and beluga whales showed that none of these animals were colonized with MRSA. Overall, MRSA was isolated on ≥1 occasions from 5 dolphins (n = 6, 83.3%) and 3 walruses (n = 6, 50%) (Table). All strains were indistinguishable on PFGE and were consistent with the CMRSA2 (USA100) strain. They were also *spa* type t002 and did not possess the PVL toxin genes.

This report of MRSA shows colonization in several dolphins and walruses, with apparent transmission between species. The direction of transmission cannot be determined because of the sampling method; however, a human origin is suspected because the clone that was isolated is a predominant human clone. The failure to identify a concurrently colonized person does not preclude a human source. Since the time MRSA was introduced into the facility is unknown, the source of infection may have been decolonized by the time of sampling or was not sampled. Furthermore, park visitors occasionally have contact with these animals so the origin could have been from the general public. Whether colonization of multiple animals was due to repeated instances of human-to-animal transmission or whether animal-to-animal transmission may have occurred is not clear. For the dolphins, the second scenario is most likely, considering the social nature of these animals and the inability to isolate colonized dolphins. These factors may have resulted in the circulation of MRSA among these animals. Although no water samples were obtained for testing, waterborne transmission cannot be dismissed.

Colonization was eliminated without antimicrobial agents; however, long-term (15 months) MRSA colonization was found in 1 dolphin. With patience and continued use of infection control measures, MRSA was apparently eradicated from this facility without the need for active decolonization. This study shows the impressive ability of MRSA to colonize diverse animal species and provides further evidence suggesting that interspecies transmission of human epidemic clones can occur between persons and animals. This study also provides evidence suggesting that MRSA colonization in many animal species can be transient and that application of appropriate infection control and hygiene measures may be critical control tools for the management of MRSA in animals.

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Parachlamydia and *Rhabdochlamydia* in Premature Neonates

To the Editor: New members have recently been recognized in the order Chlamydiales (*1*). The family *Rhabdochlamydiaceae* includes *R. porcellionis* (a parasite of *Porcellio scaber*) and *R. crassificans* (a pathogen of the cockroach *Blatta orientalis*) (*2*,*3*); their pathogenic role in humans has not yet been investigated. *Parachlamydia acanthamoebae* and *Protochlamydia naegleriophila* belong to the family *Parachlamydiaceae* (*1*,*4*). Increasing evidence indicates that these obligate intracellular bacteria infecting free-living amebae may cause respiratory diseases in humans (*1*). Recent findings also suggest a role for *Parachlamydia* in miscarriage, stillbirth, and preterm labor (*5*–*7*). Whether these bacteria may contaminate the newborns of infected mothers is unknown.

The aims of this study were to 1) develop a real-time PCR for detecting *Rhabdochlamydia* spp. and 2)

apply this PCR, and those previously described for *Parachlamydia* and *Protochlamydia* (*4*,*8*), to respiratory samples from premature neonates. Using the GenBank database (www.ncbi.nlm.nih.gov), we selected primers RcF (5′-GACGCTGCGTGAGTGATGA-3′) and RcR (5′-CCGGTGCTTCTTTACGCAGTA-3′), and probe RcS (5′-6 carboxyfluorescein-CTTTCGGGTT-GTAAAACTCTTTCGCGCA-Black Hole Quencher 1-3′), which amplify parts of the 16S rRNA encoding gene, to specifically amplify *Rhabdochlamydia* spp. The 5′-FAM probe (Eurogentec, Seraing, Belgium) contained locked nucleic acids (underlined) to improve specificity. Reactions were performed with 0.2 μM of each primer, 0.1 μM of probe, and iTaq Supermix (Bio-Rad, Rheinach, Switzerland). PCR products were detected with ABI Prism 7000 (Applied Biosystems, Rotkreuz, Switzerland). Inhibition, negative PCR mixture, and extraction controls were systematically tested.

To enable quantification, a plasmid containing the target gene was constructed as described (*4*,*9*). The analytical sensitivity of the real-time PCR for *Rhabdochlamydia* spp. was ≤10 copies DNA/μL. No cross-amplification was observed when the analytical specificity was tested with human, amebal (*Acanthamoeba castellanii* ATCC 30010), and bacterial DNA (online Technical Appendix, available from www.cdc.gov/EID/content/15/12/2072-Techapp.pdf). Intrarun and interrun reproducibility were excellent (online Technical Appendix).

This PCR and those previously described for *Parachlamydia* and *Protochlamydia* (*4*,*8*) were retrospectively applied to 39 respiratory samples from 29 neonates admitted in the neonatology unit of our institution (median 1 sample per patient, range 1–4 sample). All but 1 patient had a gestational age at birth ≤36 weeks (median 28.6, range 24.6–41.2 weeks). Respiratory

Table. Characteristics of 29 newborns with positive PCR results for *Parachlamydia acanthamoebae* or *Rhabdochlamydia* spp. and controls*

Characteristics	Positive PCR result, n = 12	Negative PCR result, n = 17	p value†
Sex, M/F	8 (67)/4 (33)	6 (35)/11 (65)	0.14
Gestational age at birth, wk, median (range)	27 (24–36)	30 (25–41)	0.16
Weight <10th percentile	4 (33)	4 (24)	0.68
Height <10th percentile	3 (25)	6 (35)	0.69
Primary adaptation			
First Apgar score (1 min), median (range)	2.5 (0–7)	8 (2–9)	0.0017
First 3 Apgar scores,‡ median (range)	18.5 (8–27)	27 (17–29)	0.0023
Cardiac massage in first 48 h	6 (50)	0 (0)	0.002
Endotracheal intubation in first 48 h	11 (92)	8 (47)	0.019
Respiratory distress syndrome	11 (92)	14 (82)	0.62
Hyaline membranes disease	9 (75)	8 (47)	0.25
Bradypneic syndrome	7 (58)	11 (65)	1.00
Bronchopulmonary dysplasia	8 (67)	9 (53)	0.70
Amniotic fluid aspiration	1 (8)	3 (18)	0.62
Invasive mechanical ventilation, d, median (range)	12 (2–50)	3 (0–14)	0.005
Endotracheal intubation during hospital stay	12 (100)	11 (65)	0.028
Infectious complications			
Lung infection	5 (42)	7 (41)	1.00
Other systemic infection	7 (58)	5 (29)	0.15
Other complications			
Intraventricular hemorrhage	4 (33)	6 (35)	1.00
Persistent artery canal	7 (58)	6 (35)	0.27
Necrotizing enterocolitis	2 (17)	1 (6)	0.55
Congenital malformations	1 (8)	1 (6)	1.00
Hospitalization			
Stay in neonatology ward, d,§ median (range)	113.5 (9–435)	48 (7–131)	0.003
Death	3 (25)	0 (0)	0.06
Pregnancy			
Premature membranes rupture	5 (42)	6 (35)	1.00
Placental detachment	2 (17)	1 (6)	0.55
Preeclampsia	4 (33)	2 (12)	0.20
Systemic infection	7 (58)	9 (53)	1.00
Cesarean delivery	9 (75)	10 (59)	0.45

*Results are given as no. (%) except as indicated.
†Fisher exact test and nonparametric Mann-Whitney rank sum test were used for the analysis of proportions and continuous variables, respectively.
‡Sum of the 3 scores at 1, 5, and 10 min after birth.
§If survived.

distress syndrome was present in 25 (86%) of these 29 neonates. Samples had been drawn a median of 14 days (range 1–229 days) after birth, when clinically indicated. Results of PCR for *Parachlamydia*, *Protochlamydia*, and *Rhabdochlamydia* were positive for 9 (31%), 0 (0%), and 4 (14%) neonates, respectively. Positive results were obtained on the first sample drawn after birth for all but 2 neonates (initial negative results). One patient had positive PCR results for *Parachlamydia* and *Rhabdochlamydia*. These 12 newborns with positive PCR results for *Parachlamydia* and/or *Rhabdochlamydia* were compared with the 17 who had negative PCR results (Table).

Newborns with a *Chlamydia*-related organism documented in the respiratory tract had a significantly worse primary adaptation score (Apgar). These patients experienced more resuscitation maneuvers at birth. Durations of invasive mechanical ventilation and hospital stay were also longer among them. Three newborns died, compared with no deaths among the 17 with negative PCR results ($p = 0.06$). Pneumonia was documented in 5 of the 12 patients with positive *Parachlamydia* and/or *Rhabdochlamydia* PCR results but was concomitant to PCR positivity for only 3 of them. An alternative etiology was documented in all 3 (online Technical Appendix).

Parachlamydia and *Rhabdochlamydia* have thus been detected in a population of premature neonates. Most of these patients had severe respiratory distress syndrome, and the role of these bacteria as a causal agent of pneumonia could not be clearly assessed. The longer duration of mechanical ventilation for newborns with positive PCR results may suggest an occult superinfection with a *Chlamydia*-related bacterium contributing to the severity of the initial respiratory disease.

Our results also raise a question about the mode of acquisition of these microorganisms. A recent study reported a higher seroprevalence of *Parachlamydia* in women experiencing miscarriage (*5*,*6*), and DNA of this bacterium has been detected in the amniotic fluid of a woman with premature delivery (*7*). Whether neonatal infection results from a systemic infection during pregnancy or an inoculation at delivery is unknown. Because of the retrospective design of the study, no samples from the mothers were available for additional molecular or serologic analyses. Hospital water supplies are an important reservoir of free-living amebae and may represent another mode of acquisition because patients undergoing mechanical ventilation are exposed to aerosolized particles (*10*). Simultaneous detection of Parachlamydia and Rhabdochlamydia in 2 patients with initial negative results and their simultaneous detection in 1 neonate supports the latter hypothesis.

In conclusion, *Parachlamydia* and *Rhabdochlamydia* DNA were detected in respiratory secretions of premature newborns with more severe conditions at birth, more mechanical ventilation requirements, and a trend toward a higher mortality rate. The pathogenic role of these *Chlamydia*-related bacteria in neonates deserves further investigations.

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Porcine Kobuvirus in Piglets, Thailand

To the Editor: To date, the genus *Kobuvirus* has consisted of 2 officially recognized species, *Aichi virus* and *Bovine Kobuvirus* (*1*). Aichi virus has been shown to be associated with acute gastroenteritis in humans (*2*–*4*), and bovine kobuvirus has been detected only in cattle (*5*,*6*). Most recently, a third candidate species of *Kobuvirus* has been described in pigs by 2 different groups of investigators from Hungary and the People's Republic of China (*7*,*8*). This new candidate species was serendipitously recognized in stool specimens from pigs when PCR products (≈1,100 bp) were amplified by using a primer pair for the detection of caliciviruses (*7*).

Nucleotide sequences of these nonspecific PCR products were similar to those of the U-1 bovine kobuvirus and Aichi virus A846/88 reference strains; sequence identities ranged from 73% to 79% at the nucleotide level and from 69% to 70% at the amino acid (*7*). The representative strain of a new candidate species of porcine kobuvirus, S-1-HUN (Porcine kobuvirus/swine/S-1-HUN/2007/Hungary), has been analyzed to determine its complete genome sequence and genetic organization (*9*). The RNA genome of the S-1-HUN strain comprises 8,210 nt, with a genome organization analogous to that of picornaviruses. Therefore, this strain is tentatively classified as a new species of the genus *Kobuvirus*, and named porcine kobuvirus (*7*,*9*).

Currently, 2 reports have described the epidemiologic feature of porcine kobuvirus in healthy piglets. Thirty-nine (65%) of 60 stool samples collected from pigs in Hungary were positive for porcine kobuvirus by reverse transcription–PCR (RT-PCR) (*9*). Another report from China found that the prevalence of porcine kobuvirus was 30% (97 of 322 piglets) (*8*). These findings suggested that porcine kobuvirus infections are common in piglets. However, whether this agent is associated with particular diseases, including gastroenteritis, in piglets was not clear.

We conducted an epidemiologic survey of porcine kobuvirus and report the detection of this virus in the stool specimens of piglets with diarrhea. Sequence and phylogenetic analyses of the porcine kobuvirus strains were carried out to determine their evolutionary relationships with kobuvirus strains previously reported.

A total of 98 stool specimens were collected from piglets with diarrhea from 6 farms in Chiang Mai Province, Thailand, during 2001–2003. Age of the piglets ranged from 7 to 49 days old. Porcine kobuvirus was detected in fecal specimens by RT-PCR (*9*). The representative strains of porcine kobuvirus detected in our study were analyzed further by direct sequencing of their PCR amplicons (216 bp) by using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences of these fragments were compared with those of reference strains available in the NCBI GenBank database by using BLAST server (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA 4 (*10*). Nucleotide sequences of porcine kobuvirus strains described in this study were deposited in GenBank under accession nos. GQ152093–GQ152122.

Prevalence of porcine kobuvirus was exceptionally high in piglets with diarrhea, 99% (97 of 98 specimens). Thirty representative strains of porcine kobuvirus detected in this study were randomly selected, sequenced, and analyzed to determine their evolutionary relationships with other kobuvirus reference strains. The partial 3D region among all 30 porcine kobuvirus strains was highly conserved, with nucleotide sequence identities >90%. In addition, our strains were most closely related to 2 porcine kobuvirus reference strains (S-1-HUN and Swine/2007/CHN) available in GenBank, with the nucleotide sequence identity ranging from 91.5% to 96.3%. Phylogenetic analysis of partial 3D nucleotide sequences of our porcine kobuvirus strains, together with published sequences of porcine kobuvirus reference strains (and those of Aichi virus and bovine kobuvirus), is shown in the Figure. The phylogenetic tree confirmed that all strains we identified belonged to the porcine kobuvirus species and formed a tight cluster in a monophyletic branch with the other 2 porcine kobuvirus reference strains (S-1-HUN and Swine/2007/CHN). These strains are also distantly related to standard strains of Aichi virus and bovine kobuvirus. Recently, 18 sequences of partial 3D region of the porcine kobuvirus strains detected in China have been deposited in GenBank. Unfortunately, the specific position of PCR amplification of the strains found in China was different from that of our strains (*8*). Therefore, the relationship between these strains could not be analyzed.

Porcine kobuviruses have previously been reported only in healthy pigs (*7*–*9*). In our study, the exceptionally high prevalence of porcine kobuviruses (99%) has been observed in piglets with acute gastroenteritis; those samples were negative for rotavirus infection as determined previously by RT-PCR. However, associations of this agent with enteric diseases in pigs

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Technical Appendix

Table 1. Bacterial species used in determining the specificity of the real-time PCR for *Rhabdochlamydia* spp.*

Species	Source of strain
Bordetella pertussis	Clinical specimen
Chlamydia trachomatis	Clinical specimen
Chlamydophila pneumoniae	ATCC VR-1310
Criblamydia sequanensis	Strain CRIB-18
Enterococcus faecalis	ATCC 29212
Escherichia coli	ATCC 35218
Gardnerella vaginalis	Clinical specimen
Haemophilus influenzae	ATCC 49247
Klebsiella pneumoniae	ATCC 27736
Lactobacillus spp.	Clinical specimen
Legionella pneumophila	Clinical specimen
Neisseria lactamica	Clinical specimen
Neochlamydia hartmanellae	ATCC 50802
Parachlamydia acanthamoebae	Strain Hall's coccus
Parachlamydia acanthamoebae	ATCC VR-1476 (strain BN9)
Protochlamydia amoebophila	ATCC PRA-7 (strain UWE25)
Protochlamydia naegleriophila	Strain KNic
Pseudomonas aeruginosa	ATCC 27853
Simkania negevensis	ATCC VR-1471
Staphylococcus epidermidis	Clinical specimen
Streptococcus agalactiae	ATCC 13813
Streptococcus pneumoniae	Clinical specimen
Waddlia chondrophila	ATCC VR-1470

*ATCC, American Type Culture Collection.

Table 2. Clinical characteristics of 12 neonates with positive PCR results for *Parachlamydia acanthamoebae* or *Rhabdochlamydia* spp.

Patient (sex)	Gestational age at birth, wk	APGAR score*	Cardiac massage (first 48 h)	Duration of invasive ventilation, d	Species identified (d from birth)	Infections† (d from birth)	Other isolated pathogen	Hospital stay, d/ outcome
1 (F)	26	19	No	5	*Parachlamydia (*14)	Pneumonia (21)	*Enterobacter aerogenes*	90/Survived
2 (M)	35	25	No	9	*Parachlamydia* (20)	–	–	110/Survived
3 (M)	24	15	Yes	50	*Parachlamydia* (15)	Pneumonia (5)	*Ureaplasma urealyticum*	140/Survived
4 (M)	29	11	No	14	*Parachlamydia* (1)	Chorioamnionitis (5)	*Streptococcus* (gr. G), *U. urealyticum*	113/Survived
5 (M)	27	8	Yes	9 (Died)	*Parachlamydia* (7)‡	Pneumonia and chorioamnionitis (1)	*Streptococcus pneumoniae*§	9/Died
6 (F)	27	18	Yes	23	*Parachlamydia* (10)	–	–	203/Died
7 (M)	36	22	No	3	*Parachlamydia* (3)	–	–	9/Survived
8 (M)	25	11	Yes	18	*Parachlamydia* (149)	–	–	255/Survived
9 (M)	27	24	No	2	*Parachlamydia/ Rhabdochlamydia* (18)	Enterocolitis (12)	–	90/Survived
10 (M)	30	14	Yes	9	*Rhabdochlamydia* (155)‡	–	–	255/Survived
11 (F)	28	27	No	26	*Rhabdochlamydia* (229)	–	–	435/Survived
12 (F)	27	22	Yes	45	*Rhabdochlamydia* (22)	Enterocolitis (12)	–	114/Died

*Sum of the 3 Apgar scores (at 1, 5, and 10 min after birth).
†Diagnosis ≤ or ≥10 days after positive sample.
‡Previous sample with negative PCR result (for the mentioned species).
§isolated from placenta.

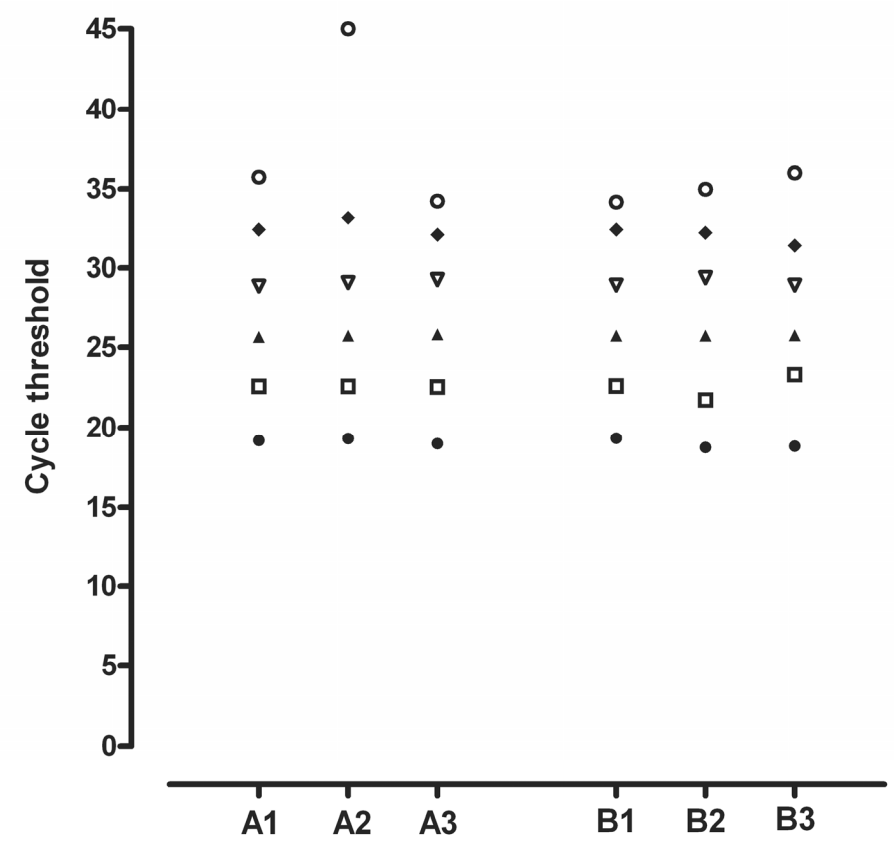

Figure. Intrarun and interrun reproductibilities of real-time PCR for *Rhabdochlamydia* spp. assessed by testing 10-fold dilutions (1–10^5 plasmid copy/μL) of plasmidic positive controls in 2 consecutive runs (A and B), each consisting of 3 duplicates. One duplicate at 10^5 plasmid/μL was negative.