

Novel *Chlamydia*-like Organisms as Cause of Bovine Abortions, UK

To the Editor: Despite the worldwide economic impact of, and welfare issues associated with, infectious bovine abortifacients, as well as potential zoonotic threats to human health, accurate diagnosis of the causes of abortion is uncommon (1). This poor diagnosis could be explained in part by the lack of identification of infectious abortifacient agents.

Although *Chlamydomydia abortus* is a known etiologic agent of ruminant abortion, several novel species of *Chlamydia*-like organisms have recently emerged as putative ruminant abortifacients. *Waddlia chondrophila* was isolated from the brain and nervous tissue of an aborted bovine fetus in Germany (2), and *Parachlamydia acanthamoebae* and other unidentified *Chlamydia*-like species were identified in 18.3% of bovine placenta samples in Switzerland (3,4). Given the paucity of information about the causes of infectious bovine abortion and the high prevalence of *Chlamydia*-like organisms in the samples from Switzerland, we attempted to determine whether such organisms can be detected in bovine fetal tissues in the United Kingdom.

Pooled tissue samples comprising brain, heart, and/or placenta (depending on availability) were obtained from bovine fetuses submitted for diagnosis to the Scottish Agricultural College Disease Surveillance Centre, Dumfries, Scotland, UK, during 2008. Tissue pools were homogenized by using a Precellys bead mill homogenizer (Bertin Technologies, Ann Arbor, MI, USA), and DNA was extracted by using the Wizard Genomic DNA Purification Kit (Promega, Southampton, UK) according to manufacturer's instructions. A pan-Chlamydiales PCR for the 16S rDNA sequence was per-

formed by using forward primer 16S FOR2 (5'-CGT GGA TGA GGC ATG CAA GTC GA-3') and reverse primer 16S REV2 (5'-CAA TCT CTC AAT CCG CCT AGA CGT CTT AG-3') to generate amplicons of \approx 260 bp (5). Negative-control reactions contained DNA-free water instead of extracted DNA. PCR products were purified (QIAquick PCR Purification Kit; QIAGEN, Crawley, UK) before direct sequencing by using the PCR primers and dideoxy chain termination/cycle sequencing on an ABI 3730XL DNA sequencer (MWG Operon, Ebersberg, Germany).

After the initial PCR, 22 (26.5%) of the 83 fetal samples tested were Chlamydiales positive. Serologic, bacteriologic, and histopathologic examination of fetal tissues identified no other infectious abortifacient agents in the Chlamydiales-positive samples. Sequence information was successfully obtained for 15 of these 22 samples with forward and reverse primers; sequences ranged from 140 bp to 194 bp (European Molecular Biology Laboratory/GenBank accession nos. GQ919016–GQ919030). These 15 short sequences were carefully aligned to a representative set of 22 similar Chlamydiales 16S rDNA

sequences, identified by a BLAST (www.ncbi.nlm.nih.gov/BLAST) similarity search of the European Molecular Biology Laboratory/GenBank database, plus alignment of an outgroup of 7 non-Chlamydiales sequences. A Bayesian phylogenetic tree (Markov Chain Monte Carlo settings: 2 runs of 625,000 generations; burn-in of 125,000 generations; trees sampled every 100 generations) was then estimated with a general time reversible + Γ nucleotide substitution model by using the MrBayes program (6) launched from the TOPALi v2 package (7).

Despite the short sequence length of the 15 samples, the tree was well resolved with the Chlamydiales sequences and formed 3 clusters (*Chlamydiaceae*, *Rhabdochlamydiaceae*/*Simkaniaceae*, and *Parachlamydiaceae*/*Waddliaceae*/*Criblamydiaceae*) (Figure). Two of these sequence clusters represented 10 and 5 of the samples, whereas no samples were represented in the cluster containing the *Chlamydiaceae*, which includes *C. abortus*. Most (10/15) sequences were found in the cluster containing the *Parachlamydiaceae*. This finding agrees with those of the aborted bovine placenta studies in Switzerland (3,4) and provides further evidence



Figure. Bayesian phylogenetic tree demonstrating the relationship of 15 isolated organisms from the older chlamydiales samples to known chlamydial species. *Cand.*, *Candidatus*; *R.*, *Rhabdochlamydia*; *P.*, *Protochlamydia*.

that *Parachlamydia*-like species may play a substantial role in bovine abortion in mainland Europe and the United Kingdom. Four of the remaining 5 samples clustered with members of the family *Rhabdochlamydiaceae*; the fifth sequence (CLBUK3), although present in the same *Rhabdochlamydiaceae/Simkaniaceae* cluster, appeared to be more distinct from other family members.

The identification of these organisms in such a large percentage of the bovine fetal tissue samples tested may indicate a role for these organisms in undiagnosed bovine abortions in the United Kingdom and Europe and may be a zoonotic source of infection for humans. Indeed, considerable evidence supports a role for *Parachlamydia* spp. in human pneumonia, whereas *Rhabdochlamydia* spp. is a suspected cause (8). In addition, evidence suggests that *P. acanthamoebae* crosses the human placenta to the unborn fetus (9). Also, the presence of both *parachlamydia* and *rhabdochlamydia* DNA in the lung secretions of hospitalized premature human neonates recently correlated with increased medical interventions and increased duration of hospital stay (10).

We demonstrate the presence of *Parachlamydiaceae* and *Rhabdochlamydiaceae* species in bovine abortions in the United Kingdom. Given the zoonotic potential and the economic and welfare impacts of bovine abortion on the agricultural sector, further studies are required to understand the incidence and pathogenic roles of these organisms in both humans and animals. These studies should include broader molecular epidemiologic studies, as well as detailed histologic/immunohistochemical investigations and organism recovery through culture of infected placental and fetal tissues.

Acknowledgment

We thank the Scottish Agricultural College Disease Surveillance Centre, Dumfries, for sample collection.

This work was funded by the Scottish Government Rural and Environment Research and Analysis Directorate and the Biological and Biotechnology Sciences Research Council.

Nicholas Wheelhouse, Frank Katzer, Frank Wright, and David Longbottom

Author affiliations: Moredun Research Institute, Edinburgh, Scotland, UK (N. Wheelhouse, F. Katzer, D. Longbottom); and Scottish Crop Research Institute, Dundee, Scotland, UK (F. Wright)

DOI: 10.3201/eid1608.091878

References

1. Cabell E. Bovine abortion: aetiology and investigations. In *Pract*. 2007;29:455–63.
2. Henning K, Schares G, Granzow H, Polster U, Hartmann M, Hotzel H, et al. *Neospora caninum* and *Waddlia chondrophila* strain 2032/99 in a septic stillborn calf. *Vet Microbiol*. 2002;85:285–92. DOI: 10.1016/S0378-1135(01)00510-7
3. Borel N, Ruhl S, Casson N, Kaiser C, Pospischil A, Greub G. *Parachlamydia* spp. and related *Chlamydia*-like organisms and bovine abortion. *Emerg Infect Dis*. 2007;13:1904–7.
4. Ruhl S, Casson N, Kaiser C, Thoma R, Pospischil A, Greub G, et al. Evidence for *Parachlamydia* in bovine abortion. *Vet Microbiol*. 2009;135:169–74. DOI: 10.1016/j.vetmic.2008.09.049
5. Ossewaarde JM, Meijer A. Molecular evidence for the existence of additional members of the order Chlamydiales. *Microbiology*. 1999;145:411–7. DOI: 10.1099/13500872-145-2-411
6. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 2003;19:1572–4. DOI: 10.1093/bioinformatics/btg180
7. Milne I, Lindner D, Bayer M, Husmeier D, McGuire G, Marshall DF, et al. TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics*. 2009;25:126–7. DOI: 10.1093/bioinformatics/btn575
8. Greub G. *Parachlamydia acanthamoebae*, an emerging agent of pneumonia. *Clin Microbiol Infect*. 2009;15:18–28. DOI: 10.1111/j.1469-0691.2008.02633.x
9. Baud D, Goy G, Gerber S, Vial Y, Hohlfeld P, Greub G. Evidence of maternal-fetal transmission of *Parachlamydia acanthamoebae*. *Emerg Infect Dis*. 2009;15:120–1. DOI: 10.3201/eid1501.080911
10. Lamoth F, Aeby S, Schneider A, Jatton-Ogay K, Vaudaux B, Greub G. *Parachlamydia* and *Rhabdochlamydia* in premature neonates. *Emerg Infect Dis*. 2009;15:2072–75. DOI: 10.3201/eid1512.090267

Address for correspondence: Nicholas Wheelhouse, Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Scotland EH26 0PZ, UK; email: nick.wheelhouse@moredun.ac.uk

West Nile Virus Knowledge among Hispanics, San Diego County, California, USA, 2006

To the Editor: West Nile virus (WNV), spread by infected mosquitoes, is a serious public health threat throughout the United States and can cause life-altering and even fatal disease (1). In San Diego County, California, the human infection rate was 0.18 per 100,000 persons during 2003–2006 (5 cases, 1 locally acquired) and then increased to 0.52 and 1.17 per 100,000 persons in 2007 and 2008, respectively, despite few changes in surveillance activities (2). Community-based mosquito control programs, adoption of personal protective behavior (PPB), and education are the most effective ways to prevent human WNV infection because no specific antiviral drug treatment or vaccine exists (1,3). Although WNV-associated illness has occurred in all racial and ethnic groups, Hispanics are potentially at risk because of language and cultural barriers to obtaining information regarding WNV prevention (4). San Diego County Department of