SHORT REPORT
Chlamydia gallinacea: a widespread emerging *Chlamydia* agent with zoonotic potential in backyard poultry

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SUMMARY

Chlamydia gallinacea, a new chlamydial agent, has been reported in four European countries as well as Argentina and China. Experimentally infected chickens with *C. gallinacea* in previous study showed no clinical signs but had significantly reduced gains in body weight (6·5–11·4%). Slaughterhouse workers exposed to infected chickens have developed atypical pneumonia, indicating *C. gallinacea* is likely a zoonotic agent. In this study, FRET-PCR confirmed that *C. gallinacea* was present in 12·4% (66/531) of oral–pharyngeal samples from Alabama backyard poultry. Phylogenetic comparisons based on *ompA* variable domain showed that 16 sequenced samples represented 14 biotypes. We report for the first time the presence of *C. gallinacea* in North America, and this warrants further research on the organism's pathogenicity, hosts, transmission, and zoonotic potential.

Key words: *Chlamydia gallinacea*, backyard poultry, FRET-PCR.

Chlamydia spp. are obligate intracellular bacterial pathogens that cause a number of important diseases in animals and humans. Novel members of the group have been discovered in the last few years [1–5] including *Chlamydia gallinacea*, which was found primarily in chickens, but was also shown to infect cattle [6–8]. Experimentally infected chickens exhibit no clinical disease symptoms but grow more slowly and have significantly reduced gains in body weight (6·5–11·4%) [7]. Importantly, French slaughterhouse workers exposed to *C. gallinacea*-infected chickens developed respiratory clinical symptoms, suggesting that *C. gallinacea* may also infect humans and may represent a zoonotic

threat [6]. The organism appears to be widely distributed and has been reported in four European countries as well as in Argentina and China [1,6–8]. To determine if this emerging agent might also be present in North America, we used PCR to determine if backyard poultry from Alabama is infected with *C. gallinacea*.

All samples used in this study were collected for the avian influenza and avian paramyxovirus type-1 surveillance program in avian species organized by the US Department of Agriculture (USDA). Sample collections followed the recommendation for collecting swab specimens from poultry for viral diagnostic testing (USDA SOP WI-AV-0020). In total, 1929 oral–pharyngeal swabs from apparently healthy poultry were collected into 531 tubes (1–5 swabs from the same bird and same owner/per tube) containing 3 ml brain–heart infusion (BHI) medium and transported

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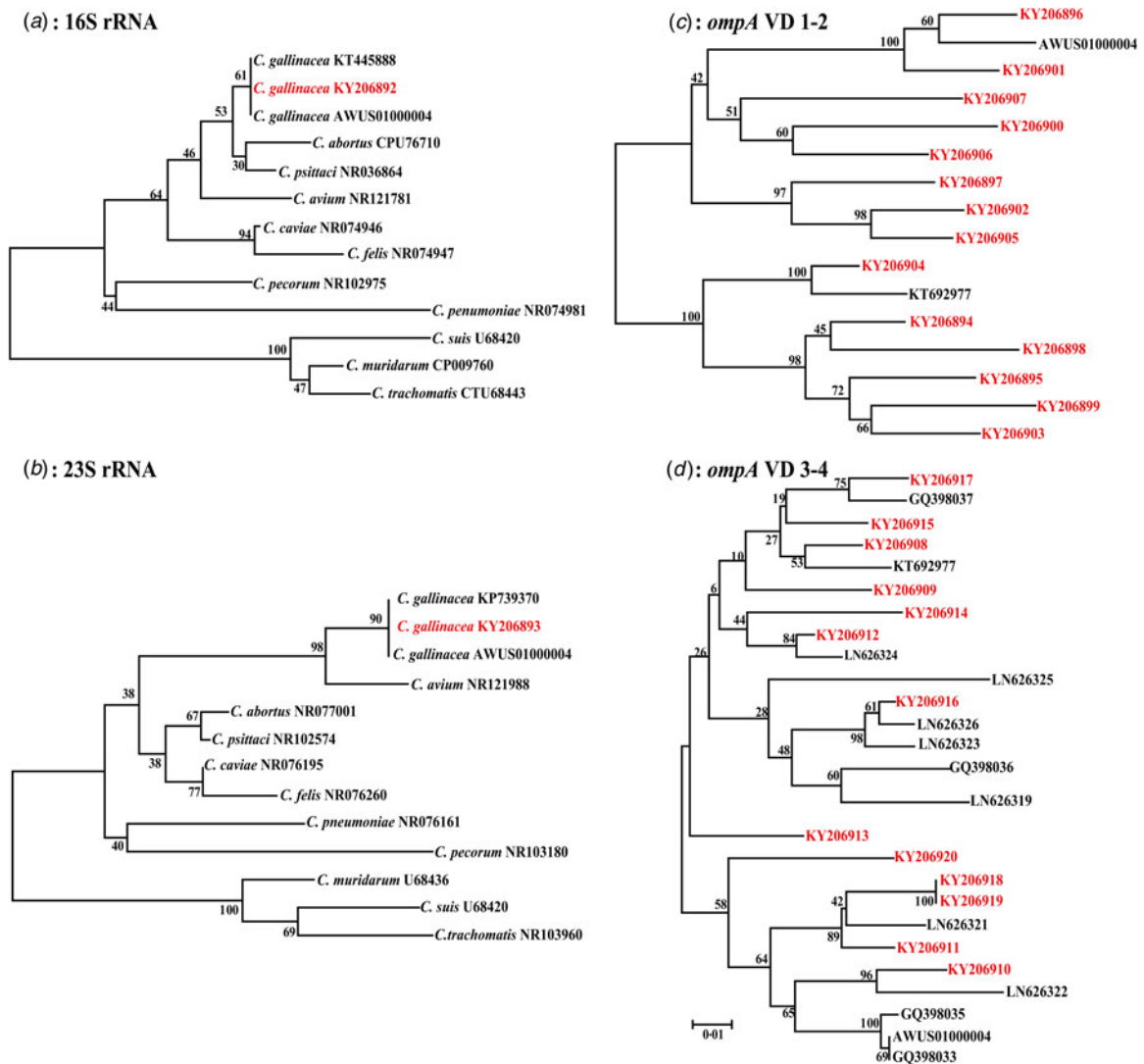(Email: wangche@auburn.edu)

Fig. 1. Phylogenetic comparison of *Chlamydia gallinacea* from backyard poultry in this study. The 16S rRNA (301 bp) and 23S rRNA (329 bp) gene sequences (in red font and accession number) found in the current study are compared with those of other representative *Chlamydia* spp. (in black font with GenBank accession number) using the Clustalx 1.83 alignment software. In addition, the *ompA* VD 1–2 (478 bp) and *ompA* VD 3–4 (311 bp) of *C. gallinacea* strains identified in in this study are compared with other *C. gallinacea* from poultry in European countries and China. Based on these alignments, phylogenetic trees were constructed by the neighbor-joining method using the Kimura 2-parameter model with MEGA 6.0. Branch lengths are measured in nucleotide substitutions and numbers show branching percentages in bootstrap replicates. Scale bar represents the percent sequence diversity.

on ice to the Alabama State Diagnostic Laboratory. For DNA extraction, BHI for each tube was thawed at room temperature and a 50 µl aliquot processed using the MagMax 96 Viral RNA isolation kit (ThermoFisher Scientific, Waltham, MA, USA). The extracted DNA was collected in 50 µl elution buffer and tested for *Chlamydia* spp. using a previously described *Chlamydia* spp. real-time FRET-PCR [7]. This PCR amplifies a 168-bp fragment of the 23S rRNA gene of *Chlamydia* spp. and can detect all 11 *Chlamydia* species with a detection sensitivity of a

single copy/reaction. High-resolution melting curve analysis enables differentiation of the *Chlamydia* spp. detected [7].

To further characterize the organisms present, we analyzed high-copy *C. gallinacea*-positive specimens by standard PCRs targeting a variable region of the 16S rRNA (301 bp) of the recognized 11 *Chlamydia* spp. and a highly variable domain (VD) of the 23S rRNA (329 bp) [8,9]. In addition, *C. gallinacea*-specific PCRs targeting the *ompA* VDs 1–2 (478 bp) and VD 3–4 (311 bp) [8] were used to investigate the

polymorphism of *C. gallinacea* in the backyard poultry studied (Fig. 1). All PCR products were verified by gel electrophoresis and automated DNA sequencing (Elim Biopharmaceuticals, Inc., Hayward, California, USA).

Altogether 12·4% (66/531) of the tubes containing the 1929 oral–pharyngeal samples that we collected from backyard poultry in Alabama were positive in the *Chlamydia* FRET-PCR. The positives comprised 64 of the 472 tubes containing swabs from chickens (13·6%) and two of the six tubes containing swabs from guinea fowl (33·3%). None of the tubes with swabs from turkeys, geese, ducks, quail, pheasants, peafowl, and pigeons were positive. The 16S rRNA and 23S rRNA gene sequences showed that only *C. gallinacea* was detected, and the strains found in this study were identical to those from Europe and China (Fig. 1*a* and *b*). Sixteen samples positive in the genus-specific *Chlamydia*-FRET-PCR with over 1000 copies of the 23S rRNA/reaction were further tested with standard PCRs targeting the *ompA* VD 1–2 and *ompA* 3–4 domains of *Chlamydia* spp. [7,8]. Phylogenetic comparisons showed that 16 sequenced samples represented 14 biotypes (Fig. 1*c* and *d*), and the sequences were deposited in GenBank (KY206892, KY206893, KY206894-KY206907, and KY206908-KY206920) (Fig. 1*a–d*). The value of phylogenetic comparisons based on partial gene sequences of *ompA* is limited. Research is in progress to isolate *C. gallinacea* from swabs of backyard poultry, which allows amplification of the whole *ompA* sequences for complete phylogenetic comparisons.

Avian chlamydiosis is an important infectious disease of domestic poultry as well as companion and wild birds that can be associated with acute, subacute, or chronic respiratory, digestive, or systemic signs. Although *C. psittaci* was considered to be the sole causative agent of avian chlamydiosis, recent studies indicated that prevalence of *C. gallinacea* may be higher than that of *C. psittaci* [7,10]. A survey conducted over 1 year in a French abattoir demonstrated 80·0% of the *Chlamydiaceae*-positive samples contained *C. gallinacea* [6]. Our study in the USA adds to the growing evidence that *C. gallinacea* is a common and widespread chlamydial agent in poultry.

In conclusion, we report for the first time the presence of *C. gallinacea* in North America. The findings from our study should alert the poultry industry in North America to the possibility of a newly emerging pathogen, *C. gallinacea*, in their flocks. Further studies are indicated to more definitively establish the organism's pathogenicity, hosts, transmission, and zoonotic potential. Such studies will be aided by *Chlamydia* species-specific peptide ELISAs that have recently become available [11].

DECLARATION OF INTEREST

The authors declare that no competing financial interests exist.

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