecção respiratória viral. (Lehtinen et al., 2006). Além disso, a maioria das crianças com sibilância recorrente e asma na idade escolar apresentou o primeiro episódio de sibilância associada à IRA nos primeiros

anos de vida. (Jackson & Lemanske, 2010). O fator de risco mais significativo para o desenvolvimento da sibilância no pré-escolar é a ocorrência de infecção sintomática por rinovírus durante a infância (Lemanske et al. Al. 2005). Os vírus penetram e se replicam nas células epiteliais das vias aéreas, causando um processo inflamatório e remodelação da mucosa. As manifestações clínicas são secundárias à liberação de mediadores pró-inflamatórios pelas células danificadas ou pelo efeito citotóxico do próprio vírus (Jackson & Jonhston, 2010). Para muitas crianças, os episódios de sibilância associados à infecção respiratória diminuem com a idade, mas para outras, sibilância na infância podem ser fator de risco para asma (Busse et al., 2010). Além disso, a diminuída capacidade da criança para montar uma defesa antiviral adequada, assim como uma resposta Th2 prolongada, uma diminuição da resposta imune inata ou defeitos na imunidade local aumentam a probabilidade de propagação do vírus para as vias aéreas inferiores e resultam em infecções mais severas (Sly et al., 2010).

Em relação ao primeiro episódio de sibilância, foi demonstrado anteriormente que fatores ambientais como a frequência à creche e a presença de pássaro em casa são fatores associados à sibilância (Bouzas et al, 2014). Ao contrário de estudos que associam infecções na infância com morbidades respiratórias subsequentes, a teoria da higiene propõe que

infecções na infância, são protetores para o desenvolvimento de doenças alérgicas e possivelmente asma. Esta relação é apoiada em estudo que mostra uma baixa prevalência de alergias e asma em crianças que frequentam creche e aquelas com irmãos mais velhos em casa. Foi proposto que este efeito protetor se relaciona com um maior número de infecções, incluindo aquelas causadas por patógenos respiratórios e a subsequente estimulação protetora da imunidade Th1. (Busse et al., 2010). Notadamente tem sido postulado que a exposição às endotoxinas bacterianas (Martinez & Halt, 1999) e animais (Hesselmar et al, 1999, Svanes et al, 1999) no início da vida pode conferir proteção contra o desenvolvimento de asma e alergias.

IV 4. Complicações das IRA

As IRA geralmente são autolimitadas e os sintomas incluem corrimento nasal, obstrução nasal, febre e tosse; porém podem evoluir para complicações graves, particularmente em crianças menores de 2 anos (Budge et al., 2014; Marcone et al., 2013). Estas complicações são principalmente otite média aguda (OMA), pneumonia e hospitalização (Chonmaitree et al., 2008; Antonova et al., 2012; Neuzil et al., 2002). Heikkinen e colaboradores relataram a maior frequência para OMA (39,7%), seguida de pneumonia (2,4%) e hospitalização por complicação do quadro respiratório (0,8%) em crianças após infecção pelo vírus

influenza (Heikkinen et al., 2004). Em geral, a desnutrição e o aleitamento materno inadequado foram indicados como os principais fatores de risco envolvidos na ocorrência de complicações devido a IRA (Ujunwa et al., 2014). A infecção pneumocócica tem sido considerada potencialmente chave na ocorrência de complicações das IRA (Klugman et al., 2009). Observou-se um aumento de colonização pneumocócica durante as IRA devido às alterações anatômicas nas vias aéreas superiores, produzidas pela infecção viral que facilitam o isolamento ou aquisição do pneumococo (Klugman et al., 2009).

V. ARTIGOS

ARTIGO I

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Frequency of complications and the effects of pneumococcal vaccination in young children with acute respiratory tract infection

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ABSTRACT

Background: Acute respiratory infection (ARI) is the most frequent reason for children being seen by doctors worldwide. We aimed to estimate the frequency of complications in children aged 6–23 months during ARI episode and to evaluate risk factors present on recruitment associated with complications after the universal implementation of pneumococcal vaccine (PCV10) in our region.

Methods: This prospective cohort enrolled children who had shown ARI for up to 7 days and who were subsequently followed up 14–21 days after, in Salvador, Brazil. Data on recruitment were registered. The vaccine card was personally checked. Complication was defined when hospitalization, pneumonia or acute otitis media (AOM) were informed during the follow-up visit. Pneumonia and AOM were diagnosed by a doctor. Multiple logistic regression analysis was performed.

Results: Of 576 children, 422 (73%) returned and 79 (19%; 95%CI: 15–23%) had complications. The mean interval between admission and follow-up was 23 ± 13 days. Pneumonia (n = 47; 11%), hospitalization (n = 28; 7%), and AOM (n = 17; 4%) were reported. Most of the patients presented one complication (n = 66; 84%) followed by two (n = 13; 16%). Report of fever (92% versus 79%; OR [95%CI]: 2.90 [1.18–7.14]), bird at home (24% versus 14%; OR [95%CI]: 2.13 [1.07–4.26]), ronchi (48% versus 36%; OR [95%CI]: 2.06 [1.16–3.67]) or crackles (17% versus 7%; OR [95%CI]: 2.36 [1.04–5.38]) on auscultation were directly associated with complications whereas PCV10 (59% versus 75%; OR [95%CI]: 0.46 [0.26–0.82]) was inversely associated. Bird at home (OR [95%CI]: 5.80 [1.73–19.38]) and ronchi (OR [95%CI]: 6.39 [1.96–20.85]) were associated with AOM; PCV10 was inversely associated with AOM (OR [95%CI]: 0.16 [0.05–0.52]). Crackles were associated with pneumonia (OR [95%CI]: 2.55 [1.01–6.40]).

Conclusions: One fifth of the children presented complications. PCV10 was independently associated with lower odds of development of AOM. Bird at home and ronchi are risk factors of otitis. Crackles are associated with pneumonia.

1. Introduction

One of the most common problems that affect children within their first years of life is the occurrence of acute respiratory infections (ARI). It has been reported an adjusted frequency of 6.2 ARI episodes per child-year among children aged under three years [1]. ARI has been recognized as the main reason for families seeking pediatric assistance [2].

ARI symptoms generally include runny nose, nasal blockage, low-grade fever, cough and the episode can evolve to severe complications, particularly in young children [1,3]. These complications are mainly acute otitis media (AOM), pneumonia, and hospitalization [4–6]. Heikkinen et al. reported the highest frequency for AOM (39.7%), followed by pneumonia (2.4%) and hospitalization (0.8%) in children after an influenza infection [7]. In general,

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malnutrition, inadequate breast feeding and poor immunization have been indicated as the main risk factors involved in the occurrence of complications due to ARI [8]. Pneumococcal infection has been considered as potentially key in the occurrence of ARI complications [9]. There is an increasing frequency of pneumococcal colonization during clinical ARI and the physical changes produced by viral infection in the nasal passages facilitate the isolation or acquisition of pneumococci [9].

We aimed to estimate the frequency of complications in children aged between 6 and 23 months during an ARI episode. We further evaluated risk factors present on recruitment associated with complications after the universal implementation of pneumococcal vaccine (PCV10) in our region.

2. Methods

Our prospective cohort evaluated children aged from 6 to 23 months with symptoms of ARI between September 2009 and October 2013 at an Emergency Department in Salvador, Northeastern Brazil. Children with ARI defined as report of either fever, sneeze, running nose, nasal blockage, or cough for up to seven days were evaluated in the study. Children transferred from other hospitals or reporting previous episode of wheeze were excluded. Parents or legal guardians were invited to sign the written informed consent and, for the eligible children, they were invited to answer a standardized questionnaire.

The questionnaire contained data on the present illness, obstetric, neonatal, family morbidities, as well as the child's lifestyle, and physical examination performed by the emergency pediatrician. Between 14 and 28 days after the study's admission, children returned for a follow up visit and parents or legal guardians answered a questionnaire about the ARI evolution. Complications comprised the following events: hospitalization, pneumonia and AOM. Pneumonia was diagnosed when the child presented cough or fever along with difficulty breathing AND pulmonary infiltrate, consolidation, or pleural effusion in the chest radiograph. AOM was diagnosed when the child presented earache along with tympanic alterations found by otoscopy (bulging position, decreased or absent mobility, abnormal color or opacity, or air-fluid interfaces PLUS distinct erythematous patches or streaks or increased vascularity). Pneumonia and AOM were diagnosed by a doctor.

Nutritional status evaluation was performed by using the software Anthro, version 3.2.2; severe malnutrition, malnutrition, overweight and obesity were defined as *z*-score for weight-for-height index, respectively, lower than −3, lower than −2, higher than 2, higher than 3, by using the World Health Organization (WHO) standard [10]. Fever was defined as axillary temperature higher than 37.4 °C [11], and tachypnea as respiratory rate (RR) equal to or higher than 50 breaths/min in children aged 6–11 months and RR equal to or higher than 40 breaths/minute in children from 12 months of age onwards [12].

Pneumococcal vaccine (PCV10: Synflorix, GlaxoSmithKline Biologicals, Rixensart, Belgium) was introduced in Salvador, Brazil, in July 2010 for children aged <2 years [13]. Every child included in the study who could have received PCV10 had the vaccine card checked personally by one of the researchers upon recruitment.

Data were entered into Epi Info version 6.04. Data analyses were performed using STATA version 11.0. Descriptive statistics with their 95% confidence intervals (95% CI) were calculated. Independent variables that appeared to be significant in bivariate analyses were firstly included in a robust Poisson model. All the other independent variables and possible interactions were also assessed before the final model to estimate their independent effects on development of complications. The study was approved by the Ethics Committee from the Federal University of Bahia.

3. Results

Among 1154 evaluated children, 504 (43.7%) reported previous episode of wheeze, 11 (1%) came from other hospitals and 63 (5.4%) did not consent. Thus, 576 (49.9%) children were recruited, out of which 422 (73.3%) returned for reevaluation and were included in this study group. Table 1 in the supplementary material shows the comparison of children who returned with children who did not return.

The baseline characteristics of the study group ($n = 422$) are described in Table 1. The median age was 10 months (mean 11.4 ± 4.5), 278 (65.9%) were younger than 1 year, 167 (39.6%) were in the range of 6–9 months of age, 206 (48.8%) were males, and 407 (96.4%) were born in Salvador. Most children 391 (92.6%) had been breastfed, and 207 (49%) were still being breastfed at the time of recruitment. The vaccination card could be checked for 384 (91%) children out of which 277 (72.1%) and 168 (43.8%) received PCV10 or influenza vaccine, respectively. The numbers of PCV10 doses that had been given were: 1 dose (36 patients; 13.0%), 2 doses (96 patients; 34.7%), 3 doses (116 patients; 41.9%), 4 doses (29 patients; 10.4%). Presence of environmental factors at home was reported by informants: smokers in the household ($n = 80$; 19%), cockroaches 278 (65.9%), insects 271 (64.2%), dust 233 (55.2%), mold 139 (32.9%), rats 108 (25.6%), dogs 93 (22%), bird 68 (16.1%), cats 27 (6.4%) and chicken 15 (3.6%). On physical examination, fever 161 (39.1%), ronchi 162 (38.4%) and tachypnea 99 (23.8%) were the most frequent findings.

The mean interval between admission and the follow-up visit was 23 ± 13 days, median [interquartile range]: 19 [15–27] days. Overall, complications were detected in 79 (19%; 95% CI: 15–23%) patients and this frequency was similar among children aged under 1 year or 1 year and above (17.3% vs. 21.5%; $p = 0.3$). Pneumonia ($n = 47$; 11.1%), hospitalization ($n = 28$; 6.6%) and AOM ($n = 17$; 4.0%) were reported. Most of the patients presented one complication ($n = 354$; 84%) followed by two ($n = 67$; 16%). Overall, 28 patients were hospitalized, 10 with pneumonia and 3 with AOM; the other 15 had other respiratory discomfort.

Tables 2–4 depict the comparison of medical history, environmental factors and clinical presentation of children with or without complications. Differences were significant in bivariate analysis when report of fever, report of wheeze, duration of hoarseness, use of PCV10, chicken or bird at home, ronchi or crackles at physical examination upon recruitment, were all assessed. Table 5 presents the multivariate analysis of factors associated with complications in bivariate analysis. Directly independent association was found between history of fever (OR [95%CI]: 3.30 [1.38–7.89]), bird at home (OR [95%CI]: 1.90 [1.04–3.45]), ronchi (OR [95%CI]: 1.64 [0.99–2.68]) or crackles (OR [95%CI]: 2.62 [1.27–5.41]) at physical examination and complications. Conversely, use of PCV10 was inversely associated with complications. We further assessed the independent association of factors on recruitment in regard to each one of the complications: bird at home (OR [95%CI]: 5.80 [1.73–19.38]) and ronchi (OR [95%CI]: 6.39 [1.96–20.85]) were associated with AOM; PCV10 was inversely associated with AOM (OR [95%CI]: 0.16 [0.05–0.52]). Crackles were associated with pneumonia (OR [95%CI]: 2.55 [1.01–6.40]).

4. Discussion

Our study showed that development of complications during an ARI episode is common among young children as one fifth of them had complications. Pneumonia was the most frequent complication. An environmental factor (bird at home) was an independent risk factor of AOM. On the other hand, PCV10 was independently associated with lower odds of development of AOM. Auscultatory

Table 1
Baseline characteristics of 422 children with acute respiratory infection on recruitment.

Characteristics	*n* (%)	Duration mean ± SD, min–max
History		
Cough	369 (87.4)	3.6 ± 1.8, 1–7[b]
Running nose	359 (85.1)	3.6 ± 1.7, 1–7[b]
Fever	342/421 (81.2)[a]	2.9 ± 1.5, 1–7[b]
Sneeze	331 (78.4)	3.5 ± 1.7, 1–7[b]
Wheeze	168 (39.8)	2.5 ± 1.6, 1–7[b]
Hoarseness	128 (30.3)	2.8 ± 1.6, 1–7[b]
Dyspnea	115 (27.3)	2.8 ± 1.6, 1–7[b]
Vomiting	54 (12.8)	2.0 ± 1.5, 1–7[b]
Earache	32/420 (7.6)[a]	2 (1, 4), 1–7[b,c]
Thoracic retraction	44 (10.4)	2.6 ± 1.9, 1–7[b]
Obstetric		
Low birth weight	54/406 (13.3)[a]	
Early respiratory distress	25/420 (6.0)[a]	
Prematurity	47 (11.1)	
Neonatal oxygen	22/419 (5.3)[a]	3.0 (1, 3), 1–60[b,c]
Mechanical ventilator	10/420 (2.4)[a]	
Smoking mother during pregnancy	19 (4.5)	
Smoking mother nowadays	26 (6.2)	
Flu vacine during pregnancy	255/396 (64.4)[a]	
Vaccination		
Pneumococcal (PCV10)	277/384 (72.1%)[a]	
Influenza	168/384 (43.8%)[a]	
Breastfeeding		
Exclusive	353/420 (84.0)[a,d]	4.7 ± 1.9, 0.1–13[d,e]
Mixed	253/421 (60.1)[a,d]	8.8 ± 4.2, 0.5–21[d,e]
Family history		
Rhinitis	247/421 (58.7)[a]	
Asthma	121/420 (28.8)[a]	
Skin allergy	85/421 (20.2)[a]	
Infant lifestyle		
Shared bed	311 (73.7)	
Smokers at home	80 (19)	
Absence of ventilation at home	138 (32.7)	
Chicken at home	15 (3.6)	
Bird at home	68 (16.1)	
Physical examination		
Severe malnutrition	1 (0.2)	
Malnutrition	4 (0.9)	
Overweight	33 (7.8)	
Obesity	9 (2.1)	
Tachypnea	99/416 (23.8)[a]	
Fever	161/412 (39.1)[a]	
Ronchi	162 (38.4)	
Wheezing	68 (16.1)	
Crackles	37 (8.8)	

[a] The denominator was not 422 because there was missing information.
[b] Duration in days.
[c] Median (25th percentile, 75th percentile), min–max.
[d] Mixed breastfeeding was informed for 1 child but the informant did not know if this child had been exclusively breastfed.
[e] Duration in months.

findings (ronchi and crackles) were independently associated with AOM or pneumonia, respectively.

Although most ARI in children are self-limited, complications can happen. Young children (<2 years of age) are more susceptible to complications [6]. In our study we found complications in 19% of the patients evaluated, a frequency close to the one found in a French Pediatric Emergency Department (12%) where children younger than 36 months were evaluated [14]. Herein, the most frequently observed complication was pneumonia (11.1%) followed by hospitalization (6.6%) and AOM (4.0%). In a longitudinal study in children <2 years conducted in Bangladesh, out of 1322 episodes of ARI, pneumonia (29%), AOM (2%) and hospitalization (0.3%) were reported [15]. The difference in the frequency of pneumonia and hospitalization between the two studies may be explained by differences in the management of ARI cases in these two countries (Brazil and Bangladesh). Pneumonia was diagnosed only on clinical grounds in Bangladesh whereas it is usually confirmed by chest radiograph in Brazil [16]. Diagnosis of pneumonia based solely on clinical grounds overestimates pneumonia diagnosis [17]. On the other hand, admission to hospital in Bangladesh is more difficult than in Brazil due to limited number of hospital beds [18].

Auscultatory findings such as ronchi and crackles can be regarded as warning signs for complications. As such, close follow-up must be provided to patients showing these signs upon admission, in order to detect complications as soon as possible. Ronchi on pulmonary auscultation is a sign of secretion in the airways and had association with AOM in our study. It has been shown that transient negative middle ear pressure occurs in two thirds of uncomplicated colds in healthy children [19], who have secretion in the airways. This negative pressure may facilitate secondary AOM [19].

Bird at home was a risk factor of AOM. Allergen exposure may occur from contact with bird feathers or droppings. In a study conducted among school-age children to determine whether exposure to pets plays a significant role in the development of allergic diseases, rhinitis was more common among children who had a pet at home, including poultry, other birds, and cats in comparison with children living in homes without pets (33.3% vs. 18.0%, $p < 0.001$)

Table 2
Comparison of medical history variables among children with acute respiratory infection with or without development of complications during evolution.

Characteristics	Complications		p
	Yes (n = 79)	No (n = 343)	
Age (months, mean ± SD)	11.4 ± 4.6	10.7 ± 4.4	0.2
History			
Cough	70 (88.6%)	299 (87.2%)	0.73
Duration of cough (days, mean ± SD)	3.7 ± 1.7	3.6 ± 1.8	0.80
Running nose	72 (91.1%)	287 (83.7%)	0.09
Duration of running nose (days, mean ± SD)	3.8 ± 1.6	3.5 ± 1.7	0.18
Fever	73 (92.4%)	269/342 (78.7%)[a]	0.005
Duration of fever (days, mean ± SD)	3.1 ± 1.6	2.8 ± 1.5	0.11
Sneeze	63 (79.7%)	268 (78.1%)	0.75
Duration of sneeze (days, mean ± SD)	3.7 ± 1.8	3.5 ± 1.7	0.40
Wheeze	40 (50.6%)	128 (37.3%)	0.03
Duration of wheeze (days, mean ± SD)	2.8 ± 1.6	2.4 ± 1.6	0.16
Hoarseness	24 (30.4%)	104 (30.3%)	1.00
Duration of hoarseness (days, mean ± SD)	2.0 ± 1.3	2.9 ± 1.6	0.01
Dyspnea	26 (32.9%)	89 (25.9%)	0.21
Duration of dyspnea (days, mean ± SD)	3.1 ± 1.8	2.8 ± 1.6	0.41
Vomiting	10 (12.7%)	44 (12.8%)	1.00
Duration of vomiting (days, mean ± SD)	2.7 ± 1.9	1.9 ± 1.3	0.13
Earache	4/78 (5.1%)[b]	28/342 (8.2%)[a]	0.40
Duration of earache (days, median (IQR))	2.5 (2–3.8)	2.0 (1–4.0)	0.60
Thoracic retraction	10 (12.7%)	34 (9.9%)	0.50
Duration of thoracic retraction (days, mean ± SD)	2.7 ± 2.0	2.5 ± 1.9	0.80
Obstetric			
Low birth weight	7/73 (9.6%)[b]	47/333 (14.1%)[a]	0.30
Early respiratory distress	7 (8.9%)	18/341 (5.3%)[a]	0.29
Prematurity	7 (8.9%)	40 (11.7%)	0.50
Neonatal oxygen	5 (6.3%)	17/340 (5.0%)[a]	0.60
Duration of neonatal oxygen (days, median (IQR))	3.0 (1.5–5)	3.0 (1–3)	0.83
Mechanical ventilator	2 (2.5%)	8/341 (2.3%)[a]	1.00
Smoking mother during pregnancy	2 (2.5%)	17 (5.0%)	0.55
Smoking mother during nowadays	2 (2.5%)	24 (7.0%)	0.20
Flu vaccine during pregnancy	44/77 (57.1%)[b]	211/319 (66.1%)[a]	0.14
			Vaccination
Pneumococcal (PCV10)	43/73 (58.9%)[b]	234/311 (75.2%)[a]	0.005
Influenza	30/73 (44.1%)[b]	138/311 (44.4%)[a]	0.61
Breastfeeding			
Exclusive	69 (87.3%)	284/341 (83.3%)[a]	0.38
Duration of breastfeeding exclusive (months, mean ± SD)	4.9 ± 2.0	4.6 ± 1.9	0.23
Mixed	46 (58.2%)	207/342 (60.5%)[a]	0.70
Duration of breastfeeding mixed (months, mean ± SD)	9.1 ± 4.8	8.7 ± 4.1	0.70

[a] The denominator was not 343 because there was missing information.
[b] The denominator was not 79 because there was missing information.

[20]. Patients with rhinitis have nasal inflammation, and Eustachian tube dysfunction, which lead to increased negative pressure in the middle ear and improper ventilation, with enhanced nasal protein transudation. As a result, respiratory secretions are produced and can lead to AOM [21].

The universal implementation of pneumococcal conjugate vaccines has been a major public health intervention to prevent pneumococcal disease. In our country, evidence has been provided that the introduction of PCV10 in the routine immunization program has effectively lowered rates of hospitalization due to pneumonia among children [22]. As there is increasing frequency of pneumococcal colonization during clinical ARI [9], the use of PCV probably impacts on the frequency of such colonization. For those vaccinated, the risk of hospitalization due to influenza-associated pneumonia has decreased to approximately half the risk among children who had not received this vaccine [23]. Herein, PCV10 did not protect children against pneumonia. The vast majority of our

Table 3
Comparison of environmental factors among children with acute respiratory infection with or without development of complications during evolution.

Evironmental factors	Complications		p
	Yes (n = 79)	No (n = 343)	
Shared bed	58 (73.4%)	253 (73.8%)	1.00
Smokers at home	17 (21.5%)	63 (18.4%)	0.52
Absence of ventilation at home	26 (32.9%)	112 (32.7%)	1.00
Chicken at home	7 (8.9%)	8 (2.3%)	0.01
Bird at home	19 (24.1%)	49 (14.3%)	0.03

Table 4
Comparison of clinical presentation factors among children with acute respiratory infection with or without development of complications during evolution.

Clinical presentation	Complications		p
	Yes (n = 79)	No (n = 343)	
Severe malnutrition	1 (1.3%)	0	0.2
Malnutrition[c]	1/78 (1.3%)	3 (0.9%)	0.6
Overweight[d]	7/76 (9.2%)	26/337 (7.7%)	0.7
Obesity	3 (3.8%)	6 (1.7%)	0.4
Tachypnea	18 (22.8%)	81/337 (24.0%)[a]	0.81
Fever	35/77 (45.5%)[b]	126/335 (37.6%)[a]	0.20
Ronchi	38 (48.1%)	124 (36.2%)	0.049
Wheezing	18 (22.8%)	50 (14.6%)	0.07
Crackles	13 (16.5%)	24 (7.0%)	0.007

[a] The denominator was not 343 because there was missing information.
[b] The denominator was not 79 because there was missing information.
[c] The patient with severe malnutrition was excluded from this analysis.
[d] The patients with obesity were excluded from this analysis.

Table 5
Multivariate analysis of factors associated with complications among 384 children with acute respiratory infection.

Factors	Bivariate analysis		Multivariate analysis	
	Unadjusted OR (95% CI)	p	Adjusted OR (95% CI)	p
Male gender	1.33 (0.81–2.18)	0.3	1.58 (0.91–2.73)	0.1
Age	1 (0.99–1.00)	0.3	0.99 (0.99–1.00)	0.7
History of fever	3.30 (1.38–7.89)	0.007	2.90 (1.18–7.14)	0.02
History of wheeze	1.72 (1.05–2.82)	0.03	1.11 (0.62–1.97)	0.7
Duration of hoarseness	0.65 (0.45–0.93)	0.02	0.88 (0.73–1.05)	0.2
Pneumococcal vaccination (PCV10)	0.47 (0.28–0.80)	0.006	0.46 (0.26–0.82)	0.009
Chicken at home	4.07 (1.43–11.58)	0.008	2.30 (0.74–7.18)	0.15
Bird at home	1.90 (1.04–3.45)	0.04	2.13 (1.07–4.26)	0.03
Ronchi	1.64 (0.99–2.68)	0.05	2.06 (1.16–3.67)	0.01
Crackles	2.62 (1.27–5.41)	0.009	2.36 (1.04–5.38)	0.04

cases were not hospitalized. That said, it is possible to infer that PCV10 protects against more severe cases of pneumonia.

A recent Cochrane Database Systematic Review about efficacy of pneumococcal conjugate vaccines for preventing pneumonia reported modest beneficial effects in healthy infants with a low baseline risk of AOM gained from the licensed 7-valent CRM197-PCV7 [24]. From an internationally recognized transparent Excel-based model, it was possible to predict that PCV10 will decrease morbidity by reducing the outpatient care due to AOM in Georgia [25]. In the present study, PCV10 was inversely associated with complications during ARI. To the best of the authors' knowledge, this is the first cohort to demonstrate this finding. Reduction in the frequency of tympanostomy tube placements in children attributable to PCV10 has been newly reported [26].

This study has limitations. Approximately one quarter (26.7%) of the recruited patients did not return for the follow-up evaluation. However, the only difference found between patients who returned or not for follow-up was the higher frequency of vomiting among those who did not return (Table 1 in the Supplementary material). That means, none of the differences found between children who did or did not have complications were also detected among the patients excluded due to lost follow-up. Another limitation is that not all followed-up patients had the vaccination card available to be checked. However, this loss was less than 10%, as 384 (90.1%) had the vaccination card with them. Moreover, statistical differences were found in the multivariate analysis despite the fact that the sample size had been decreased due to absence of information retrieved from the vaccination card. Furthermore, no result from laboratory test to investigate the causative agents was available.

5. Conclusion

We conclude that one fifth of children aged 6–23 months presented ARI complications, and PCV10 immunization is independently associated with lower odds of development of AOM. Bird at home and ronchi are risk factors of otitis. Crackles are associated with pneumonia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.04.015.

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ARTIGO II

Cell Host & Microbe
"Environmental factors, airway microbiome and the first wheezing episode among young children with acute respiratory infection: a prospective study"
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Cell Host & Microbe

Environmental factors, airway microbiome and the first wheezing episode among young children with acute respiratory infection: a prospective study

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Environmental factors, airway microbiome and the first wheezing episode among young children with acute respiratory infection: a prospective study

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Abstract

Wheezing during acute respiratory infection (ARI) is frequently linked to respiratory virus infection, but large studies combining environmental and comprehensive viral/bacterial pathogen profiling are lacking. We report viral and bacterial community profiling of nasopharynx aspirates across a prospective cross-sectional study, including children aged 6-23 months with ARI with and without the first episode of wheezing. We also assessed environmental factors. Comprehensive viral/bacterial respiratory pathogen profiling reveals the first wheezing episode is not associated with respiratory virus, but rather with deleterious (bird at home) and protective bacterial (*Haemohpilus influezae*) and environmental (dog at home) factors. Unlike other studies, this study recruited children with ARI with and without the first wheezing episode and comprehensively quantified bacterial and viral respiratory pathogens. Our data identify one bacterium (*Haemohpilus influezae*) in the nasopharynx as a protector for the first episode of wheezing. The airway microbiome plays a role in the development of wheezing in children.

Keywords: Acute respiratory infection, bird, dog, Haemophilus influenzae, infant, microbiome, nasopharyngeal aspirates, respiratory virus.

Introduction

Acute respiratory infection (ARI) is the most common disease among young children; as such, it is a source of significant morbidity (1). Respiratory viruses are major causative agents of ARI (2). Moreover, respiratory virus infection can cause acute wheezing in young children (3). It has been reported that the most significant risk factor for the development of preschool childhood wheezing is the occurrence of symptomatic rhinovirus illnesses during infancy (4). Besides that, virus-induced respiratory wheezing illnesses are associated with the development of asthma (5). In regard to the first wheezing episode, we have previously shown that environmental factors like day care center attendance and birds at home are associated factors for wheezing detected upon physical examination (6). Herein, we aimed to assess the possible association between environmental factors, respiratory viruses and bacteria detected in nasopharyngeal aspirates (NPA) and the first episode of wheezing detected upon physical examination among children with ARI.

Results

Recruitment and baseline characteristics

Overall, 1154 children were evaluated, out of which 504 (43.7%) reported at least one previous episode of wheezing, 16 (1.4%) did not have NPA collected successfully, 11 (1.0%) came from other hospitals, 1 (0.09%) had insufficient amount of NPA collected, and 63 (5.4%) did not consent. Therefore, this study group comprised 559 (48.4%) cases. The mean age was 11.4±4.5 months and 286 (51.2%) were female. The baseline characteristics of the study group are described in Table 1. The most frequent complaints were cough (86.8%), runny nose (83.9%), fever (82.6%), and sneeze (76.4%). On physical examination, the most frequent findings were fever (40.3%), ronchi (36.9%), eye edema (29.2%), and tachypnea (23.6%). Wheezing was detected in 92 children (16.5%; 95% CI: 13.6%-19.7%).

Comparison of personal and environmental characteristics between patients with and without the first wheezing episode

Table 2 shows the comparison of personal and environmental data between children with or without the first episode of wheezing detected on physical examination. Having a dog at home was associated with absence of a first episode of wheezing. Day care center attendance and bird at home presented borderline results ($0.05 < p < 0.10$).

Detection of virus and bacteria in nasopharyngeal aspirates

At least one virus or one bacterium was detected, respectively, in 456 (81.6%) or 558 (99.8%) children. Table 3 depicts the frequency of detection of the complete respiratory viral and bacterial panel and compares these frequencies with detection among 20 asymptomatic adults (controls). Among these controls, the mean age was 34 ±10 years and there were 75% females. Among the studied cases, Rhinovirus (48.1%), Parainfluenza virus 1 (32.0%), and Adenovirus 2 (20.4%) were the most frequently found viruses. *S. aureus* (98.0%), *H. influenzae* (97.1%), and *M. catarrhalis* (79.1%) were the most common bacteria. Rhinovirus, Parainfluenza virus 1, Adenovirus 2, *M. catarrhalis, M. pneumoniae*, and *S. pneumoniae* were significantly more frequent among the cases in comparison with the controls.

Comparison of viral and bacterial detection between patients with and without the first wheezing episode

Table 4 shows the comparison of the frequency of detected viruses and bacteria among children with or without wheezing upon physical examination. *H. influenzae* was significantly more common in NPA of cases without wheezing.

Independent association between environmental factors, viral and bacterial detection in nasopharyngeal aspirates and the first wheezing episode

Table 5 presents the results of the multivariable logistic regression. Having a dog or bird at home, as well as the presence of *H. influenzae* in nasopharynx were independently associated with the first wheezing episode detected upon physical examination.

Discussion

We detected respiratory virus in 81.6% of our cases with ARI. Rhinovirus (48.1%), Parainfluenza virus 1 (32.0%), and Adenovirus 2 (20.4%) were the most frequently found viruses whereas *S. aureus* (98.0%), *H. influenzae* (97.1%), and *M. catarrhalis* (79.1%) were the most common bacteria at RNA level. Surprisingly, none of the viral pathogens was significantly associated with the first wheezing episode. However, the presence of *H. influenzae* RNA in NPA and having a dog at home independently protect against the first wheezing episode; conversely, having a bird at home is a risk factor for it.

Herein, the first episode of wheezing was detected upon physical examination in one sixth (16.5%) of our studied cases. In the Netherlands (KOALA Birth Cohort Study), new onset wheeze was reported by caregivers in 10% of 3454 children followed-up from the 7th to the 24th month of life (7). We have previously verified that reporting of wheezing by the caregiver of a child which has never wheezed before requires confirmation by physical examination performed by the pediatrician, as reporting was 2.7 times higher than the detection upon physical examination (8). Thus, our frequency of detected wheezing was considerably higher than the frequency of reported wheezing in the Netherlands, which is in agreement with a higher prevalence of wheezing in developing countries (9-11).

Notably, we did not find a significant association, neither in bivariate nor in multivariate analysis, between detection of respiratory viruses in the nasopharynx and wheezing detected upon physical examination. Noteworthy, an Italian study reported 88.2% overall detection of 18 investigated respiratory viruses among 85 cases <1-year-old hospitalized with the first acute episode of wheezing (12). In a study enrolling Thai children aged <2 years hospitalized

with a first episode of acute wheezing, respiratory viruses (only 5 viruses were investigated) were found in 80.6% of 170 cases (13). In a Finnish study, respiratory viruses were found in 100% of samples, when 111 first-time wheezing children aged 3-23 months were enrolled and 14 viruses were investigated (3). All three studies employed RT-PCR to detect respiratory viruses. Taken together, detection of respiratory viruses was very high when children with first episode of wheezing were studied; however, when children with ARI were enrolled, with or without the first episode of wheezing, association between respiratory viruses and the first wheezing episode was not found, as in the present study.

In our study, we identified the presence of a bird at home as a potential risk factor for the first wheezing episode detected upon physical examination. Although exposure to bird at home is not a well-known associated factor, allergen exposure may occur from contact with bird feathers or dropping (14). In a study conducted in Qatar, birds at home were associated with allergic rhinitis (27.1%vs 13.8%; $p<0.001$) and eczema (22.8% vs 16.4%; $p< 0.001$) (15). Thus, we hypothesize that having a bird at home can sensitize young children to wheezing during an ARI episode, independent of the causative viral or bacterial pathogen.

We also identified potential protective factors: having a dog at home and the presence of *H. influenzae* RNA in NPA. It has been postulated that exposure to bacterial endotoxins (16) and animals (17,18) early in life might confer protection against the development of asthma and allergies. Similar to our results, the presence of a dog at home was associated with a decreased risk for wheezing in a 4-year longitudinal study (19). Increased microbial burden early in life has been associated with decreased prevalence of sensitization and possibly asthma (20). One of the substances under investigation to explain such association is endotoxin. Increased endotoxin exposure has been linked to decreased sensitization rates and possibly lower prevalence of wheezing (21). Endotoxin is a component of the cell wall of gram-negative bacteria, like *H. influenza* (22), which can modify immune responses as it is a

potent inducer of TH1 cytokines (23). Additionally, emerging data have suggested that parallel host-bacteria interactions during infancy might play a role in modulating the development of aeroallergens sensitization (24).

Our study has both strengths and limitations. Strengths include the sample size, the prospective recruitment made by trained pediatricians, the outcome variable defined on the basis of detected wheezing upon physical examination performed by pediatricians, and the previously validated sensitive and specific transcriptomic profiling detecting a wide range of viruses and bacteria in NPA. Regarding limitations, atopy was not investigated by laboratory tests, e.g. by quantification of IgE or IgE RAST. Nevertheless, these biological markers are uncommon among children aged under-2 years, the age strata of our study (25). Another limitation of the study is the lack of paired healthy controls, due to the study design, powered to detect risk factors for wheezing among children with ARI, specifically. However, we have collected a small number of samples of healthy adults (n=20), as controls for respiratory pathogen detection. As expected, we found a significant decrease in respiratory pathogens (Table 3) but not *S. aureus* or *H. influenzae*. The presence of *H. influenzae* in 95% of healthy, asymptomatic adults further strengthens its case as a protective factor, in agreement with the recent demonstration of its predominance in one of five bacterial clusters in the healthy nasal microbiome (26). In turn, these results are in favor of our choice for comprehensive transcriptional pathogen profiling by nCounter.

In conclusion, the presence of *H. influenzae* in NPA and having a dog at home independently protect against the first wheezing episode, whereas having a bird at home is a risk factor for it among young children with ARI.

Experimental Procedures

Study design

We conducted this prospective cross-sectional study at the Pediatric Emergency Room of the Federal University of Bahia Hospital, in Salvador, Northeastern Brazil. Community-dwelling children aged from 6 to 23 months with report of either fever, sneeze, runny nose, nasal blockage, or cough for up to seven days were considered to have ARI and then were recruited from September 2009 to October 2013. Exclusion criteria comprised transfer from other hospitals or report of previous episode of wheezing. Written informed consent was obtained from parents or legal guardians. Afterwards, pre-defined forms were filled out with data on demographics, the current illness, obstetric, neonatal, personal, and family morbidities, as well as aspects of the child's lifestyle including environmental factors present or absent where the child lived, all collected from the caregiver. A thorough physical examination was performed by a pediatrician and collected data were registered in a specific pre-defined form. We recruited a convenience sample of 20 asymptomatic adults from our research team. Eligibility requirements for controls included absence of fever, cough, sore throat, sneeze, nasal blockage, rhinorrhea, and of nasal medicines use for the past 14 days.

Clinical definitions

Tachypnea was defined as respiratory rate (RR) equal to or higher than 50 breaths/minute in children aged 2-11 months and RR equal to or higher than 40 breaths/minute in children from 12 months of age onwards (27). Fever was defined as axillary temperature higher than 37.4°C (28). Low birth weight was defined as <2500 g and prematurity was defined as gestational age fewer than 37 weeks (29).

Laboratory procedures

Following physical examination, a NPA sample was collected from the cases for pathogen diagnostics using a standardized procedure: after having measured the distance between the entrance of the nostril and the ear lobe to estimate the distance from the entrance of the nostril to the nasopharynx, an aseptic plastic sputum catheter was inserted into the nostril until

reaching the nasopharynx; approximately 2 mL of nasal secretions were collected by applying negative pressure and placed in a sterile tube with 1 mL of Nuclisens Lysis Buffer (Biomeriux, Boxtel, The Netherlands). From healthy adult controls, a nasopharyngeal aspirate was collected after instillation of isotonic saline. The collection was performed in a supine position when 2 mL of isotonic saline was instilled into each nostril and immediately aspirated into a specimen trap by inserting a flexible plastic suction catheter. After washing each nostril, 1 mL of saline was aspirated through the catheter and placed in a sterile tube with 1 mL of NucliSens Lysis Buffer.

Total RNA (10–50 ng) was extracted using RNEasy (Qiagen, Hilden, Germany) following manufacturer´s instructions and was subsequently hybridized against probes targeting Rhinovirus, Respiratory Syncytial Virus A and B, Parainfluenza Virus 1, 2 and 3, Influenza A and B, Human Metapneumovirus, Adenovirus 2 and 5, Human Bocavirus, Coronavirus OC 43 and 229E, *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Staphylococcus aureus.* The probe set list for microbial detection is presented in Supplemental Table 1. Then, specimens were simultaneously quantified for respiratory viruses and bacteria using the NanoString nCounter gene expression system, which captures and counts individual mRNA transcripts (30). The laboratory tests were performed by trained technicians (VIB-Nucleomics, Leuven, Belgium), sample processing, quality control and nCounter data analysis by researchers blinded to clinical information (Laboratory for Clinical and Epidemiological Virology, Rega Institute for Medical Research, KU Leuven, Belgium). NPA were kept at -70°C prior to analysis.

Raw data were processed using nSolver 2.0 software (Nanostring Technologies), sequentially correcting three factors: technical variation between batches (positive control RNA), background correction (negative control) and RNA content by adjusting the counts geometric mean for 15 housekeeping genes (Supplemental Table 2). This method allows identification

of multiple pathogens in NPA samples of ARI patients in a single reaction, which present low RNA yield (10-50 ng) (31).

Statistical analysis

Data analyses were performed using SPSS 17.0. Descriptive statistics with their 95% confidence intervals (95% CI) were calculated. In bivariate analyses, categorical variables were compared by using chi-square or Fisher's exact test, as appropriate and continuous variables were assessed by using Student t test. Multivariable logistic regression analysis by enter method assessed the potential impact of viral and or bacterial detection (predictor variables) on wheezing detected on physical examination (outcome variable) in an age-adjusted model. Statistical tests were two-tailed, at significance level of 0.05. Personal and environmental factors which presented $p<0.10$ in bivariate analysis were included in the multivariable analysis.

Exclusion criteria were chosen to address potential confounders. Blinding at laboratory test procedure was performed to address potential bias. Cases with missing biological sample were excluded. As the frequency of missing information collected from the caregiver was very low, we chose to handle the missing data by excluding the cases with missing information.

A sample of 560 cases of ARI without previous episode of wheezing was planned to be recruited. This sample size would provide the study with a power of 80% to detect as statistically significant (at the 5% level) an odds ratio of 1.85 or more for a range of frequency of exposure among non-cases from 40% to 70%.

Ethical approval

The study was carried out in accordance with The Code of the World Medical Association and it was approved by the Ethics Committee from the Federal University of Bahia (Approval N°.067/2009).

Author contributions

JRO and MLB participated in the acquisition of data. KFF performed RNA/DNA extractions and nCounter experiments. AB, DS, MRAC, and CIO took part in data interpretation. JVW designed and coordinated nCounter and 16S metagenomics experiments. CMNC designed the study. JRO and CMNC analyzed the data. JRO drafted the manuscript. All authors revised the manuscript critically for important intellectual content.

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TABLE I. Frequency of baseline characteristics among 559 children with acute respiratory infection

Characteristics	n (%)
History	
Cough	485 (86.8)
Runny nose	469 (83.9)
Fever	461/558 (82.6)[a]
Sneeze	427 (76.4)
Hoarseness	163 (29.2)
Difficulty breathing	148 (26.5)
Vomiting	86 (15.4)
Diarrhea	31 (5.5)
Ocular alteration	11 (2.0)
Cyanosis	4/554 (0.7)[a]
Obstetric	
Low birth weight	62/535 (11.6)[a]
Early respiratory distress	40/556 (7.2)[a]
Prematurity	57 (10.2)
Neonatal oxygen	33/553 (6.0)[a]
Mechanical ventilator	17/554 (3.1)[a]
Smoking mother during pregnancy	28 (5.0)
Smoking mother nowadays	37 (6.6)
Drug addicted mother	8 (1.4)
Flu vaccine during pregnancy	339/529 (64.1)

Family history	
Rhinitis	319/557 (57.3)[a]
Asthma	153/556 (27.5)[a]
Skin allergy	103/557 (18.5)[a]
Child Morbidity	
Rhinitis	65/555 (11.7)[a]
Bronchopulmonar dysplasia	3/555 (0.5)
Asthma	0
Eczema	15 (2.7)
Gastroesophageal reflux disease	48 (8.6)
Heart disease	8/558 (1.4) [a]
Infant lifestyle	
Shared bed	425/558 (76.2)[a]
Cockroach	372/558 (66.7) [a]
Insect at home	363 (64.9)
Dust at home	314 (56.2)
Dust in the room	247 (44.2)
Absence of ventilation at home	182 (32.6)
Mold at home	172 (30.8)
Mouse at home	155 (27.7)
Mold in the room	143 (25.6)
Pollution at home	138 (24.7)
Dog at home	120 (21.5)

Smokers at home	116 (20.8)
Day care center attendance	46 (8.2)
Cat at home	38 (6.8)
Chicken at home	18 (3.2)
Bird at home	88 (15.7)
Physical examination	
Fever	221/548 (40.3)[a]
Ronchi	206 (36.9)
Eye edema	163 (29.2)
Tachypnea	130/420 (23.6)[a]
Eye redness	114 (20.4)
Wheezing	92 (16.5)
Crackles	50 (8.9)
Chest retraction	32 (5.7)

[a] The denominator was not 559 because there was missing information.

TABLE II. Comparison of children with acute respiratory infection with and without wheezing detected upon physical examination on admission

Characteristics	Wheezing on physical examination		*P*
	Yes n=92	**No n=467**	
Age (months)	11.6 ± 5.1	11.4 ± 4.4	0.6
Obstetric			
Low birth weight	10/85 (11.8%) [a]	52/450 (11.6%) [b]	1.0
Early respiratory distress	9/91 (9.9%) [a]	31/465 (6.7%) [b]	0.3
Prematurity	10 (10.9%) [a]	47 (10.1%) [b]	0.8
Neonatal oxygen	6/90 (6.7%) [a]	27/463 (5.8%) [b]	0.8
Mechanical ventilator	2/90 (2.2%) [a]	15/464 (3.2%) [b]	1.0
Smoking mother during pregnancy	6 (6.5%)	22 (4.7%)	0.4
Smoking mother nowadays	8 (8.7%)	29 (6.2%)	0.4
Drug-addicted mother	2 (2.2%)	6 (1.3%)	0.6
Flu vaccine during pregnancy	59/87 (67.8%) [a]	280/442 (63.3%) [b]	0.4
Family history			
Rhinitis	57/91 (62.2%)	262/466 (56.3%)	0.3
Asthma	30/91 (33.0%)	123/465 (26.5%)	0.2
Skin allergy	18/91 (19.8%)	85/466 (18.2%)	0.7
Child Morbidity			
Rhinitis	11/91 (12.1%)	54/464 (11.6%)	0.9
Bronchopulmonar dysplasia	1 (1.1%)	2/463 (0.4%) [b]	0.4
Eczema	3 (3.3%)	12 (2.6%)	0.7
Gastroesophageal reflux disease	10 (10.9%)	38 (8.1%)	0.4
Heart disease	1 (1.1%)	7/466 (1.5%)	1.0

Infant lifestyle			
Shared bed	68/91 (74.7%) [a]	357 (76.4%)	0.7
Cockroach	59/91 (64.8%) [a]	313 (67.0%)	0.7
Dust at home	52 (56.5%)	262 (56.1%)	0.9
Dust in the room	41 (44.6%)	206 (44.1%)	0.9
Absence of ventilation at home	26 (28.3%)	156 (33.4%)	0.3
Mold at home	34 (37.0%)	132 (29.6%)	0.2
Mold in the room	22 (23.9%)	121 (25.9%)	0.7
Dog at home	12 (3.0%)	108 (23.1%)	0.03
Smokers at home	25 (27.2%)	91 (19.5%)	0.1
Pollution at home	24 (26.1%)	114 (24.4%)	0.7
Day care center attendance	12 (13.0%)	34 (7.3%)	0.07
Cat at home	6 (6.5%)	32 (6.9%)	0.9
Chicken at home	4 (4.3%)	14 (3.0%)	0.5
Bird at home	20 (21.7%)	68 (14.6%)	0.08
Mouse at home	25 (27.2%)	130 (27.8%)	0.9
Insect at home	60 (65.2%)	303 (64.9%)	1.0

[a] The denominator is not 92 because data were missing

[b] The denominator is not 467 because data were missing

TABLE III. Comparison of the frequency of detected viruses and bacteria in nasopharyngeal aspirates from children with acute respiratory infection (cases) and asymptomatic adults (controls)

Pathogen	**Cases**	**Controls**	
	n=559 (%)	**n=20 (%)**	***P***
Viruses			
Rhinovirus	269 (48.1)	3 (15.0)	0.004
Parainfluenza virus 1	179 (32.0)	2 (10.0)	0.04
Adenovirus 2	114 (20.4)	0	0.02
Influenza A	88 (15.7)	0	0.06
Coronavirus OC 43	78 (14.0)	1 (5.0)	0.5
Respiratory Syncytial Virus A	63 (11.3)	0	0.2
Respiratory Syncytial Virus B	63 (11.3)	0	0.2
Human Bocavirus	55 (9.8)	1 (5.0)	0.7
Coronavirus 229E	55 (9.8)	0	0.2
Adenovirus 5	53 (9.5)	0	0.2
Human Metapneumovirus	50 (8.9)	0	0.2
Parainfluenza virus 3	47 (8.4)	0	0.4
Parainfluenza virus 2	27 (4.8)	1 (5.0)	1.0
Influenza B	24 (4.3)	0	1.0
Bacteria			
S. aureus	548 (98.0)	20 (100.0)	1
H. influenzae	543 (97.1)	19 (95.0)	0.5
M. catarrhalis	442 (79.1)	6 (30.0)	<0.001
M. pneumoniae	268 (47.9)	1 (5.0)	<0.001
C. pneumoniae	189 (33.8)	3 (15.0)	0.08
S. pneumoniae	135 (24.2)	0	0.006

Results in n (%)

TABLE IV. Comparison of the frequency of detected pathogens in nasopharyngeal aspirates from children with acute respiratory infection with and without wheezing

Pathogen	Wheezing upon physical examination		*P*
	Yes (n=92)	No (n=467)	
Viruses			
Detected virus	79 (85.9%)	377 (80.7%)	0.2
Viral co-detection	50/79 (63.3%)[a]	271/377 (71.9%)[b]	0.1
Adenovirus 5	4 (4.3%)	49 (10.5%)	0.07
Adenovirus 2	17 (18.5%)	97 (20.8%)	0.6
Human Bocavirus	10 (10.9%)	45 (9.6%)	0.7
Coronavirus 229E	6 (6.5%)	49 (10.5%)	0.2
Coronavirus OC 43	13 (14.1%)	65 (13.9%)	1.0
Influenza A	15 (16.3%)	73 (15.6%)	0.9
Influenza B	3 (3.3%)	21 (4.5%)	0.8
Parainfluenza virus 1	28 (30.4%)	151 (32.3%)	0.7
Parainfluenza virus 2	6 (6.5%)	21 (4.5%)	0.4
Parainfluenza virus 3	7 (7.6%)	40 (8.6%)	0.8
Rhinovirus	47 (51.1%)	222 (47.5%)	0.5
Respiratory Syncytial virus A	6 (6.5%)	21 (4.5%)	0.8
Respiratory Syncytial virus B	7 (7.6%)	40 (8.6%)	0.1
Human Metapneumovirus	47 (51.1%)	222 (47.5%)	0.5
Bacteria			
Detected bacterium	92 (100%)	466 (99.8)	1.0
Bacterial co-detection	88 (95.7%)	457/466 (98.1%)[c]	0.3
S. aureus	90 (97.8%)	458 (98.1%)	0.7
H. influenzae	86 (93.5%)	457 (97.9%)	0.03
M. catarrhalis	74 (80.4%)	368 (78.9%)	0.7
M. pneumoniae	48 (52.2%)	220 (47.1%)	0.4
C. pneumoniae	30 (32.6%)	159 (34.0%)	0.8
S. pneumoniae	27 (29.3%)	108 (23.1%)	0.2

[a] The denominator is not 92 because virus was detected in 79 samples
[b] The denominator is not 467 because virus was detected in 377 samples
[c] The denominator is not 467 because bacterium was detected in 466 samples

TABLE V. Multivariate analysis of factors associated with wheezing among 559 children with acute respiratory infection

Factors	Multivariate analysis	
	Adjusted OR (95% CI)	***P***
Dog	0.48 (0.25-0.92)	0.03
Bird	1.83 (1.02-3.27)	0.04
Day Care Center	2.03 (0.96-4.28)	0.06
H. influenzae	0.32 (0.11-0.93)	0.04
Adenovirus 5	0.41 (0.14-1.16)	0.09
Age	0.99 (0.94-1.05)	0.8

Supplemental Table 1. Probe sets for microbial detection by nCounter

Pathogen and target	Probe Sequence	Target Region
AV type 2	TCTGAGTCTCCAGATATCAACCCAATAAAAACTAAAATTGGCTCTGGCATTGATTACAATGAAAA	1045
	CGGTGCCATGATTACTAAACTTGGAGCGGGTTTAA	-1144
AV type 5	CAACTGCGCCTGAAACACCTGGTCCACTGTCGCCGCCACAAGTGCTTTGCCCGCGACTCCGGTGA	21-
	GTTTTGCTACTTTGAATTGCCCGAGGATCATATCG	121
CoV 229E	AAAGGGCCTTTGTGTGTTGACACATCACACTTCACTACCCAATTCGTTGGTGCCAAGTTTGATAGG	1002
S	TGGAGTGCTAGTATTAACACGGGAAATTGCCCTT	-1102
CoV OC43 complete	AATGGCTCCGCACAAGCTACAGCTTTTTGTAAATCTGGTAGTTTAGTCCTTAATAACCCTGCTTAT	456
genome	ATAGCTCCTCAAGCTAACTCTGGGATTATTACTA	-556
PIV 1	GACATCAACGACAACAGGAGATCATGTTCTGTAATAGCTGCAGGAACAAGGGGTTATCAGTTAT	803
HN	GCTCCTTGCCCACTGTGAATGAGACTACAGATTACT	-903
PIV 3	GGTTCCAAAAAGCTGCAGTTAATCATAATTAACCGCAATATGCATTAACCTATCTATAATACAAG	1715
HN	TATATGATAAGTAATCAGCAATCAGACAATAGACA	-1815
RSV A HQ699285	CTCAACCACCTCCGAAGGCAATCTAAGCCCATCACAAGTCTATACAACATCCGAGTACCTATCAC	797
	AATCTCCATCTTCATCCAACACAACACACCTGTAG	-897
RSV B HQ699309	CCCACACCACCAATCCACACAAACTCAGCCACAATATCACCTAATACAAAATCAGAAACACACC	318
	ATACAACAGCACAAACCAAAGGCAGAACCTTTACTC	-418
IV A	TAGCTACTCAACCGGTGCACTTGCCAGCTGCATGGGTCTCATATACAATAGGATGGGAACGGTAA	350
H1N1 - M	CCACAGAAGTGGCTTTTGGCCTAGTGTGTGCCACT	-450
IV B	CGATGTCTGTTTCCAAAGATCAAAGGGACTGAAAAGGGTTGGACTTGACCCTTCATTAATCAGTA	671
NP	CTTTTGCCGGAAGCACACTACCCAGAAGATCAGGT	-771
RV	TGCATACACACCACCTGGTATTGATGAACCAACAACTAGAAAGGATGCAATGCTAGGGACACAT	2014
A1 polyprotein	GTTGTGTGGGATGTTGGATTGCAGTCTACTATATCT	-2114
hMPV	ATCCGGCTTGAAGGTGAAGTCACAGCAATTAAGAATGCCCTCAAAACGACCAATGAAGCAGTAT	381
F	CTACATTGGGGAATGGAGTTCGAGTGTTGGCAACTG	-481
BoV	ATAAAACAAATACTTTCAAATGGTGCTGACACAACATACAACAATGACCTCACAGCTGGCGTTCA	402
VP2	CATCTTTTGTGATGGAGAGCATGCTTACCCAAATG	-502
H. influenzae	GTGAGTAATGCTTGGGAATCTGGCTTATGGAGGGGGATAACGACGGGAAACTGTCGCTAATACC	112
16S	GCGTATTATCGGAAGATGAAAGTGCGGGACTGAGAG	-212
M. pneumoniae	GCAAGGGTTCGTTATTTGATGAGGGTGCGCCATATCAGCTAGTTGGTGGGGTAACGGCCTACCAA	175
16S	GGCAATGACGTGTAGCTATGCTGAGAAGTAGAATA	-275
S. pneumoniae	AGCGGAGTCTGTTATAAAGGAACTCAGTTCAGTCTCATTGCAGTCAATTCAGACATGTCATTAGG	2193
LytA	AAGGTAAAGATTTTCTTTAGCACATGAACTGTATC	-2292
S. aureus-	TGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACT	413
S33 R complete	GTGCACATCTTGACGGTACCTAATCAGAAAGCCAC	-512

sequence

C. penumoniae 16S	GAAGGGTTAGTAGTACATAGATAATCTGCCCTCAACTTGGGGATAACGGTTGGAAACGATCGCTA	99
	ATACCGAATGTAGTGTAATTAGGCATCTAATATAT	-199
M. catarrhalis UspA2	GGACTCTATAGTAGAGCAGGGCAAGACAAAAACAGTTTATTCTGTCACCACCAAAACCGCTACTG	363
	CAGATGATGTCAATAGTGCATATTCACGAGGCATT	-462

Supplemental Table 2: List of housekeeping genes used for nCounter data normalization

Name	Accession number
EEF1G	NM_001404.4
TUBB	NM_178014.2
TBP	NM_001172085.1
POLR2A	NM_000937.2
GUSB	NM_000181.1
HPRT1	NM_000194.1
GAPDH	NM_002046.3
SDHA	NM_004168.1
OAZ1	NM_004152.2
PPIA	NM_021130.2
G6PD	NM_000402.2
RPL19	NM_000981.3
POLR1B	NM_019014.3
ABCF1	NM_001090.2
ALAS1	NM_000688.4

V. Discussão

V. 1. Etiologia da infecção respiratória aguda,

Os vírus respiratórios foram detectados em 81,6% de todos os casos com IRA estudados. Os vírus mais frequentemente isolados foram: o rinovírus (48,1%), o vírus Parainfluenza 1 (32,0%) e o adenovírus 2 (20,4%). Enquanto as bactérias mais comumente isoladas foram: o *S. aureus* (98,0%), o *H. influenzae* (97,1%) e a *M. catarrhalis* (79,1%). Em um estudo realizado no Vietnã, as crianças foram acompanhadas desde o nascimento até 1 ano de idade; as famílias foram convidadas a participar do estudo levando a criança sempre que a mesma não estivesse bem. No total, dos 566 episódios de IRA, com aspirado nasofaríngeo disponível, os vírus respiratórios foram pesquisados por PCR multiplex e foram encontrados em 347 (61%) casos. É importante destacar que foram investigados 5 vírus adicionais (PIV 4, CoV NL63, CoV HKU1, enterovírus, paraecovírus) nesse estudo (Anders et al., 2015). É notado que nossa taxa de detecção viral foi maior que a taxa de detecção no estudo de Vietnamitas; possivelmente, diferentes testes laboratoriais utilizados podem explicar essa diferença (nCounter e multiplex PCR)

V. 2. IRA e sibilância

Em nosso estudo a sibilância foi detectada no exame físico em um sexto (16,5%) dos casos de IRA estudados. Nos países baixos (KOALA

Birth Cohort Study), foi relatada, pelo cuidador, primeiro episódio de sibilância em 10% das 3454 crianças acompanhadas do 7º ao 24º mês de vida (Mommers et al, 2010). Já observamos previamente que o relato de sibilância pelo cuidador de uma criança que nunca sibilou antes requer confirmação por exame físico realizado pelo pediatra, pois sibilância referida foi 2,7 vezes maior que a detectada no exame físico de criança pelo pediatra (Bouzas et al., 2014). Assim, nossa frequência de sibilância detectada foi consideravelmente maior do que a frequência de sibilância relatada nos Países Baixos, o que está de acordo com uma maior prevalência de sibilos em países em desenvolvimento (Mallol et al., 2010; Garcia-Marcos et al., 2010).

Várias hipóteses tentam explicar a relação entre infecção por vírus respiratório e sibilância. Uma das hipóteses seria que a infecção viral pode afetar o desenvolvimento da resposta imunológica, interferir no desenvolvimento pulmonar normal e na regulação do tônus do trato respiratório. Outra hipótese seria que o vírus desencadearia a obstrução no trato respiratório por resposta inflamatória em indivíduos que já apresentam alterações na função primária ou estrutural nas vias aéreas ou susceptibilidade em desenvolver uma resposta imunológica que predisponha a obstrução. Uma terceira hipótese seria que a resposta a diferentes vírus depende de fatores genéticos, da exposição a fatores

ambientais e do nível de maturidade dos sistemas respiratório e imunológico (Martinez, 2009).

Notavelmente, não encontramos associação significativa, nem em análise bivariada nem em análise multivariada, entre a detecção de vírus respiratórios na nasofaringe e a sibilância detectada no exame físico. Um estudo italiano relatou 88,2% de detecção global de 18 vírus respiratórios investigados em 85 crianças menores de 1 ano de idade hospitalizados com o primeiro episódio agudo de sibilância (Bosis et al, 2008). Em um estudo envolvendo crianças tailandesas com idade menores de 2 anos de idade hospitalizadas com um primeiro episódio de sibilância aguda, foram detectados vírus respiratórios (apenas 5 vírus foram investigados) em 80,6% de 170 casos (Teeratakulpisan et al., 2014). Em um estudo finlandês, vírus respiratórios foram encontrados em 100% das amostras, quando foram incluídas 111 crianças com primeiro episódio de sibilância entre crianças de 3-23 meses e 14 vírus estudados (Turunen et al., 2015). Todos os três estudos empregaram RT-PCR para detectar vírus respiratórios. Em conjunto, a detecção de vírus respiratórios foi muito elevada quando crianças com primeiro episódio de sibilância foram estudadas; entretanto, quando as crianças com IRA foram matriculadas, com ou sem o primeiro episódio de sibilância, não foi encontrada

associação entre vírus respiratórios e o primeiro episódio de sibilância, como no presente estudo.

V. 3. Fatores associados ao primeiro episódio de sibilância

Em nosso estudo, identificamos pássaro em casa como um fator de risco para o primeiro episódio de sibilância detectado no exame físico. Embora a exposição ao pássaro em casa não seja um fator associado conhecido, a exposição ao alérgeno pode ocorrer por contato com penas de pássaro ou seus excrementos, contaminando o ar da casa, e que podem ser facilmente inaladas e depositadas nas vias aéreas, provocando um episódio subsequente de sibilância (Quirce et al, 2001). Em um estudo realizado no Catar, passarinhos em casa, foram associadas à rinite alérgica (27,1% vs 13,8%, p <0,001) e eczema (22,8% vs 16,4%; p <0,001) (Janahi et al., 2006). Assim, nós hipotetizamos que ter um pássaro em casa pode sensibilizar as crianças a apresentar sibilos durante um episódio de IRA, independentemente do agente causador viral ou bacteriano.

Também identificamos possíveis fatores protetores: cão em casa e a presença de RNA de *H. influenzae* em NPA. Tem sido postulado que a exposição às endotoxinas bacterianas (Martinez & Halt, 1999) e animais (Hesselmar et al., 1999, Svanes et al., 1999) no início da vida pode conferir proteção contra o desenvolvimento de asma e alergias. Semelhante aos nossos resultados, a presença de um cão em casa foi associada a um menor

risco de sibilância em um estudo longitudinal de 4 anos (Litonjua et al, 2002). O aumento da carga microbiana no início da vida tem sido associado à diminuição da prevalência de sensibilização e possivelmente de asma mais tardiamente (Matricardi et al, 2000). Uma das substâncias sob investigação para explicar essa associação é a endotoxina. O aumento da exposição à endotoxina tem sido associado a taxas de sensibilização diminuída e possivelmente menor prevalência de sibilância (Braun-Fahrlander et al., 2002). A endotoxina é um componente da parede celular de bactérias gram-negativas, como H. influenzae, (Befford & Gern , 2005), que pode modificar as respostas imunes, pois é um potente indutor de citocinas TH1 (Lapa e Silva et al, 2000). Além disso, dados emergentes sugeriram que as interações paralelas entre hospedeiro e bactéria durante a infância podem desempenhar um papel na modulação da resposta imune e o desenvolvimento da sensibilização dos aeroalérgenos (Halt, 2015).

V. 4. Complicações

Embora a maioria das IRA em crianças sejam autolimitadas, podem ocorrer complicações principalmente em menores de 2 anos de idade; sendo OMA, pneumonia e hospitalização as principais complicações observadas (Neuzil et al., 2002). Em nosso estudo encontramos complicações em 19% dos pacientes avaliados, uma frequência próxima à encontrada em um Serviço de Emergência Pediátrica Francês (12%), onde

menores de 36 meses foram avaliados (Ploin et al.,2007). A complicação mais frequentemente observada foi pneumonia (11,1%) seguida de internação por agravamento do quadro respiratório (6,6%) e OMA (4,0%). Em um estudo longitudinal realizado em Bangladesh, dos 1322 episódios de IRA, pneumonia (29%), OMA (2%) e hospitalização (0,3%) foram observados (Homaira et al., 2012). A diferença na frequência de pneumonia e hospitalização entre os dois estudos pode ser explicada pelas diferenças no manejo dos casos de IRA nesses dois países (Brasil e Bangladesh). A pneumonia foi diagnosticada apenas em bases clínicas em Bangladesh, enquanto que no Brasil, geralmente, é confirmada pela radiografia de tórax (Nascimento-Carvalho et al.,2004). O diagnóstico de pneumonia baseado exclusivamente em motivos clínicos superestima o diagnóstico de pneumonia (Hazir et al., 2006). Por outro lado, a hospitalização em Bangladesh é mais difícil do que no Brasil devido ao número limitado de leitos hospitalares

Achados na ausculta respiratória como roncos e crépitos podem ser considerados sinais de alerta para complicações. Como tal, deve ser prestado um acompanhamento cuidadoso aos pacientes que apresentem esses sinais clínicos na avaliação inicial, a fim de detectar as complicações o mais rapidamente possível. Roncos na ausculta pulmonar, podendo ser roncos de transmissão por secreção nas vias aéreas, teve associação com

OMA em nosso estudo. Tem sido demonstrado que a pressão transitória negativa na orelha média ocorre em dois terços de resfriados, não complicados, em crianças previamente hígidas (Winther et al., 2002), que apresentam secreção nas vias aéreas; esta pressão negativa pode facilitar a OMA secundária em crianças com IRA (Winther et al., 2002).

Foi observado, em nosso estudo, que pássaro em casa é um fator de risco para OMA. Já foi descrito anteriormente que a exposição à alérgenos pode ocorrer devido ao contato com penas de pássaro ou a seus excrementos depositados no ambiente (Quirce et al, 2001). Em um estudo conduzido entre crianças em idade escolar para determinar se a exposição a animais de estimação desempenha um papel significativo no desenvolvimento de doenças alérgicas, a rinite foi mais comum entre as crianças que tinham contato domiciliar com passarinhos, gatos e galinhas, em comparação com crianças que vivem em lares sem animais (33,3% vs. 18,0%, p <0,001) (Janahi et al, 2006). Pacientes com rinite têm inflamação da mucosa nasal e disfunção da Trompa de Eustáquio, que levam ao aumento da pressão negativa no ouvido médio e à ventilação inadequada, com transudação de proteína e aumento da secreção. Como resultado, as secreções respiratórias são produzidas e podem levar a complicação como OMA (Mir et al., 2012).

V. 5. Vacina conjugada pneumocócica (PCV10)

A implementação universal de vacinas pneumocócicas conjugadas tem sido uma importante intervenção de saúde pública para prevenir a doença pneumocócica. A vacina pneumocócica (PCV10: Synflorix, GlaxoSmithKline Biologicals, Rixensart, Bélgica) foi introduzida em Salvador, Brasil, em julho de 2010, para crianças menores de 2 anos (Secretaria de Saúde do Estado da Bahia, 2010). Em nosso país, tem sido provado que a introdução de PCV10 no programa de imunização de rotina efetivamente reduziu taxas de hospitalização devido à pneumonia entre crianças (Afonso et al., 2013). Como há uma crescente frequência de colonização pneumocócica durante a IRA clínica, (Klugman et al., 2009) o uso de PCV provavelmente impactou na frequência de tal colonização. Para os vacinados, o risco de hospitalização por pneumonia associada à infecção por influenza diminuiu para aproximadamente a metade do risco em comparação as crianças que não receberam a vacina (Klugman et al., 2009). Aqui em nosso estudo, PCV10 não protege as crianças contra a pneumonia, a grande maioria dos nossos casos não foram hospitalizados; dito isto, é possível inferir que PCV10 protege contra casos mais graves de pneumonia.

Uma revisão recente da Cochrane Database Systematic Review sobre a eficácia das vacinas pneumocócicas conjugadas para a prevenção da

pneumonia relatou efeitos benéficos modestos em lactentes saudáveis com baixo risco de OMA adquirida após licenciado a vacina 7-valente CRM197-PCV7 (Fortanier et al., 2014). A partir de um modelo internacionalmente reconhecido, foi possível prever que PCV10 irá diminuir a morbidade (Komakhidze et al., 2015). No presente estudo, PCV10 foi inversamente associado com complicações durante a IRA. Para o melhor conhecimento, esta é a primeira coorte a demonstrar esse achado. Redução na frequência de colocações de tubos de timpanostomia em crianças atribuíveis a PCV10 tem sido recentemente relatado (Palmu et al., 2015).

VI. Perspectivas de estudo

Acompanhar as crianças que apresentaram infecção respiratória aguda com ou sem chiado para avaliar sibilância recorrente, fatores ambientais relacionados e história familiar de atopia.

Avaliar o papel do microbioma das vias aéreas identificados em aspirado de nasofaringe e as complicações observadas em vigência IRA.

VII. Limitações do estudo

A atopia não foi investigada por testes laboratoriais, por quantificação de IgE ou IgE RAST. No entanto, estes marcadores biológicos são incomuns entre crianças menores de 2 anos, faixa etária do nosso grupo de estudo. (Eysink et al.,2015).

Outra limitação do estudo é a falta de controles saudáveis pareados, devido ao desenho do estudo, propiciado para detectar fatores de risco para sibilância em crianças com IRA, especificamente. No entanto, coletamos um pequeno número de amostras de adultos saudáveis (n = 20), como controles para a detecção de patógenos respiratórios. Como esperado, encontramos uma diminuição significativa nos patógenos respiratórios no grupo de adultos saudáveis, mas não S. aureus ou H. influenzae. A presença de H. influenzae em 95% dos adultos saudáveis e assintomáticos fortalece ainda mais seu caso como fator protetor, de acordo com a recente demonstração de seu predomínio em um de cinco aglomerados bacterianos no microbioma nasal saudável (de Steenhuijsen Piters et al., 2016). Por sua vez, estes resultados são a favor da nossa escolha para o perfil transcripcional abrangente de patógenos por nCounter.

Aproximadamente um quarto dos pacientes recrutados (26,7%) não retornou para a avaliação de acompanhamento. No entanto, a única diferença encontrada entre os pacientes que retornaram e não retornaram

para o seguimento foi a maior freqüência de vômitos entre aqueles que não retornaram (ANEXOS-Tabela 1 em material suplementar). Isso significa que nenhuma das diferenças encontradas entre crianças com ou sem complicações foram detectadas entre os pacientes excluídos por perda de seguimento.

Outra limitação é que nem todos os pacientes recrutados tiveram o cartão de vacinação disponível para ser verificado. No entanto, esta perda foi inferior a 10%, pois 384 (90,1%) dos pacientes tiveram o cartão de vacinação verificado. Além disso, foram excluidos na análise multivariada os pacientes com situação vacinal desconhecida e as diferenças estatisticas foram encontradas.

VIII. Conclusões

1. *Haemophilus influenzae* em NPA e cão em casa protegem de forma independente contra o primeiro episódio de sibilância em crianças com IRA, enquanto pássaro em domicílio é um fator de risco.

2. Um quinto das crianças com idades entre 6-23 meses com IRA apresentou complicações.

3. Passaro em casa e roncos na auscuta respiratória estão associados a OMA enquanto crépitos na auscuta pumonar está associado com pneumonia

4. A imunização com PCV10 é independentemente associada a menor probabilidade de desenvolvimento de OMA em crianças de 6-23 meses em vigencia de IRA

X. SUMMARY

TITLE: The first wheezing episode, respiratory bacteria and viruses and associated complications among young children with acute respiratory infection.

Acute respiratory infection (ARI) is the most common disease among young children; as such, it is a source of significant morbidity. Wheezing during acute respiratory infection (ARI) is frequently linked to respiratory virus infection, but large studies combining environmental factors and comprehensive viral/bacterial pathogen profiling are lacking. **Objective**: We aimed to assess the association between environmental factors, respiratory pathogens detected in nasopharyngeal aspirates (NPA) and first wheezing episode detected upon physical examination and associated complications among children with ARI. **Methods:** This prospective cross-sectional study enrolled children aged 6-23 months with fever, sneeze, runny nose, nasal blockage, or cough for ≤ 7 days between September 2009 and October 2013 at an Emergency Department in Salvador, Northeastern Brazil. Children transferred from other hospitals or reporting previous episode of wheezing were excluded. Data on complaints, physical examination findings, and NPA were collected upon enrollment. Patients were subsequently followed up within 14-21 days after recruitment. Poor evolution was defined when hospitalization, pneumonia or acute otitis

media (OAM) occurred. A previously validated custom-designed nCounter probeset was used to detect 14 virus and 6 bacterial-targets in NPA. Multivariable logistic regression analysis by enter method was performed. **Results**: Of 559 enrolled children, 92 (16.5%) presented wheezing; 456 (81.6%) and 558 (99.8%) had at least one virus or bacterium detected, respectively. Overall, mean age was 11.4 ± 4.5 months, 120 (21.5%) and 88 (15.7%) reported dog or bird at home, respectively. *Staphylococcus aureus* (98%), *Haemophilus influenzae* (97%), and *Moraxella catarrhalis* (79%) were the most common bacteria. By multivariable logistic regression, *Haemophilus influenzae* (AdjOR=0.32; 95%CI: 0.11-0.93), dog (AdjOR=0.48; 95%CI: 0.25-0.92) and Bird (AdjOR=1.83; 95%CI: 1.02-3.27) at home were independently associated with wheezing. Of the 422 children who returned, 79 (19%, 95%CI: 15-23%) had poor evolution. Bird at home (OR[95%CI]:0.16[0.05-0.52]) and ronchi (OR [95%CI]:6.39[1.96-20.85]) were associated with AOM; PCV 10 was inversely associated with AOM (OR [95%CI]: 0.16[0.05-0.52]). **Conclusions**: *Haemophilus influenzae* in NPA and dog at home independently protect against the first wheezing episode whereas bird at home is a risk factor for it. Pneumococcal vaccination independently protects against AOM. Bird at home and ronchi are risk factors of otitis.

IX. Referências

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X. ANEXOS

ANEXO 1

Table 1. Comparison of children with acute respiratory infection on recruitment who returned with children who did not return

Characteristics	Follow-up visit		P
	Yes (n= 422)	No (n= 154)	
Age (months, mean ± SD)	11.4 ± 4.5	11.4 ± 4.6	1.00
Male Sex	206 (48.8%)	76 (49.4%)	0.90
Nutritional assessment			
Malnutrition	5 (1.2%)	4 (2.6%)	0.30
History			
Cough	369 (87.4%)	128 (83.1%)	0.20
Duration of cough (days, mean ± SD)	3.7 ± 1.8	3.5±1.9	0.40
Running nose	359 (85.1%)	122 (79.2%)	0.09
Duration of running nose (days, mean ± SD)	3.6 ± 1.7	3.5±1.8	0.75
Fever	342/421 (81.2%) [a]	133 (86.4%)	0.20
Duration of fever (days, mean ± SD)	2.9 ± 1.5	3.0±1.7	0.60
Sneeze	331 (78.4%)	109 (70.8%)	0.06
Duration of sneeze (days, mean ± SD)	3.5 ± 1.7	3.5±1.7	1.00
Wheeze	168 (39.8%)	60 (39.0%)	0.90
Duration of wheeze (days, mean ± SD)	2.5 ± 1.6	2.7±2.1	0.60

Hoarseness	128 (30.3%)	41 (26.6%)	0.34
Duration of hoarseness (days, mean ± SD)	2.8 ± 1.6	3.0±1.7	0.30
Dyspnea	115 (27.3%)	38 (24.7%)	0.50
Duration of dyspnea (days, mean ± SD)	2.8 ± 1.6	3.2±2.0	0.30
Vomiting	54 (12.8%)	32 (20.8%)	0.02
Duration of vomiting (days, mean ± SD)	2.0 ± 1.5	2.1±1.3	0.80
Ear ache	32/420 (7.6%) [a]	15 (9.7%)	0.40
Duration of ear ache (days, median (IQR))	2 (1 – 4)	3 (1–4)	0.50
Thoracic retraction	44 (10.4%)	21 (13.6%)	0.30
Duration of thoracic retraction (days, mean ± SD)	2.6 ± 1.9	2.5±1.9	0.90
Vaccination			
Pneumococcal (PCV10)	277/384 (72.1%)[a]	85/123 (69.1%)[b]	0.50
influenza	168/384 (43.8%)[a]	49/123 (39.8%)[b]	0.40
Breastfeeding			
Exclusive[c]	353/420 (84.0%)[a]	133 (86.4%)	0.50
Duration of breastfeeding exclusive (months, mean ± SD)	4.7±1.9	4.5±2.0	0.40
Mixed[c]	253/421 (60.1%)[a]	83/153 (54.2%)[b]	0.21
Duration of breastfeeding mixed (months, mean ± SD)	8.8±4.2	9.3±4.2	0.38

Family history			
Rhinitis	247/421 (58.7%)[a]	80/153 (52.3%)[b]	0.20
Asthma	121/420 (28.8%)[a]	36/153 (23.5%)[b]	0.20
Skin allergy	85/421 (20.2%)[a]	20/153 (13.1%)[b]	0.051
Infant lifestyle			
Shared bed	311(73.7%)	127/153 (83%)[b]	0.02
Smokers at home	80 (19.0%)	39 (25.3%)	0.10
Absence of ventilation at home	138 (32.7%)	49 (31.8%)	0.80
Chicken at home	15 (3.6%)	3 (1.9%)	0.40
Bird at home	68 (16.1%)	21 (13.6%)	0.50
Physical examination			
Tachypnea	99/416 (23.8%)[a]	37/151(24.5%)[b]	0.90
Fever	161/412 (39.1%)[a]	65/153 (42.5%)[b]	0.50
Ronchi	162 (38.4%)	50 (32.5%)	0.20
Wheezing	68 (16.1%)	27 (17.5%)	0.70
Crackles	37 (8.8%)	14 (9.1%)	0.90

[a] The denominator was not 422 because there was missing.

[b] The denominator was not 154 because there was missing.

[c] Mixed breastfeeding was informed for 1 child but the informant did not know if this child had been exclusively breastfed.

ANEXO 2

Termo de Consentimento Livre e Esclarecido

Projeto de Pesquisa: Prevalência de chiado, infecção respiratória viral

{ID}: ___ ___ ___

Eu,……., fui procurado(a) por Maiara Lanna Bouzas orientanda da Profa. Cristiana Nascimento de Carvalho, Professora da Universidade Federal da Bahia, e convidado (a) para conversar sobre a possibilidade da criança ……. poder participar sob minha inteira responsabilidade, do projeto de pesquisa de nome: **PREVALÊNCIA DE CHIADO E INFECÇÃO POR VÍRUS INFLUENZA EM LACTENTES.** Fui informado que a infecção por Influenza, ou seja, gripe é extremamente comum em todo o mundo, e que ela leva a várias complicações, como pneumonias, otites, convulsões, principalmente em crianças pequenas, além de fazer com que nós, os pais ou responsáveis, percam nosso trabalho para cuidar da nossa criança. No entanto, ainda não se sabe a quantidade exata de crianças doentes por ano e para isso é necessário fazer esta pesquisa coletando secreção do nariz da criança e sangue. Além disso, após 14 dias, eu serei contatado (a) por telefone para que minha criança seja reavaliada por Dr Juliana R de Oliveira, quando coletará a segunda amostra de sangue. Se eu concordar, minha criança poderá participar deste estudo, e se eu não concordar a minha criança será tratada com o que apresenta agora, de maneira igual a qualquer outro paciente. As informações clínicas e laboratoriais registradas na pesquisa serão usadas de forma totalmente anônima. A qualquer momento poderei tirar minha criança da pesquisa sem que isso venha a prejudicar ou impedir seu atendimento no CPPHO.

CONSENTIMENTO

Autorizo a inclusão da criança_____

Sob minha responsabilidade no estudo acima citado, sob condução de Maiara Lanna Bouzas, tel.(71) 8895-2221.

_____ _____

Assinatura de um dos pais/guardiões Assinatura do médico

_____ _____ _____

Assinatura da testemunha Local Data

DOCUMENTO EM 2 VIAS, SENDO UMA PARA SER ENTREGUE AO RESPONSÁVEL PELA CRIANÇA QUE PARTICIPARÁ DA PESQUISA

ANEXO 3

Ficha de Avaliação Pesquisa Clinica Inicial

Projeto de Pesquisa: Prevalência de chiado, infecção respiratória viral e desenvolvimento de chiado recorrente em lactentes

Sexo {CSEXO}: (1) Masculino (2) Feminino

No. do Prontuário{CPRONT}: _____ Data Avaliação {CDATAV} _____/_____/_____

Nome (Iniciais) {CNOME}: _____

Naturalidade {CNATUR}: _____ Procedência{CPROC}: _____

Data Nascimento{CNASC}: ___ / ___ / ___ Idade(anos /meses) {CIDADE}:__ __a/ __ m

Cor {CCOR}: _____ Peso (g){CPESO}:__ __ __ __ __ (99999) NS/NR

Altura (cm) {CALTU} __ __ __ (999) NS/NR

Ocup. Mãe/Resp.{COCMAE}: _____

1. Doença Atual

	Sim Há quantos dias?	Não	NS/NR
Febre {CFEBRE}		0	9
Tosse {CTOSSE} **Se sim, {CTOSSET} (1) Seca (2) Cheia**		0	9
2.3. Espirros {CESPIRR}		0	9
2.4. Coriza {CCORIZA}		0	9
2.5. Hialina {CHIALI} Se sim, {CHIALIC} (1) Amarela (2) Verde		0	9
2.6. Rouquidão {CROUQ}		0	9
2.7. Otalgia {COTALG }		0	9
2.8. Secreção ouvido? {CSOUV}		0	9
2.9. Chiado ou Sibilância {CCHIAD}		0	9
2.10. Dispnéia {CDISP} Se sim há quanto tempo? {CDISP} _____		0	9
2.11. Tiragem {CTIRAG} Se sim há quanto tempo? {CDISPT} _____		0	9
2.12. Convulsão {CCONVU} Se sim há quanto tempo? {CCONVUT} _____		0	9
2.13. Cianose {CCIANO} Se sim há quanto tempo? {CCIANOT} _____		0	9

2.14. Outros sinais / sintomas: {COUTR}		0	9
Qual? {CQUAL}			

3. Antecedentes

3.1. Obstétricos e Neonatais

	Sim	Não	NS/NR
3.1.1. Pré-Natal {CPRENAT}	1	2	9
3.1.2. Parto {CPARTO}	(1) Natural	(2) Cesário	9
3.1.3. Peso ao Nascer: _____ {CPESNAT}		(99) NS/NR	
3.1.4. Doença ao nascer {CDONAT}	1	2	9
Qual? {CQDONAT} _____			
3.1.5. Permaneceu internado após o nascimento? {CINTNAT}	1	2	9
Quantos dias ? {CTEMPINT} _____			
3.1.6. Prematuridade {CPREMAT} Caso sim, Idade gestacional: _____	1	2	9
3.1.7. Desconforto respiratório precoce? {CDRSPNAT}	1	2	9
3.1.8. Uso de O_2 {CUO2}	1	2	9
Quantos dias ? {CTEMPO2} _____			
3.1.9. Halo {CHALONAT}	1	2	9
3.1.10. Respirador {CRESPNAT}	1	2	9
3.1.11. Mãe Fumante {CMAEFUMG} durante a gestação	1	2	9
3.1.12. Mãe Fumante {CMAEFUMA} atualmente	1	2	9
3.1.13. Mãe Usuária de drogas {CMAEDROG}	1	2	9

3.2. Fisiológicos

3.2.1. Andou com quantos meses? (meses) {CTEMPAND}		99
3.2.2. Sentou com quantos meses? (meses) {CTEMPSNT}		99

3.3. Contatos com IRA – Rede de contatos doentes

3.3.1. Quem acha que contaminou a criança {CCONTCRN}? (0) Irmão Maior (1) Irmão do meio (2) Irmão Menor (3) Pai / Mãe (4) Outro (5) Colega creche (6) Indeterminado (7) Acha que foi em casa (8) Acha que foi na creche (9) NS/NR

3.4. Alimentares

3.4.1. Foi amamentado? {CAMAMENT}	(1) Sim	(2) Não	(9) NS/NR
Se não, por quê? {CNAMMOT}			
3.4.2. Amamentação exclusiva? {CAMEXC}	(1) Sim	(2) Não	(9) NS/NR
Se sim, até quando? (meses) {CTAMEXC}			
3.4.3. Amamentação mista? {CAMMIS}	(1) Sim	(2) Não	(9) NS/NR
Se sim, até quando? (meses) {CTAMMIS}			

3.6 Antecedentes Familiares

3.6.1. Alergia de pele {CALPL}	(1) Sim	(2) Não	(9) NS/NR
Se sim, quem na família? {CALPLFAM}	(0) Pai (1) Mãe (2) Irmãos (3) Primos (4) Tios (5) Outros (9) NS/NR		
3.6.2. Asma {CASMAF}	(1) Sim	(2) Não	(9) NS/NR
Se sim, quem na família? {CASMAFAM}	(0) Pai (1) Mãe (2) Irmãos (3) Primos (4) Tios (5) Outros (9) NS/NR		
3.6.3. Rinite {CRINIF}	(1) Sim	(2) Não	(9) NS/NR
Se sim, quem na família? {CRINIFAM}	(0) Pai (1) Mãe (2) Irmãos (3) Primos (4) Tios (5) Outros (9) NS/NR		
3.6.4. A mãe fez uso de vacina para Influenza na gravidez? {CINFVMAEG}	(1) Sim (2) Não (9) NS/NR		

3.7. Patológicos Pessoais – Doenças Associadas

Doenças Respiratórias

	Sim	Não	
3.7.1. Rinite {CRINI}	1	2	(9) NS/NR
3.7.2. Displasia Bronco-Pulmonar {CDBP}	1	2	(9) NS/NR
3.7.3. Outras {COTRSP}	1	2	(9) NS/NR
Se sim, quais? {COTRSPQ}			
3.7.4. Asma {CASMA}	1	2	(9) NS/NR
3.7.5. Eczema {CECZ}	1	2	(9) NS/NR
3.7.6. Rinite {CRINI}	1	2	(9) NS/NR

3.7.7. Refluxo Gastroesofágico {CREFL}	1	2	(9) NS/NR
3.7.8. Cardiopatia {CCARD}	1	2	(9) NS/NR
3.7.9. Neuropatia {CNEUR}	1	2	(9) NS/NR
3.7.10. Anemia Falciforme {CANEFA}	1	2	(9) NS/NR
3.7.11. Malformações {CMALFORM}	1	2	(9) NS/NR
3.7.12. Outras {COTPAT} Qual: _____	1	2	(9) NS/NR
3.7.13. Fez teste do pezinho? {CTESTPZ}	1	2	(9) NS/NR

3.8. Hábitos de Vida

3.8.1. Casa – número de habitantes {CCASAQ}: _____

Nome	Idade
	{CID1}
	{CID2}
	{CID3}
	{CID4}
	{CID5}
	{CID6}
	{CID7}

3.8.2. Cama compartilhada? {CCAMCOMP}	(1) Sim	(2) Não		(9) NS/NR
Se sim, com quem? {CCAMCOMPQ}				
3.8.3. Há poeira em casa? {CPOEIRC}	(1) Sim	(2) Não		(9) NS/NR
3.8.4. Há poeira no quarto? {CPOEIRQ}	(1) Sim	(2) Não		(9) NS/NR
3.8.5. Há mofo em casa? {CMOFOC}	(1) Sim	(2) Não		(9) NS/NR
3.8.6. Há mofo no quarto? {CMOFOQ}	(1) Sim	(2) Não		(9) NS/NR
3.8.7. Há baratas em casa? {CBRT}	(1) Sim	(2) Não		(9) NS/NR
3.8.8. Há fumantes em casa? {CFUMA}	(1) Sim	(2) Não		(9) NS/NR
Se sim, quem? {CFUMAQ}				
Se sim, quanto cigarros por dia {CFUMANC}				
3.8.9. Há poluição em casa? {CPOLUI} Que tipo: _____	(1) Sim	(2) Não		(9) NS/NR
3.8.10. Há ventilação em casa? {CVENTILA}	(1) Sim	(2) Não		(9) NS/NR
3.8.11. Freqüenta creche? {CCRECHE}	(1) Sim	(2) Não		(9) NS/NR
Se sim, há quanto tempo (meses)? {CCRECHEQ}				(9) NS/NR
Se sim, em que turno(s)? {CCRECHET}	(0) Manhã	(1) Tarde	(2) Manhã e Tarde	(9) NS/NR
3.8.12. Número de habitantes no mesmo quarto? {CNHQ}				

Caso sim, quais as idades:			
3.8.13. Tem cão em casa? {CCAO}	(1) Sim	(2) Não	(9) NS/NR
3.8.14. Tem gato em casa? {CGATO}	(1) Sim	(2) Não	(9) NS/NR
3.8.15. Tem galinha em casa? {CGALINHA}	(1) Sim	(2) Não	(9) NS/NR
3.8.16. Tem passarinho em casa? {CPASSARO}	(1) Sim	(2) Não	(9) NS/NR
3.8.17. Tem rato em casa? {CRATO}	(1) Sim	(2) Não	(9) NS/NR
3.8.18. Tem inseto em casa {CINSET} Caso sim, qual:	(1) Sim	(2) Não	(9) NS/NR
3.8.19. Tem outro animal em casa? {COUTROAN} Caso sim, qual: _____	(1) Sim	(2) Não	(9) NS/NR

Exame Clínico

4.1.FC {CFC} (bpm)			
4.2. FR {CFR} (ipm)			
4.3. TEC {CTEC} (s)			
4.4. Temperatura Axilar {CTAXI} (ºC)			
4.5. Apresenta tosse? {CTOSSE}	(1) Sim	(2) Não	
Se sim, qual a característica? {CTOSSEC}	(0) Seca	(1) Produtiva	
4.6. Apresenta hiperemia nos olhos? {CHIPEREM}	(1) Sim	(2) Não	
4.7. Apresenta edema nos olhos? {CEDEMOL}	(1) Sim	(2) Não	
4.8. Apresenta rinorréia? {CRINORREIA}	(1) Sim	(2) Não	
4.9. Apresenta prurido nasal? {CPRURIDO}	(1) Sim	(2) Não	
4.10. Apresenta obstrução nasal? {COBSTNAS}	(1) Sim	(2) Não	
4.11. Apresenta rouquidão? {CROUQUI}	(1) Sim	(2) Não	

Aparelho Respiratório

4.12. Apresenta Tiragem? {CTIRAG}	(1) Presente	(2) Ausente	
Se sim, qual? {CTIRAGQ}	(1) Intercostal	(2) Subdiafragmática	
4.12. Apresenta Murmúrio Vesicular? {CMURMU}	(0) Normal	(1) Aumentado	(2) Diminuído
4.13. Apresenta crépitos no pulmão esquerdo? {CCREPEQ}	(1) Presentes	(2) Ausentes	
4.14. Apresenta crepitos no pulmão direito? {CCREPDR}	(1) Presentes	(2) Ausentes	
4.15. Apresenta sibilos no pulmão esquerdo? {CSIBEQ}	(1) Presentes	(2) Ausentes	
4.16. Apresenta sibilos no pulmão direito? {CSIBDR}	(1) Presentes	(2) Ausentes	

4.17. Apresenta roncos no pulmão esquerdo? {CRONEQ}	(1) Presentes	(2) Ausentes
4.18. Apresenta roncos no pulmão direito? {CRONDR}	(1) Presentes	(2) Ausentes
4.19 Outros sinais {COTSIN}		

ANEXO 4

COMITÊ DE ÉTICA EM PESQUISA – CEP/MCO/UFBA
MATERNIDADE CLIMÉRIO DE OLIVEIRA
UNIVERSIDADE FEDERAL DA BAHIA
IORG0003460. Assurance FWA00002471, October 26, 2010
IRB00004123, October 5, 2007 - October 4, 2010

PARECER/RESOLUÇÃO N.º 067/2009

Registro CEP: 070/09 (Este nº deve ser citado nas correspondências referentes a este projeto)

Título do Projeto. "Prevalência de Chiado, infecção respiratória viral e desenvolvimento de chiado recorrente em lactantes."

Patrocínio/Financiamento. Solicitado financiamento à FAPESB – Fundação de Amparo à Pesquisa da Bahia.

Pesquisadora Responsável. Professora, **Cristiana Maria Costa Nascimento de Carvalho**. Pesquisadoras associadas: Professoras **Aldina Maria Prado Barral e Maria Regina Alves Cardoso**. *"Currícula Vitae"* anexos.

Instituição. Centro Pediátrico Professor Hosannah de Oliveira, Complexo Hospitalar Universitário Professor Edgar Santos, Universidade Federal da Bahia (CPPHO/C-HUPES/UFBA).

Área do Conhecimento. 4.00, Ciências da Saúde; 4.01, Medicina; Nível E; Grupo III.

Objetivos. Geral — estimar a carga de infecção pelos vírus Influenza (Flu) e Sincicial Respiratório (VSR). **Específicos** — estimar a prevalência de chiado pregresso, sibilos no atendimento, complicações e hospitalização associadas à infecção por esses vírus; descrever a sazonalidade da infecção por Flu/VSR; estimar a incidência de chiado após o episódio índice de infecção por Flu ou VSR.

Resumo. Estudo de corte transversal seguido de coorte prospectiva para avaliar 550 (quinhentos e cinqüenta) crianças, com idade entre 6 a 23 meses de vida, diagnosticadas com Infecção Respiratória Aguda. Exclusão daquelas que apresentarem passado de **"chiado"**. Após recrutamento, as crianças serão avaliadas em três momentos. Através de seus responsáveis, serão obtidos, na **1ª avaliação**, os dados da história clínica e procedido o exame físico com colheita de amostras da secreção da nasofaringe e de sangue para realização de hemograma e sorologia para vírus **Flu** e **VSR**. Subseqüentemente, após 14 (quatroze) dias da visita inicial, ocorrerá a **2ª avaliação** mediante o preenchimento da Ficha de Seguimento que conterá informações acerca da evolução, internamento, uso de antibióticos, desenvolvimento de chiado, pneumonia, otite, entre outras complicações e nova colheita de sangue (soros pareados). Em seguida, os familiares serão contatados mensalmente através de telefonemas ou presença ambulatorial, para levantamento de informações sobre o desenvolvimento de chiado com utilização da Ficha de Seguimento. Completados os dois anos de vida,

ANEXO V

Cell Host & Microbe

Information for Authors 31/01/2017

JCR 2015 12.552

Cell Host & Microbe is a monthly journal from Cell Press launched in March 2007. The journal publishes research articles and review materials with a focus on understanding microbes in relationship to their host. Working closely with authors, reviewers, and the scientific editorial board, the editorial staff maintains the high standards expected of a Cell Press journal to publish timely results of broad interest to the field. In keeping with Cell Press policies, editorial decisions at *Cell Host & Microbe* are made independently by its editorial staff, and *Cell Host & Microbe* content is freely available online 12 months after publication.

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The roles and responsibilities of corresponding author include:

1. Being responsible for all data, figures and text
2. Ensuring authorship is granted appropriately to contributors
3. Ensuring all authors approve the content and submission of the paper
4. Ensuring adherence to all editorial and submission policies
5. Identifying and declaring conflicts of interest on behalf of all authors
6. Identifying and disclosing related work by any co-authors under consideration elsewhere

7. Archiving unprocessed data and ensuring that figures accurately represent the original data (see data archiving section below)

8. Acting as the primary contact for all communication during consideration and after publication, acting as the primary contact for reagent and resource sharing, and acting as the arbiter of decisions and disputes

Additional Corresponding Authors and Lead Contact: If you feel strongly and have good reasons, additional corresponding authors can be designated. In this case, each corresponding author would hold responsibilities 1–7 above, and we would expect that each also contributed significantly to supervision of the work being described. If you indicate more than one corresponding author, we may ask you to clarify the reasons and make sure that each understands the responsibilities. We ask that you describe each corresponding author's specific contributions in the Author Contributions section and encourage use of the CRediT taxonomy.

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Authors should make every attempt to reduce the amount of postacquisition processing of data. Some degree of processing may be unavoidable in certain instances and is permitted provided that the final data accurately reflect that of the original. In the case of image processing, alterations must be applied to the entire image (e.g., brightness, contrast, color balance). In rare instances for which this is not possible (e.g., alterations to a single color channel on a microscopy image), any alterations must be clearly stated in the figure legend and in the Experimental Procedures section. Groupings and consolidation of data (e.g., cropping of images or removal of lanes from gels and blots) must be made apparent and should be explicitly indicated in the appropriate figure legends. Data comparisons should only be made from comparative experiments, and individual data should not be utilized across multiple figures. In cases in which data are used multiple times (e.g., multiple experiments were performed simultaneously with a single control experiment), this must be clearly stated within each figure legend. In the event that it is deemed necessary for proper evaluation of the manuscript, authors will be required to make the original unprocessed data available to the editors of the journal. All accepted manuscripts will be taken through a data presentation image screening process before publication.

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When a paper reports a new chemical entity or use of a known compound, the authors should use systematic nomenclature to refer to the chemical compounds, based on the guidelines of the International Union of Pure and Applied Chemistry (IUPAC). Use of informal common names, such as rapamycin, cholesterol, and penicillin, is also acceptable. Any standard or unconventional abbreviation should be defined at the point where it first occurs in the text.

For graphical representation of chemical structures, we suggest that authors refer to the IUPAC recommendations. There are a range of available molecular editor programs for creating images of chemical structures. Regardless of the program used, when inserting chemical structures into figures and/or tables, please ensure that they are exported from the molecular editor program as high-resolution files and that the final figure/table files follow general Cell Press Figure Guidelines).

Distribution of Materials and Data

One of the terms and conditions of publishing with Cell Press is that authors be willing to distribute any materials, data sets, and protocols used in the published experiments to qualified researchers for their own use. Materials include but are not limited to cells, DNA, antibodies, reagents, organisms, and mouse strains or, if necessary, the relevant ES cells. These must be made available with minimal restrictions and in a timely manner, but it is acceptable to request reasonable payment to cover the cost of maintenance and transport of materials. If there are restrictions to the availability of any materials, data, or information, these must be disclosed in the cover letter and in the Experimental Procedures section of the manuscript at the time of submission.

Data sets must be made freely available to readers from the date of publication. When requested, data must also be provided to editors and peer reviewers for the purposes of evaluating the manuscript.

For the following types of data, submission of the full data set to a community-endorsed, public repository is mandatory. Accession numbers must be provided in the paper (see "Database Linking" below for specific formatting instructions). Examples of appropriate public repositories are listed below.

DNA and Protein Sequences

Protein Sequences: Uniprot

DNA and RNA Sequences: Genbank/European Nucleotide Archive (ENA)/DDBJ, Protein DataBank, UniProt

DNA Sequencing Data (traces and short reads): NCBI Trace and Short-Read Archive, ENA's Sequence Read Archive

Deep Sequencing Data: Deposit in GEO or ArrayExpress upon submission to the journal

The sequences of all RNAi, antisense, and morpholino probes must be included in the paper or deposited in a public database with the accession number provided in the paper.

Human genomic data reporting newly described SNPs and CNVs identified in control samples should be deposited in an appropriate repository such as dbSNP, the Database of Genomic Variants Archive (DGVa), or the Database of Genomic Structural Variation (dbVAR).

We encourage but do not require the deposition of human sequence data in an appropriate repository such as dbGaP. We expect that, if data collected for a published paper cannot be included in the paper or made accessible in a public repository, then authors will accommodate legitimate requests for sharing of human genetics data provided that there are no IRB restrictions.

Structures of Biological Macromolecules

The atomic coordinates and related experimental data (structure factor amplitudes/intensities and/or NMR restraints) must be deposited at a member site of the Worldwide Protein Data Bank. Electron microscopy-derived density maps must be deposited into the EMDB through one of the partner sites (Protein Data Bank in Europe or EMDataBank). Atomic coordinates fitted to EM maps must also be deposited to a wwPDB member site. The corresponding database IDs must be included in the manuscript. Authors must agree to release the atomic coordinates and experimental data when the associated article is published. Additionally, Cell Press now recommends that authors include the PDB validation report as part of the Supplemental Information for all new submissions describing results of X-ray and NMR structure determination.

Microarray Data

MIAME-Compliant Microarray Data: GEO or ArrayExpress upon submission to the journal

Data must be MIAME compliant, as described at the MGED website specifying microarray standards.

Other Data Sets

In addition to the information that must be deposited in public databases as detailed above, authors are encouraged to contribute additional information to the appropriate databases. Authors are also encouraged to deposit materials used in their studies in the appropriate repositories for distribution to researchers.

Examples of repositories that facilitate sharing large data sets, including some that offer the option of anonymous referee access to data before publication, include:

For proteomics data: PRIDE, PeptideAtlas

For protein interaction data: IMEx consortium of databases, including DIP, IntAct, and MINT

For chemical compound screening and assay data: PubChem

For flow cytometry data: Flow Repository

If your paper makes use of genomic data generated from HeLa cells, we encourage you to comply with the NIH HeLa Genome Data Use Agreement by affirming that the NIH has approved use of the data and acknowledging the contributions of Henrietta Lacks and her family to the research.

Where there is no public repository and if the data sets are too large to submit to the journal online, authors should either consult the journal editorial office for advice or provide five separate copies of these data to the editors in an appropriate format (for example, CD or DVD) for the purposes of peer review.

Database Linking

Cell Press encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: "Database: xxxx" for single accession numbers and "Database: xxxx, yyyy, zzzz" for multiple accession numbers (e.g., "Genbank: NM_000492"; "GEO: GSE6364"; "PDB: 1TUP, 1KW4, 3H5X"). See http://www.elsevier.com/books-and-journals/content-innovation/data-base-linking for more information and a full list of supported databases.

Open Archive

Cell Press papers are freely available starting 12 months after publication.

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Prepublication Publicity

Work intended for submission to a Cell Press journal, currently under consideration at a Cell Press journal, or in press at a Cell Press journal may not be discussed with the media before publication. All Cell Press papers will remain under media embargo until 12 PM NOON (US, EST) on their date of publication. Providing preprints, granting interviews, discussing data with members of the media, or participating in press conferences in advance of online publication without prior approval from the Cell Press editorial office may be grounds for rejection. With regards to the scientific literature, papers in press with a Cell Press journal should not be discussed in reviews elsewhere prior to the date of online publication. Authors are welcome to present and discuss material intended for publication with Cell Press at scientific meetings; however, we do require that they refrain from discussing the findings with members of the press beyond those included in the formal presentation. In addition, we encourage meeting organizers to contact our editorial office in advance of a meeting if they have any questions regarding this

policy. Any questions or issues regarding prepublication publicity should be directed to Press Officer Joseph Caputo (jcaputo@cell.com).

Interaction with Members of the Press for Papers in Press

Authors are free to talk with the press one week prior to online publication, provided any information exchanged is embargoed until 12 PM NOON (US, EST) on the day of online publication. Authors may also discuss their work in press under embargo with other scientific journals for purposes of coverage in review material. Please refer any inquiries from media contacts and other journals to us. If your institutional press office wishes to issue a press release, they should contact us in advance for final embargo information. Any questions or issues regarding interaction with the press should be directed to Press Officer Joseph Caputo (jcaputo@cell.com).

Research Article Formats

Research Articles, Short Articles, and Resource Articles are all full-length articles that are handled through our online submission system, Editorial Manager. A brief description of each article type is provided below.

Research Articles

Research Articles present conceptual advances of unusual significance regarding a biological question of wide interest. Research papers should be as concise as possible and written in a style that is accessible to the broad *Cell Host & Microbe* readership. The total character count of an article must be under 55,000 (including spaces, references, and figure legends), and there should be no more than seven figures and/or tables. Additional display items may be published online as Supplemental Information at the discretion of the editor (please see the Supplemental Information guidelines for more information).

Short Articles

Short Articles follow the same overall criteria and general organization as full-length Research Articles but cover a discrete point of strong significance that can be presented clearly within a shorter format. The total character count must be less than 38,000 characters (including spaces, references, and figure legends) and there should be no more than four figures and/or tables. As with Research Articles, additional items may be published online as Supplemental Information.

Resource Articles

The Resource format is designed to highlight significant technical advances and/or major informational databases that are of value and interest to the broad *Cell Host &*

Microbe readership. Manuscripts reporting the development of an important technological advance should include a proof-of-principle demonstration that the new methodology will open the door for addressing important questions in a variety of biomedical research areas. Manuscripts reporting a major informational database should propose provocative new biological insights that can be derived from an analysis of the data set. Resources follow the same format and length guidelines as Research articles.

Preparation and Online Submission of Full-Length Articles

Cell Host & Microbe requires authors to submit manuscripts via our online submission system, Editorial Manager. An author tutorial regarding online submission is available at the Editorial Manager website. Authors may contact the editorial office (hostmicrobe@cell.com or +1 617 397 2851) for assistance.

Editorial Manager will prompt you to upload the individual components of your manuscript (cover letter, text, figures, supplemental data, etc.) as separate files. Alternately, you may upload your entire submission as a single combined PDF under the item type "Combined Manuscript File." Your cover letter will still need to be uploaded separately. Upon completion of this step, the website will build a composite PDF file of your entire manuscript that will contain links for the editors and reviewers to download the individual high-resolution files of each component. Please note that the version of the PDF file that is accessible to reviewers does not contain the cover letter; any information therein will remain confidential.

Please be aware that Editorial Manager will send all communications about the paper (including the request for final approval and the confirmation of submission) to the person who is checked off as corresponding author during the submission process or, if no name is designated, to the person whose account is used to submit the manuscript. If you wish to specify a different author for editorial correspondence after submission, please contact the Journal Associate at hostmicrobe@cell.com.

Cover Letter

Each submission should be accompanied by a cover letter, which should contain a brief explanation of what was previously known, the conceptual advance provided by the findings, and the significance of the findings to a broad readership. A cover letter may contain suggestions for appropriate reviewers and up to three requests for reviewer exclusions. The cover letter is confidential to the editor and will not be seen by reviewers.

General Article Organization and Text Specifications

Cell Host & Microbe full-length articles generally contain the following sections in this order: Title, Authors, Affiliations, Contact Information, Additional Title Page Footnotes, Summary,

Introduction, Results, Discussion, Experimental Procedures, Author Contributions, Acknowledgments, References, Figure and Table Legends, Figures and Tables, Graphical Abstract, and Supplemental Information. The text (title through legends) should be provided as one document, which may also contain the tables. Figures should be provided separately. Highlights and eTOC Blurb should be provided separately in a single Word document. Supplemental Information should be provided separately.

The total character count of the main text, including all sections and including spaces but excluding supplemental data, should not exceed 55,000. An article may contain up to seven figures and/or tables. Gene symbols should be italicized; protein products of the loci are not italicized. Nonstandard abbreviations should be defined when first used in the text. Use of abbreviations should be kept at a minimum. Manuscript file types that we can accept for submission include Word, RTF, and TXT. Required items differ for each article type and are specified during the submission process.

Please note that the text should be double spaced and pages should be numbered. Although summaries need to be entered as text files separate from the body of the manuscript during the online submission process, they should also be included within the manuscript file as usual.

Manuscripts that do not conform to the format guidelines may be returned to the authors for reformatting.

Preparation of Specific Sections

Title

Titles can occupy no more than three lines of type. Each line should contain no more than 50 characters, including spaces. The title should convey the conceptual significance of the paper to a broad readership.

Authors/Affiliations

Author names should be spelled out rather than set in initials. Authors should be footnoted to corresponding affiliations. Affiliations should contain the following core information: department(s)/subunit(s); institution; city, state/region, postal code; country. Note: Please check author names and affiliations carefully, as we cannot amend or correct these sections after publication.

Corresponding Author

The "Correspondence" line should include the e-mail address(es) of the corresponding author(s). One corresponding author is preferred for reasons stated in the Authorship section of Editorial Policies above. But there is no mandatory limit on the number of corresponding authors

that may be listed. Corresponding authors may also provide a Twitter handle as a secondary means of contact. Please see the corresponding author responsibilities noted above in the Editorial Policies.

Lead Contact

Every author list must identify one corresponding author as a Lead Contact, noted by a footnote in the manuscript. If there is only one corresponding author, that author will be listed as the Lead Contact. Please see the Lead Contact responsibilities noted above in the Authorship section above, under Editorial Policies.

Additional Footnotes

Footnotes are only allowed on page 1 of the text (and in tables). They may include a Lead Contact (mandatory) or a present address (optional), or they may indicate co-first authorship (optional). For more on designations of author contributions, please see the Authorship section above, under Editorial Policies.

Summary

The Summary consists of a single paragraph of fewer than 150 words. It should clearly convey the conceptual advance and significance of the work to a broad readership. In particular, the Summary should contain a brief background of the question, a description of the results without extensive experimental detail, and a summary of the significance of the findings. References should not be cited in the Summary.

Keywords

Authors are encouraged to include up to ten keywords that will be associated with the article on Cell Press platforms and on PubMed. These keywords should be listed in the manuscript after the Summary, separated by commas.

Introduction

The Introduction should be succinct, with no subheadings, and should present the background information necessary to provide a biological context for the results.

Results

This section should be divided with subheadings. Footnotes should not be used.

Discussion

The Discussion should explain the significance of the results and place them into a broader context. It should not be redundant with the Results section. This section may contain subheadings and can in some cases be combined with the Results section.

Experimental Procedures

The Experimental Procedures should, at minimum, include enough detail to allow the reader to understand the general experimental design and to be able to assess the data presented in the figures. More detailed protocols and procedures needed for readers to reproduce experiments should be included in the Supplemental Experimental Procedures. Any supplemental tables that list materials used in the study (oligonucleotides, strains, etc.) should be included within the relevant section of the Supplemental Experimental Procedures; these tables should have a title but should not be numbered. If your paper contains Supplemental Experimental Procedures, please make sure that they are referred to within the main Experimental Procedures so that it is clear to the reader that additional details are available online. This section should also include a description of any statistical methods employed in the study. A more detailed version of the procedures and details such as oligo sequences, strains, and specifics of how constructs were made can be included in the Supplemental Information, but it is not appropriate to move the majority of the Experimental Procedures to Supplemental Information in order to shorten the text. Please see our complete Supplemental Information guidelines for more information.

Author Contributions

For primary research papers, we ask you to include a dedicated Author Contributions section preceding the Acknowledgments to give information about individual author contributions to the work. Please keep this section as concise as possible and use initials to indicate author identity. All of the authors listed on the paper should be mentioned in this section at least once. We are happy for you to use a traditional format such as “A.B. and C.D. conducted the experiments, E.F. designed the experiments and wrote the paper…” but would also encourage you to use the CRediT taxonomy instead.

Acknowledgments

This section may acknowledge contributions from non-authors and/or list funding sources, and it should include a statement of any conflicts of interest. Please check this section carefully, as we cannot allow amendments or corrections after publication.

References

References should include only articles that are published or in press. For references to in press articles, please confirm with the cited journal that the article is in fact accepted and in press and include a DOI number and online publication date. Unpublished data, submitted manuscripts, abstracts, and personal communications should be cited within the text only. Personal

communication should be documented by a letter of permission. Submitted articles should be cited as unpublished data, data not shown, or personal communication.

In-text citations should be written in Harvard style and not numbered, e.g., "Smith et al., 2015; Smith and Jones, 2015."

Please use the style shown below for references. Note that "et al." should only be used after ten authors.

Article in a periodical:
Sondheimer, N., and Lindquist, S. (2000). Rnq1: an epigenetic modifier of protein function in yeast. Mol. Cell *5*, 163–172.

Article in a book:
King, S.M. (2003). Dynein motors: Structure, mechanochemistry and regulation. In Molecular Motors, M. Schliwa, ed. (Weinheim, Germany: Wiley-VCH Verlag GmbH), pp. 45–78.

An entire book:
Cowan, W.M., Jessell, T.M., and Zipursky, S.L. (1997). Molecular and Cellular Approaches to Neural Development (New York: Oxford University Press).

Figure Legends

Legends should be included in the submitted manuscript as a separate section. Each figure legend should have a brief title that describes the entire figure without citing specific panels, followed by a description of each panel. For any figures presenting pooled data, the measures should be defined in the figure legends (for example, "Data are represented as mean ± SEM."). Each legend should refer to any supporting items in the Supplemental Information (e.g., "See also Figure S1.").

Tables

When creating a table, please use the Microsoft Word Table function. Tables should include a title, and footnotes and/or legend should be concise. Include tables in the submitted manuscript as a separate section. Tables not created with the Microsoft Word table function will need to be revised by the author.

When creating tables, please adhere to the following guidelines:

- Do not submit tables in Excel or PDF format. Do not place an Excel table in a Word document.

- Format tables with Word's Table function; do not use tabs or spaces to create a table.
- Do not use line breaks or spaces to separate data within a cell. Use separate cells for all discrete data elements within a table.
- Number tables as Table 1, Table 2, Table 3, etc., rather than as Table 1a, Table 1b, Table 1c, etc.
- If bold or italic font is used within a table to indicate some feature of the data, please give an explanation of its usage in the legend.
- All abbreviations within a table must be defined in the table legend or footnotes.
- Footnotes should be listed with superscript lowercase letters, beginning with “a.” Footnotes may not be listed with numbers or symbols.

Highlights and eTOC Blurb

Highlights are a short collection of bullet points that convey the core findings of the article. *Specifications*: up to four bullet points may be included; the length of an individual bullet point should not exceed 85 characters (including spaces); only the core results of the paper should be covered.

The eTOC blurb is a short summary of the main take-home message of the paper and should describe the context and significance of the findings for the broader readership. Please see the "In Brief" links in the Table of Contents for examples. *Specifications*: This blurb should be 50 words or fewer; this blurb should be written in the third person and refer to “First Author et al.”

Both are required for all research papers and will be displayed online with the article; however, they will not appear in print. On the EM page where you are asked to upload your files, please choose "Highlights and eTOC Blurb" and upload a single Word document containing both your Highlights and the eTOC Blurb.

Supplemental Information

In general, Supplemental Information is limited to data and other materials that directly support the main conclusions of a paper but cannot be included in the main paper for reasons such as space or file format restrictions. SI should not be used to present data that are preliminary or that conceptually go beyond the main point of the paper.

Before submitting your supplemental materials, please refer to our complete instructions in the Supplemental Information guidelines. This page also contains information on submitting movie and other multimedia files.

Figures and Graphical Abstracts

Digital figure files submitted through Editorial Manager must conform to our digital figure guidelines or authors will be asked to revise them.

If you have any questions about digital files, please contact Lauren Shipp, Senior Managing Editor of *Cell Host & Microbe*, at lshipp@cell.com.

Graphical Abstract

Authors are encouraged to submit a graphical abstract with their manuscript. A graphical abstract should allow readers to quickly gain an understanding of the main take-home message of the paper and is intended to encourage browsing, promote interdisciplinary scholarship, and help readers identify more quickly which papers are most relevant to their research interests. Examples of this feature can be seen in the online version of articles published in *Cell Host & Microbe* from January 2010 onwards. Graphical abstracts are optional and may be submitted at any stage during the consideration of the paper for *Cell Host & Microbe*. Graphical abstracts can be uploaded in Editorial Manager by selecting "Graphical Abstract" when uploading files. Refer to our digital figure guidelines for graphical abstract preparation details.

Submission of Revised Manuscripts

In addition to the sections described above, revised manuscripts must also contain a detailed point-by-point response to the comments of the reviewers and/or editors. The cover letter should briefly summarize how the revised manuscript addresses these comments. In general, revised manuscripts will be reconsidered only if resubmitted within 2 months of the date of the original decision.

Checklist for Final Submission

1. Please make sure your final manuscript:

- Meets our restrictions of 55,000 characters, including spaces, and seven figures and/or tables
- A companion file containing the article Highlights and eTOC blurb
- Includes database accession numbers for new gene sequences, protein sequences, structures, or microarray data
- Includes explanation of statistical methods and sufficient detail of procedures in the Experimental Procedures section

- For any figures presenting pooled data, defines the measures in the figure legend

2. Please submit your final materials through Editorial Manager.

3. Please include all of the following when you are resubmitting your final materials:

- A modifiable electronic version of the final text (Microsoft Word .docx file is preferred)
- High-resolution digital files of the main and supplemental figures in an accepted format (see digital figure guidelines)
- Supplemental Information set and submitted as outlined in the Supplemental Information guidelines
- Additional Supplemental Information files such as movies and spreadsheets
- Signed conflict of interest form
- eTOC blurb, a paragraph of 50 words describing the context and significance of the findings
- Cover letter

Front Matter Formats

Submission of Front Matter Articles

Most of the front matter material published in *Cell Host & Microbe* is commissioned by the editors. However, proposals for appropriate review material pieces will be considered. Suggestions should be in the form of a one-page summary with a list of key references related to the proposed piece and may be sent to the Cell Host & Microbe office (hostmicrobe@cell.com). *Cell Host & Microbe* publishes the following front matter article types.

Previews

Previews highlight one or several research papers published in the same issue of *Cell Host & Microbe* or in a recent issue of another journal, placing the results in context for the journal's broad readership. Previews are 1,000 words in length with no more than 10 references and one or two figures. Most Previews are commissioned, but timely unsolicited contributions will be considered.

Reviews

Cell Host & Microbe publishes comprehensive full-length reviews on topics of interest to the journal's broad readership. Reviews are usually 5,000 to 8,000 words in length (including an abstract that is no more than 150 words), up to 100 references, and three to five figures.

Production and Proofs

After final acceptance, the manuscript will be passed to the production team to be copyedited and prepared for printing. Authors will be charged $550 for the first color figure and $275 for each additional color figure. Figures may be resized during the production process.

PDF proofs will arrive via e-mail about 2 weeks prior to publication and must be returned with vital corrections no more than 24 hours after receipt. If you will be unreachable at all during this period or anticipate any problems meeting this timeline, please contact Lauren Shipp, Senior Managing Editor (lshipp@cell.com).

Cover Submissions

After acceptance, authors are welcome to submit potential cover images related to their manuscript. Cover submissions should be informative and may be based on or resemble figures in the article, but they should also be creative and artistic. Authors may submit several different images for consideration. The editors make their selection based on the aesthetic quality of the image and the scientific scope of the study. For more information, see our Cover Submission Guidelines.