DR. MINNA KRISTIINA HONKILA (Orcid ID : 0000-0002-8288-9442)

DR. MARJO RENKO (Orcid ID : 0000-0003-0507-4773)

Article type : Brief Report

Chlamydia trachomatis, *Bordetella pertussis* and other respiratory bacteria in the aetiology of lower respiratory tract infections in young infants

Minna Honkila MD[1,2], Venla Kallinen BM[2], Marjo Renko MD PhD[1,2], Jaana Kauppila MD[3], Tytti Pokka MSc[1,2], Matti Uhari MD PhD[1,2], Terhi Tapiainen MD PhD[1,2]

[1]Department of Children and Adolescents, Oulu University Hospital, Oulu, Finland

[2]PEDEGO Research Unit and Medical Research Center Oulu, University of Oulu, Oulu, Finland

[3]NordLab Oulu, Oulu University Hospital, Oulu, Finland

Short title: Aetiology of lower respiratory tract infections in infants

Corresponding author: Minna Honkila, Department of Children and Adolescents, Oulu University Hospital, P.O. Box 23, FIN-90029 OYS, Finland. Email: minna.honkila@ppshp.fi. Tel: +358-8-3155863.

Systematic testing for *Bordetella pertussis* in young infants with lower respiratory tract infections (LRTIs) has been suggested, because the early clinical characteristics are difficult to recognise and distinguish from viral infections (1). *Chlamydia trachomatis*, a sexually transmitted disease passed on during birth, is also important (2-3). Recently, multiplex polymerase chain reaction (PCR) panels that include other respiratory bacteria have been implemented in clinical practice. Several studies have indicated that the atypical pathogens *Mycoplasma pneumoniae* and C*hlamydia pneumoniae* can cause community-acquired LRTIs in older children (4-5), but their role in young infants remains largely unknown.

This study evaluated whether young infants with LRTIs should be routinely tested for *Chlamydia trachomatis* and *Bordetella pertussis* and whether testing for other respiratory bacteria, including *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila* and *Bordetella parapertussis*, would reveal additional pathogens.

We prospectively included consecutive infants aged less than six months with LRTI symptoms: cough, wheezing, tachypnoea, dyspnoea or apnoea, with or without fever (≥ 38.0°C). They were treated in the paediatric emergency room at Oulu University Hospital, Finland, from 7 December 2016 to 6 December 2017. The Regional Ethics Committee of Northern Ostrobothnia Hospital District, Oulu, Finland provided ethical consent (EETTMK 98/2016) and written informed consent was obtained from the children's legal guardians. They completed a standard questionnaire about the infant's current illness and clinical characteristics and laboratory data were collected from hospital records. Pregnant women did not undergo universal *Chlamydia trachomatis* screening during the study period or routinely receive the acellular pertussis vaccines.

PCR (Abbott Molecular Inc, Illinois, USA) detected *Chlamydia trachomatis* from nasopharyngeal specimens and an Allplex Respiratory Panel 4 (Seegene Inc, Seoul, Korea) detected respiratory bacteria, including *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*,

Legionella pneumophila, *Bordetella pertussis*, *Bordetella parapertussis*, *Streptococcus pneumoniae* and *Haemophilus influenzae.* An Anyplex II RV16 kit (Seegene Inc, Seoul, Korea) detected respiratory viruses, including the adenovirus, bocavirus, enterovirus, influenza viruses A and B, human coronaviruses 229E, NL63 and OC43, human metapneumovirus, parainfluenza viruses 1, 2, 3 and 4, respiratory syncytial viruses A and B and rhinovirus. An in-house PCR assay detected *Bordetella pertussis*.

We regarded it as clinically significant, i.e. that testing for both *Chlamydia trachomatis* and *Bordetella pertussis* would be clinically indicated in all young infants with an LRTI, if 5%, with a 95% confidence interval (CI) of 2.5–7.5%, were caused by these pathogens. The study sample size, with a two-sided alpha error of 0.05, was 169 infants, but we decided to collect samples for at least 12 months to represent a complete epidemiological year.

The study comprised 228 infants with LRTIs with a median age at presentation of 70 days (range 6–174). Most (89%) had viral respiratory tract infections (95% CI 84–93%), notably respiratory syncytial viruses (34%), rhinoviruses (29%) and parainfluenza viruses (11%) (Table 1).

PCR testing detected *Chlamydia trachomatis* in one infant and *Bordetella pertussis* in another infant (both 0.4%; 95% CI 0.01–2.4%) (Table 1), providing an overall incidence of 0.9% (95% CI 1.1–3.1%) for either infection. *Mycoplasma pneumoniae* was detected in four infants (1.8%, 95% CI 0.5–4.6%), *Bordetella parapertussis* in two (0.9%, 95% CI 0.1–3.2%) and *Chlamydia pneumoniae* in one (0.5%, 95% CI 0.01–2.5%). *Bordetella pertussis* was not detected by the multiplex PCR panel in one infant, but diagnosed following a separate in-house PCR assay. No *Legionella* infections were detected. *Streptococcus pneumoniae* was positive in 66 cases (30%, 95% CI 24–37%) and *Haemophilus influenzae* in 42 (19%, 95% CI 14–25%). The multiplex PCR panel showed

that respiratory bacteria other than *Streptococcus pneumoniae* or *Haemophilus influenzae* were the only causative agents in 3/220 infants (1.4%), including two with *Mycoplasma pneumoniae* and one with *Bordetella parapertussis*. We found that 21 infants (9.2%) were negative for all pathogens.

The 198 cases with viral respiratory infections had paroxysmal cough (50%), whoops (16%) and staccato cough (8.1%). The infant with *Bordetella pertussis* did not have classic whooping at diagnosis and the *Chlamydia trachomatis* case did not have the typical staccato cough. One infant who only tested positive for *Mycoplasma pneumoniae* had a severe illness with dyspnoea and apnoea.

To summarise, this one-year study found a clinically important treatable bacterial pathogen in two (1%) of the 228 infants with LRTIs: one case of *Chlamydia trachomatis* and another of *Bordetella pertussis.* Multiplex PCR for respiratory bacteria identified other potential pathogens, including *Mycoplasma pneumoniae*, *Bordetella parapertussis* and *Chlamydia pneumoniae* in 3%. The infants with either *Chlamydia trachomatis* LRTI or whooping cough had similar symptoms to those with viral infections. However, routine testing for these pathogens in all young infants with LRTIs may not be warranted, but the incidence of *Bordetella pertussis* could increase rapidly during an epidemic.

ABBREVIATIONS

CI, Confidence interval; LRTI, Lower respiratory tract infection; PCR, Polymerase chain reaction.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING

This study received external funding from the Alma and K A Snellman Foundation, Finland.

References

1. Korppi M, Hiltunen J. Pertussis is common in nonvaccinated infants hospitalized for respiratory syncytial virus infection. *Pediatr Infect Dis J* 2007; 26: 316–8.
2. Hammerschlag MR, Chandler JW, Alexander ER, English M, Koutsky L. Longitudinal studies on chlamydial infections in the first year of life. *Pediatr Infect Dis* 1982; 1: 395–401.
3. Rours GI, Hammerschlag MR, Van Doornum GJ, Hop WC, de Groot R, Willemse HF, et al. Chlamydia trachomatis respiratory infection in Dutch infants. *Arch Dis Child* 2009; 94: 705–7.
4. Baer G, Engelcke G, Abele-Horn M, Schaad UB, Heininger U. Role of Chlamydia pneumoniae and Mycoplasma pneumoniae as causative agents of community-acquired pneumonia in hospitalised children and adolescents. *Eur J Clin Microbiol Infect Dis* 2003; 22: 742–5.
5. Principi N, Esposito S. Emerging role of Mycoplasma pneumoniae and Chlamydia pneumoniae in paediatric respiratory-tract infections. *Lancet Infect Dis* 2001; 1: 334–44.

Table 1. Findings from the respiratory specimens of 228 infants aged less than six months with a lower respiratory tract infection.

Test	Infants with a positive result n (%)	95% CI
***Chlamydia trachomatis* PCR** (n=228)	1 (0.4)	0.01–2.4
***Bordetella pertussis* PCR** (n=226)	1[a] (0.4)	0.01–2.4
Multiplex PCR for respiratory bacteria (N=220)		
Chlamydia pneumoniae	1[b] (0.5)	0.01–2.5
Mycoplasma pneumoniae	4[c] (1.8)	0.5–4.6
Legionella pneumophila	0 (0)	0–0.02[d]
Bordetella pertussis	0 (0)	0–0.02[d]
Bordetella parapertussis	2[e] (0.9)	0.1–3.2
Streptococcus pneumoniae	66 (30)	24–37
Haemophilus influenzae	42 (19)	14–25
Multiplex PCR for respiratory viruses (n=228)		
Adenovirus	4 (1.8)	0.5–4.4
Bocavirus	13 (5.7)	3.1–9.6
Human coronavirus 229E	1 (0.4)	0.01–2.4
Human coronavirus NL63	3 (1.3)	0.3–3.8
Human coronavirus OC43	8 (3.5)	1.5–6.8
Enterovirus	4 (1.8)	0.5–4.4
Human metapneumovirus	21 (9.2)	5.6–14
Influenza virus A	9 (3.9)	1.8–7.4
Influenza virus B	1 (0.4)	0.01–2.4
Parainfluenza virus 1	4 (1.8)	0.5–4.4
Parainfluenza virus 2	0 (0)	0–1.6[d]
Parainfluenza virus 3	19 (8.3)	5.1–13
Parainfluenza virus 4	2 (0.9)	0.1–3.1
Respiratory syncytial virus A	51 (22)	17–28
Respiratory syncytial virus B	27 (12)	8.0–17
Rhinovirus	67 (29)	24–36

Respiratory specimens contained [a] *Bordetella pertussis* plus rhinovirus and bocavirus (n=1). [b] *Chlamydia pneumoniae* plus parainfluenza virus 3 (n=1); [c] *Mycoplasma pneumoniae* plus bocavirus (n=1) and *Mycoplasma pneumoniae* plus coronavirus OC43 (n=1). [d] 97.5% one-sided CI; [e] *Bordetella parapertussis* plus rhinovirus (n=1)