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Chlamydia buteonis, a new *Chlamydia* species isolated from a red-shouldered hawk

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ABSTRACT

Chlamydiaceae are obligate intracellular bacterial pathogens for humans and animals. A recent study highlighted that a *Chlamydiaceae* intermediary between *C. psittaci* and *C. abortus* can infect hawks. Here, an isolate was obtained upon passage of cloacal and conjunctival sac material collected from a female hatch-year red-shouldered hawk (*Buteo lineatus*) in cultured cells. The diseased bird, one of 12 birds housed in a rehabilitation center, developed conjunctivitis and later died. Swabs from both sites tested positive for *Chlamydia* using the QuickVue Chlamydia test. The isolate, named RSHA, tested negative in qPCR assays specific for *C. psittaci* and *C. abortus*, respectively. Analysis of the 16S rRNA, 23S rRNA and whole genome sequences as well as MLST, ANIb and TETRA values reveal that *C. psittaci* and *C. abortus* are the closest relatives of RSHA. However, the overall results strongly suggest a phylogenetic intermediate position between these two species. Therefore, we propose the introduction of a new species designated *Chlamydia buteonis* with RSHA^T as the type strain.

Introduction

The family *Chlamydiaceae* currently comprises 11 validly published species that are pathogenic to humans and/or animals: *Chlamydia* (*C.*) *abortus*, *C. avium*, *C. caviae*, *C. felis*, *C. gallinacea*, *C. muridarum*, *C. pecorum*, *C. pneumoniae*, *C. psittaci*, *C. suis*, and *C. trachomatis* [25].

Avian chlamydiosis (ornithosis) and psittacosis, its zoonotically transmitted counterpart in humans, are well-documented diseases caused by the obligate intracellular bacterium *C. psittaci* [26]. It has been reported that more than 467 avian species can be affected by chlamydial infection [13]. In birds, the infection is usually systemic and can be fatal. The severity of clinical signs varies and depends on the species and age of the bird, as well as on the causative *C. psittaci* strain [2]. Genotypes of *C. psittaci* are based on sequences of the gene encoding the major outer membrane protein OmpA and have been tentatively associated with specific hosts, such as ducks, pigeons, psittacines and turkeys [18,27,36]. In the past decade, the broad use of DNA-based detection tools has revealed the occurrence of chlamydiae other than *C. psittaci* in birds. For example, *C. trachomatis*, *C. abortus* and *C. pecorum* along with non-classified chlamydial organisms have been detected in pigeons [23]. In the past few years, whole-genome sequencing (WGS) analysis of formerly non-classified strains has led to the identification of two new species: *C. avium* and *C. gallinacea* [10,24]. Atypical strains from wild ibises (*Threskiornis aethiopicus*) were assigned to Candidatus *C. ibidis* [37]. More recently, a group of *C. abortus* strains that are distinct from ruminant strains were isolated from wild swans and ducks [32]. Overall, this suggests that the spectrum of chlamydial lineages occurring in wild birds is wider than current knowledge indicates.

Avian chlamydiosis has been repeatedly diagnosed in raptors, including red-tailed hawks, Swainson's hawks and ferruginous hawks [4,11,16,28]. Detailed whole-genome sequence (WGS) characterization of strain RTH, isolated from a red-tailed hawk, putatively identified a new species separate from other members of the *Chlamydiaceae*, and with *C. psittaci* as its closest relative [12,21].

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Here, we describe the isolation and molecular characterization of a new chlamydial strain from a red-shouldered hawk (*Buteo lineatus*).

Materials and methods

Case description

In August 2016, a female hatch-year red-shouldered hawk (id 16-066) developed conjunctivitis and died within a week. It was the second fatal case among a group of 12 hawks kept in a flight room. The animal was in a good nutritional state based on significant internal adipose tissue stores. At necropsy, a thick beige exudate covered the conjunctival sac of one eye. The liver was slightly swollen and discolored brown. One small beige nodule, approximately 1 mm in diameter, was present in one lung lobe and the spleen was moderately enlarged. Histopathological analysis revealed ulcerations on conjunctival tissues with numerous coccobacilli and non-specific mononuclear hepatitis and splenitis.

Enterococcus faecalis and *Pseudomonas aeruginosa* were isolated from conjunctival tissue. Following a positive test obtained for a swab collected from the diseased bird using a rapid Chlamydia test, a duplicate swab was used to inoculate Vero cells and a suspected *Chlamydia* strain, designated RSHA, was isolated.

Sampling, first diagnosis and isolation procedure

Conjunctival tissue and cloaca were swabbed in duplicate from the dead bird. One swab was tested with the QuickVue Chlamydia test (Quidel) whereas the second one was inoculated onto fresh monolayers of Vero cells, with four dilutions of the inoculum tested. After inoculation, vials were centrifuged at 2000 rpm for 1 h at 30 °C. Monolayers were then washed three times with the Chlamydia Hank's solution and 1 mL of fresh medium was added to each vial. The vials were then incubated at 37 °C for 7 days and examined daily. Each dilution was passed once after 7 days of inoculation. Infected monolayers on coverslips were then fixed with acetone, and monolayers were stained with the 601-RDV *C. psittaci* fluorescent antibody conjugate (National Veterinary Service Laboratories, USA). When inclusions of typical chlamydial morphology were observed as bright apple-green spots, the sample was considered positive. The isolate was kept frozen at −80 °C until further processing.

DNA-based characterization

Species-specific real-time PCR

PCR assays targeting *C. psittaci*, *C. abortus*, *C. avium* and *C. gallinacea* species were conducted as previously described [17,19,38,39].

Multi locus sequence typing (MLST) analysis

Genotyping by MLST was carried out according to the scheme developed by Pannekoek et al. [18] targeting seven housekeeping genes: *gat*A, *opp*A, *hfl*X, *gid*A, *eno*A, *hem*N and *fum*C. Target genes were amplified and sequenced using primers and conditions described on the Chlamydiales MLST web-site (http://pubmlst.org/chlamydiales/). Sequencing of both strands was done by Eurofins (Germany). Sequences for each locus were queried against the online Chlamydiales MLST Databases to determine allelic designations and a subsequent allelic profile was used to determine the sequence type (ST). New allele sequences are accessible via the Chlamydiales MLST web-site.

Preparation of genomic DNA for WGS

Genomic DNA was prepared from 14 mL of a BGM cell culture infected with the RSHA isolate. The suspension was centrifuged at 20,000 × *g* at 8 °C for 45 min, and the supernatant was discarded. The pellet was resuspended in 40 mL of PBS buffer and centrifuged at 500 × *g* for 15 min to remove cell debris. The supernatant was transferred to a fresh tube and high-speed centrifuged as above. The pellet was resuspended in 200 μL of PBS. Prior to DNA extraction, a DNAse I (NEB, France) treatment was performed to remove potential contaminating DNA from cells. Genomic DNA was extracted using the Qiamp DNA minikit (Qiagen, France), with an additional RNase A (Roche) treatment, following the cell protocol. DNA was eluted in 50 μL of water and re-purified using the NEBNext Microbiome DNA enrichment kit (NEB, France).

Genome sequencing, assembly and draft annotation

Libraries were prepared using the Nextera XT kit (Illumina). Whole-genome sequencing was performed using an Illumina MiSeq platform (Illumina) according to the manufacturer's instructions. The MiSeq run was carried out on the DNA preparation, with paired-end reads of 150 bp using MiSeq V2 reagents. The raw reads were trimmed with a quality value of 20 and a window size of 5 using Trimmomatic [5] and assembled de novo using SPAdes v3.11.1 with the careful parameter and the automatic determination of the k-mer values [3]. To remove residual mammalian sequences from cell culture constituents, scaffolds from SPAdes were subsequently run on the nucleotide database of NCBI. The draft genome was evaluated using Quast v4.6.3 [8] and annotated using Prokka v1.13 [29]. The genome sequence of strain RSHA has been deposited at the European Nucleotide Archive (ENA) under accession number PRJEB30277.

Phylogenetic and genome analysis

The parameters of the tetranucleotide signature frequency correlation coefficient (tetranucleotide regression) and average nucleotide identity (ANIb) were determined as described previously [22] using the program JSpecies v1.2.1 with default parameters.

Nine phylogenetical markers previously described [20] were extracted from 12 *Chlamydiaceae* reference sequences and one outgroup. Amino acid sequences were aligned with mafft v7 [14] and were concatenated to build a reference phylogeny using RAxML v8 [30], with the LG + G + I model and 1000 bootstrap replicates. Pairwise amino acid sequence identities were calculated based on the mafft alignment by Geneious v10 (www.geneious.com).

Electron microscopy

BGM cells were infected with strain RSHA. Infected cells were harvested at 24, 48, 72 and 96 h post-infection. After washing, cells were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde, scrapped off the culture plate and enrobed in gelatin. Gelatin containing infected cells was trimmed into $\tilde{1}$ mm^3 cubes, post-fixed with 1% osmium tetroxide, washed and en-bloc stained with 1% uranyl acetate in water. Specimens were then washed and dehydrated using 30%, 50%, 70%, 90% and 100% ethanol in series, 10 min each. This was followed by two more 100% ethanol washes and infiltration with increasing concentrations of Spurr resin (Electron Microscopy Sciences, PA). After two exchanges of pure resin, specimens were embedded in Spurr resin and polymerized at 60 °C overnight. Silver colored ultrathin ($\tilde{7}$0 nm) sections were cut and collected using a Leica UC6 ultramicrotome (Leica Microsystems, Inc., Bannockburn, IL), counterstained with uranyl acetate and lead, and examined in a transmission electron microscope (Tecnai T12, FEI) operated at 80 kV. Digital images were acquired using an

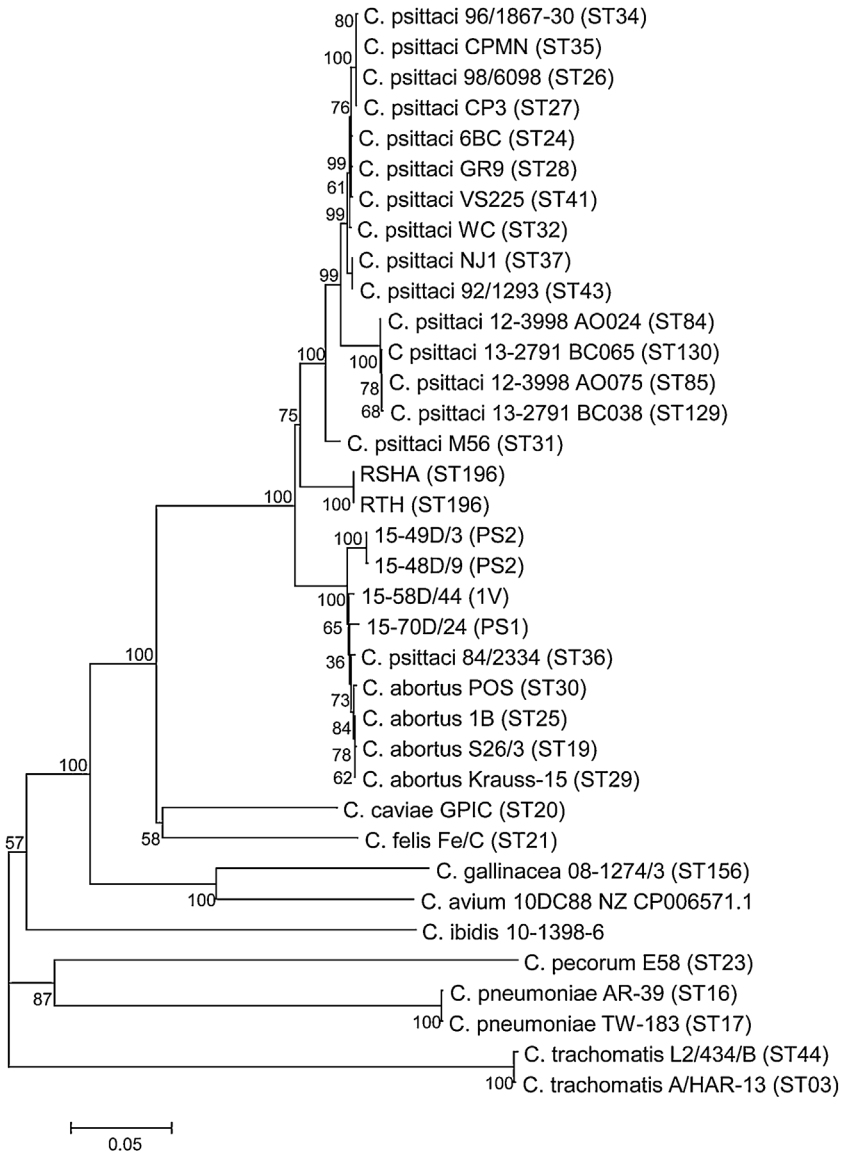

Fig. 1. Phylogenetic analyses of concatenated sequences of 7 MLST housekeeping gene fragments (*eno*A, *fum*C, *gat*A, *gid*A, *hem*N, *hlf*X, and *opp*A) for the RSHA isolate and established *Chlamydiaceae* species, including a larger number of representatives for *C. psittaci* and *C. abortus* species.

AMT bottom mount CCD camera and AMT600 software (Advanced Microscopy Techniques, MA).

Real-time PCR for detection of the new species

Specific primers RSHA-F (5′-ATTTCCAACACGCACTGCAT-3′) and RSHA-R (5′-TGGGACTAGGTGTTTCTCCCT-3′) as well as a specific probe RSHA-P (5′-FAM-GGACAACATGCCTAGATGAAGA-TAMRA 3′) were designed using the primer3 software and synthesised by Eurofins MWG Operon. Amplification was performed using the 2X TaqMan Universal PCR Master Mix (Applied Biosystems, Courtaboeuf, France). The final volume of the reaction mixture was 20 μL including 10 μL of master mix, 2 μL of DNA sample, 0.6 μM of each primer, 0.1 μM of the probe and sterile PCR water. Amplification was carried out at a Viia7 thermocycler (Applied Biosystems) using the following cycling parameters: heating at 95 °C for 10 min, 50 cycles of 95 °C for 15 s and 56 °C for 1 min. For all PCR reactions, DNA of the isolate RSHA, arbitrarily defined as the reference strain for *C. buteonis*, was used as a positive control, while sterile water was used as negative control. The specificity of the new real-time PCR was evaluated on 32 *Chlamydiaceae* DNA samples (Table S1), diluted to a Ct value around 30 when tested with the 23S rRNA-based *Chlamydiaceae*-specific real-time PCR (*Chlamydiaceae* 23S-rtPCR) [7].

Results

Molecular characterization of the RSHA isolate

DNA from the RSHA isolate tested positive using the *C. psittaci inc*A-based real-time PCR assay, but negative using *C. psittaci omp*A-based real-time PCR. The same result was obtained with primers targeting *ompA* of *C. abortus* and with species-specific *C. avium* and *C. gallinacea* real-time PCR systems.

MLST genes were successfully amplified using primers designed for *C. psittaci* [18]. Nucleotide sequences were obtained for all 7 MLST genes of the RSHA isolate, and their analysis revealed a new sequence type (sequence type number 196), which was not represented in the *Chlamydiales* database (http://pubmlst.org/chlamydiales/). Comparative phylogenetic analysis of the concatenated MLST sequences from RHSA and the corresponding MLST sequences of the RTH strain [12] places the RSHA isolate on the

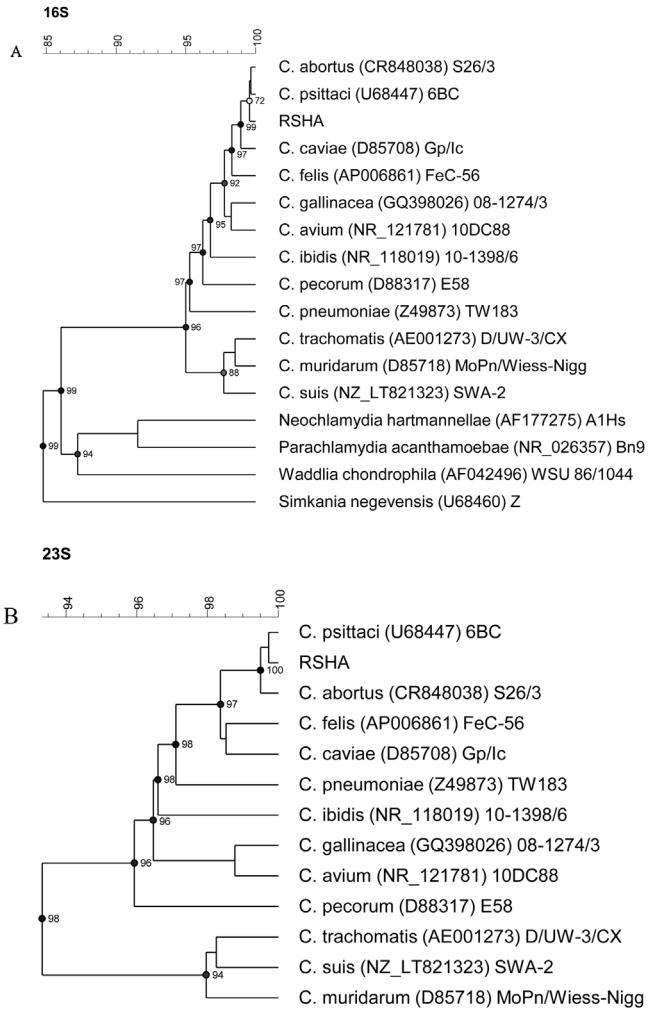

Fig. 2. Dendrograms based on the analysis of the complete 16S rRNA (A) and 23S rRNA (B) sequences from the RSHA isolate and from the type strains of twelve *Chlamydiaceae*, including the Candidatus *C. ibidis* representative. Dendrograms were constructed by UPGMA method from a similarity matrix calculated by pairwise alignment. Branch quality was calculated by cophenetic correlation. Horizontal distances correspond to genetic distances expressed in percentage of sequence similarity.

same clade as the RTH isolate, i.e. at an intermediate phylogenetic position between *C. abortus* and *C. psittaci* (Fig. 1).

In order to ascertain the species identity of the RSHA isolate, its WGS was obtained resulting in two scaffolds with a length of 770,995 and 375,551 bases and an average coverage of 117× and 106×, respectively. The full plasmid sequence of pRSHA was identified with a length of 7681 bases and an average coverage of 82×. The 16S and 23S rRNA gene sequences were extracted and then aligned with representative sequences of all established *Chlamydiaceae* spp. (Fig. 2 and Supplementary Fig. S1). Sequence homology was greater than 95.17% for 16S and greater than 93.08% for 23S rRNA genes, thus confirming the taxonomic position of strain RSHA within the genus *Chlamydia* and its close relatedness to both *C. psittaci* and *C. abortus* (Table S2). While the 23S rRNA sequence could not be extracted from the RTH genome sequence (SRA061571), comparison of the 16S rRNA sequences of the RTH and RHSA isolate revealed a single point mutation difference.

Basic genomic features of the RSHA isolate were then compared with those of established species of the *Chlamydiaceae*. The RSHA genome size is 1.14 Mbp, with 977 CDS identified and GC% 38.3. These numbers are in the same range as those for the other chlamydial species (Table 1). A plasmid similar to that of *C. psittaci* M56 was also identified in RSHA.

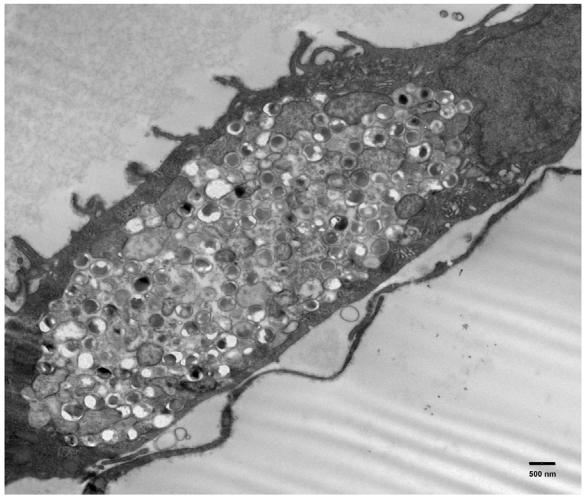

Fig. 3. Electron microscopic images of BGM cell culture infected with RSHA, after 72 h. One inclusion is depicted with a mixture of RBs and EBs.

Pairwise comparisons of the RSHA genome sequence with genomic sequences of the type strains of RHSA's closest relatives were conducted to determine average nucleotide identity (ANIb) and tetranucleotide regressions. The results in Table 2 show maximal values of 0.98725 (tetranucleotide regression) and 93.67% (ANIb) between RSHA and type strain 6BC of *C. psittaci*. A similar ANI value (93.96%) was previously reported for the RTH isolate [12].

Using a classification scheme recently proposed for the order *Chlamydiales* [20], the percentage of sequence identity was calculated for nine conserved taxonomically informative gene products (RpoN, FtsK, PepF, Adk, HemL, DnA, SucA, Hyp325 and Fabl) extracted from the RHSA genome sequence and those of the other 11 *Chlamydia* species. Although results presented in Fig. S2 reveal *C. psittaci* as the most closely related species, all values obtained for RSHA are below the accepted cut-off for species assignment.

Phenotypic characterization of the strain RSHA

RSHA can be propagated under conditions routinely used for culture of chlamydiae, such as in BGM cells or embryonated chicken eggs (not shown). The electron micrograph in Fig. 3 displays a large inclusion in an infected BGM cell with reticulate bodies (RBs), elementary bodies (EBs) and intermediate bodies (IBs). EBs were small (240–330 nm), round and electron dense. RBs appeared larger (450–1100 nm in diameter), round to oval and more electron lucent. Different stages of division by binary fission were seen in RBs. These observations support a conserved biphasic developmental cycle in RHSA as seen in all chlamydiae.

Diagnostic tools to detect the new taxon

A specific real-time PCR assay for RSHA was designed based on the *oxa*A gene locus (see Materials and methods). This gene has the particularity to be present in the genome of all *Chlamydiacea* with specific mutations identified for the hawk strain. RHSA, *C. abortus*, *C. avium*, *C. caviae*, *C. gallinacea*, *C. felis*, *C. pecorum* and *C. psittaci* strains were tested using this protocol. The results confirmed the specificity of the assay (data not shown).

Measurements on dilution series of cell culture aliquots with defined numbers of inclusion-forming units (IFUs) indicated detection limits in the range of 10 IFUs. As the target *oxa*A sequence of

Table 1
Comparison of basic genomic features of RSHA with those of established species of *Chlamydiaceae*.

	RSHA	*C. psittaci* 6BC	*C. abortus* S26/3	*C. caviae* GPIC	*C. felis* Fe/C-56	*C. avium* 10DC88	*C. gallinacea* 08-1274/3	*C. ibidis* 10-1398/6	*C. pecorum* E58	*C. pneumoniae* TW-183	*C. muridarum* MoPn/Weiss-Nigg	*C. trachomatis* D/UW-3/CX	*C. suis* SWA-2
length	1,146,546	1,171,667	1,144,377	1,173,390	1,166,239	1,041,170	1,059,583	1,146,066	1,106,197	1,225,935	1,072,950	1,042,519	1,075,789
G + C mol-%	38.29	39.1	39.9	39.2	39.4	36.9	37.86	38.3	41.1	40.6	40.3	41.3	42.06
Genes (incl. pseudogenes)	977	1034	1028	1030	1024	956	962	963	985	1120	952	935	927
5S rRNA	1	1	1	1	1	1	2	1	1	1	2	2	2
16S rRNA	1	1	1	1	1	1	1	1	1	1	2	2	2
23S rRNA	1	1	1	1	1	1	1	1	1	1	2	2	2
tRNA	38	38	38	38	38	39	39	38	38	38	37	37	37
tmRNA	1	1	1	1	1	1	1	1	1	1	1	1	1
Plasmid	1	1	0	1	1	1	1	1	0	0	1	0	1

Table 2
Pairwise comparison of the available genome sequences of the type strains of *Chlamydiaceae* species based on average nucleotide identity (ANIb; lower triangle) and tetranucleotide signature correlation index (upper triangle).

	RSHA	*C. psittaci* 6BC	*C. abortus* S26/3	*C. caviae* Gp/Ic	*C. felis* Fe/Pn-1	*C. avium* 10DC88	*C. gallinacea* 08-1274/3	*C. ibidis* 10-1398/6	*C. pecorum* E58	*C. pneumoniae* TW183	*C. muridarum* MoPn/Wiess-Nigg	*C. trachomatis* D/UW-3/CX	*C. suis* SWA-2
RSHA		0.9872	0.9663	0.9658	0.9702	0.9396	0.9477	0.9073	0.8567	0.8696	0.8550	0.8315	0.8295
C. psittaci 6BC	93.67		0.9860	0.9698	0.9665	0.9235	0.9413	0.8865	0.8831	0.8961	0.8546	0.8428	0.8332
C. abortus S26/3	92.47	92.58		0.9565	0.9567	0.8983	0.9233	0.8565	0.8832	0.9039	0.8358	0.8347	0.8213
C. caviae Gp/Ic	81.70	81.26	80.26		0.9510	0.9007	0.9114	0.8488	0.8921	0.9114	0.8441	0.8341	0.8198
C. felis Fe/Pn-1	81.20	80.84	79.82	82.00		0.9276	0.9475	0.8836	0.8739	0.8769	0.8433	0.8303	0.8365
C. avium 10DC88	73.80	73.86	73.57	74.04	73.63		0.9864	0.9329	0.8114	0.8094	0.8060	0.7658	0.7786
C. gallinacea 08-1274/3	73.24	73.34	73.33	73.71	73.19	81.09		0.9258	0.8332	0.8325	0.8217	0.7885	0.8001
C. ibidis 10-1398/6	70.92	70.87	70.55	70.81	70.64	69.93	69.74		0.7293	0.7106	0.8077	0.7698	0.7728
C. pecorum E58	68.89	68.84	68.75	69.00	68.86	68.49	68.34	67.81		0.9022	0.7761	0.7899	0.7761
C. pneumoniae TW183	69.78	69.57	69.50	69.85	69.67	68.97	68.93	68.40	69.32		0.7969	0.7883	0.7855
C. muridarum MoPn/Wiess-Nigg	68.30	68.60	68.58	68.72	68.46	68.30	68.26	67.80	67.13	67.57		0.9722	0.9770
C. trachomatis D/UW-3/CX	68.54	68.37	68.29	68.47	68.23	68.01	68.01	68.07	67.26	67.54	79.95		0.9718
C. suis SWA-2	68.02	68.36	68.38	68.22	68.21	67.80	67.93	67.68	67.06	67.35	81.53	78.84	

strain RTH is identical to that of strain RSHA, it is safe to assume that it would be detected by this assay as well.

Discussion

The present report of a new taxon underpins the widely held notion among chlamydia researchers that wildlife, and particularly wild birds, harbor yet unknown or non-classified chlamydial organisms [6,40].

In the past decade, advances in DNA-based diagnostic technology have enabled the discovery of new members of the family *Chlamydiaceae*, including *C. avium* and *C. gallinacea* [24], as well as *Candidati C. ibidis* [20,37], *C. sanzinia* [33], *C. corallus* [34], *C. serpentis*, and *C. poikilothermis* [31].

In this study, an isolate, named RSHA, obtained from a dead red-shouldered hawk (*Buteo lineatus*) was not detected using *omp*A-based qPCR assays for *C. psittaci* and *C. abortus*, but was detected using an *inc*A-based assay originally developed for *C. psittaci*, which was already known to identify certain *C. abortus* strains (unpublished data). Preliminary genotyping of this strain based on 7 MLST target genes identified a new sequence type located at an intermediate position between *C. psittaci* and *C. abortus* (Fig. 1). Intermediate strains from birds have been previously reported [1,9,35]. Recently, some of these strains were assigned to the species of *C. abortus* [32] based on 16S rRNA, *omp*A, and MLST analyses.

Analysis of WGS data of the RSHA isolate revealed that the criteria for assignment to a new chlamydial species within the family *Chlamydiaceae* were fulfilled. Indeed, according to the recent classification scheme proposed by Pillonel et al. [20], sequence identity values of ≥80% for 16S rRNA and 23S rRNA, and of ≥92.5% for 16S rRNA, ≥91% for 23S rRNA confirm the assignment of strain RHSA to the order *Chlamydiales* and family *Chlamydiaceae*, respectively. Furthermore, comparative analysis of nine taxonomically conserved proteins including a representative strain from each member of the *Chlamydia* genus yields sequence identities (RpoN < 96%, FtsK < 98%, PepF < 96%, Adk < 95%, HemL < 95%, DnA < 70%, SucA < 64%, Hyp325 ≥ 57% and Fabl < 78%) that are consistent with RSHA being assigned to a new species with *C. psittaci* as closest neighbor. A similar analysis was previously applied *a posteriori* to *C. avium* and *Candidatus C. ibidis* confirming their assignment to new species [20]. Recently, the same method was used for the description of *Candidati C. serpentis* and *C. poikilothermis* [31]. The tetra nucleotide regression and ANIb values calculated for RSHA are also below values generally considered for delineating bacterial species [22].

In the 1990s, the *C. psittaci*-like strain RTH was isolated from a wild red-tailed hawk (*Buteo jamaicensis*) showing signs of respiratory distress and diarrhea [16]. The genome of this strain was recently sequenced and further analysis suggested that RTH represents a new species in the family *Chlamydiaceae* [12]. Although the 23S rRNA sequence could not be extracted from the RTH genome sequence owing to incomplete assembly and low coverage, comparison of the RTH and RSHA 16S rRNA and MLST sequences revealed complete sequence identity, with the exception of a single nucleotide polymorphism in the 16S rRNA gene sequence, thus strongly suggesting a close evolutionary relationship between these two strains. Unfortunately, none of the nine taxonomically conserved protein sequences could be fully extracted from the published RTH genome sequence data (SRA061571). However, comparison of the incompletely assembled RTH sequences with those of RSHA revealed a strong percentage of identity between these two strains (around 99%; not shown).

In consequence, we propose classifying the two hawk strains as members of a new species to be designated as *Chlamydia buteonis* sp. nov., with strain RSHA^T as the type strain.

The epidemiological and clinical importance of the proposed new species has yet to be determined. Respiratory distress and diarrhea were reported in the red-tailed hawk (*Buteo jamaicensis*) infected by the RTH isolate [16], while conjunctivitis was observed in the red-shouldered hawk (*Buteo lineatus*) sampled in this study. Few reports have focused on clinical signs associated with chlamydial infections in predatory birds. However, lethargy, weight loss, moderate to severe leukocytosis, and radiographic evidence of splenomegaly were recently reported in a wild bird population [15]. Therefore, future studies of clinical cases in these birds should take the present findings into account. *C. psittaci*-like strains were recently detected in red-tailed and Swainson's hawks from California, with a best sequence alignment of the *omp*A gene, with the *C. psittaci* M56 genotype. It will be important to re-analyze these strains with the new PCR assay specific for *C. buteonis*. Moreover, the assay we have developed for *C. buteonis* should be applied to identify potential pathogens when diseased raptors belonging to the genus *Buteo* are sampled.

Description of Chlamydia buteonis *sp. nov*

Chlamydia buteonis (bu.te.o'nis. L. gen. n. buteonis of a buzzard, of a bird of the genus Buteo).

C. buteonis strains occur in raptor birds called buzzards in Europe and hawks in North America. The agent can be recovered from tissue of the conjunctiva and contents of the cloaca. From the cases seen so far, it seems likely that *C. buteonis* can cause conjunctivitis and/or respiratory signs, or at least be a contributing factor to these clinical manifestations.

Natural routes of transmission have yet to be investigated, but, analogous to most other chlamydiae, airborne transmission through aerosolized dry feces and dust particles seems possible. The potential for zoonotic infection of humans is unknown.

C. buteonis strains can be grown in cell culture or embryonated chicken eggs. Their growth is characterized by a biphasic developmental cycle, which is encountered in the other *Chlamydia* spp. as well. Electron microscopy is the method of choice to visualize the typical vacuole-like intracellular inclusions harboring the various morphological forms, i.e. elementary, reticulate and intermediate bodies.

Members of the species can be specifically identified using real-time PCR targeting the *oxaA* gene locus as described in Materials and methods. In addition, MLST based on 7 housekeeping genes and 16S rRNA gene sequence alignments can be used for identification. Sequence homology of *C. buteonis* 16S genes to those of other *Chlamydia* spp. is greater than 95.17%, with *C. psittaci* and *C. abortus* being the closest relatives. The size of the circular *C. buteonis* genome is 1.14 Mbp, with a G + C value of 38.3 mol-%. The type strain was found to harbor a plasmid sized 7681 bp and similar to that of *C. psittaci* M56. Strain RSHA^T isolated from a red-shouldered hawk (*Buteo lineatus)* is the designated type strain of *C. buteonis.*

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2019.06.002.

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