

Molecular detection of chlamydiales in captive zoo animals at Songkhla Zoo, southern Thailand

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Abstract

Several species of *Chlamydia* are recognized as significant zoonotic pathogens that infect a wide range of hosts, including humans. This study aimed to investigate the occurrence and genetic characteristics of Chlamydiales-related bacteria in captive animals at Songkhla Zoo, southern Thailand. A total of 92 cloacal and rectal swab samples were collected from mammals (n = 22), birds (n = 55), and reptiles (n = 15). Genomic DNA was screened using polymerase chain reaction (PCR) targeting the Chlamydiales 16S rRNA gene and the ompA gene specific for *Chlamydia psittaci*. Positive amplicons were subjected to Sanger sequencing and phylogenetic analysis. A single sample obtained from an asymptomatic giant Asian pond turtle (*Heosemys grandis*) tested positive for the Chlamydiales 16S rRNA gene, resulting in a detection rate of 1.09% (95% CI: 0.03–5.91%). Sequence analysis revealed 100% nucleotide identity to the previously reported Chlamydiales bacterium AY845420.1 and divergence from recognized pathogenic species. This study provides preliminary molecular evidence of Chlamydiales-related DNA in a captive giant Asian pond turtle in Thailand and supports the need for continued surveillance in zoological collections.

Keywords: chlamydiales, polymerase chain reaction, reptiles, zoo animals

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Received: April 1, 2026

Accepted: July 2, 2026

<https://doi.org/10.56808/2985-1130.4052>

Introduction

Chlamydia is a genus of obligate intracellular Gram-negative bacteria belonging to the family Chlamydiaceae and is responsible for both acute and chronic diseases in a wide range of animals, including humans (Elwell *et al.*, 2016; Borel *et al.*, 2018; Staub *et al.*, 2018). It is particularly important in avian and reptilian species, where infections can result in substantial economic losses.

Several *Chlamydia* species are of zoonotic importance. *Chlamydia pneumoniae* and *C. psittaci* are associated with respiratory infections in mammals, birds, and reptiles, whereas *C. abortus* is linked to abortion in humans and other mammals. However, there is currently no evidence of animal-to-human transmission of *C. pneumoniae* (Myers *et al.*, 2009; Gautam and Krawiec, 2023).

Chlamydia outbreaks have been reported worldwide, causing high morbidity and mortality in animals, including cases documented in zoos in Taiwan, the United States, Australia, and Japan. A PCR-based study conducted in Japanese zoos detected *Chlamydia* spp. in mammals, birds, and reptiles and found that reptiles had the highest infection rates. The detected species included *C. psittaci* and *C. pneumoniae*, the latter showing close genetic similarity to strains isolated from humans (Kabeya *et al.*, 2015).

In Thailand, surveillance studies have reported the presence of *C. psittaci* in 1.3% of pigeon feces (Wannaratana *et al.*, 2017), 7.87% of captive psittacine birds (Suksai *et al.*, 2016), and 4.9% of wild birds, including Asian openbill storks (Suksai *et al.*, 2019). In addition, a study at Chiang Mai Zoo detected Chlamydial infections in 9.57% of examined reptiles (Boonsri *et al.*, 2022).

Despite the potential risks to animal and public health, information on *Chlamydia* in zoo animals in Thailand remains limited. This lack of data hinders the development of effective prevention and control measures. Therefore, this study investigated the prevalence and genetic diversity of *Chlamydia* in captive animals at Songkhla Zoo, with a particular

focus on their zoonotic potential and the risk of interspecies transmission.

Materials and Methods

Animal ethics: This study has been reviewed and approved by Institutional Animal Care and Use Committee of Rajamangala University of Technology Srivijaya, based on the Ethic of Animal Experimentation of National Research Council of Thailand (IAC 05-04-2024).

Sample collection: Based on estimated population proportions, 92 of 1,097 zoo animals, including mammals (n = 22), birds (n = 55), and reptiles (n = 15), were sampled during routine health screenings from December 1, 2023, to May 31, 2024. To minimize fecal contamination, the cloacal or rectal opening was wiped 1–2 times beforehand. Cloacal or rectal mucosal surfaces were then firmly scraped 8–10 times using sterile plastic-shaft swabs. Each swab tip was placed in a 1.5 mL microcentrifuge tube, stored at 4°C, and processed within 24 h, following the instructions on the DNA extraction kit.

DNA extraction and PCR amplification: Total DNA was extracted from a 200 µL sample using the Presto™ Buccal Swab DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions. Molecular screening targeted the *Chlamydia* 16S rRNA and *C. psittaci* ompA genes using 2X PCR SuperMix (Bio-Helix Co., Ltd., Taiwan). Each 25 µL reaction mixture contained 1 µL of template DNA, 12.5 µL of SuperMix, 0.5 µL of each primer (Table 1), and 9.5 µL of nuclease-free water. Previously confirmed positive DNA samples and nuclease-free water served as positive and negative controls, respectively. The amplification profile consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 20 s, annealing for 1 min (51.5°C for 16SG; 48°C for 16Cpsi and OmpCTU), and 72°C for 2 min, with a final extension at 72°C for 5 min.

Table 1 Primers used in the present study.

Primer name	Target	Sequence (5' to 3')	Size (bp)
16SG F	16S rRNA	F 5'-GATGAGGCATGCAAGTC-3'	460
		R 5'-TTACCTGGTACGCTCAAAT-3'	
16Cpsi	16S rRNA	F 5'-ATAATGACTTCGGTTATT-3'	221
		R 5'-TGTTTTAGATGAAACAT-3'	
OmpCTU	OmpA	F 5'-ATGAAAAAACTCTTGAAATCGG-3'	1058
		R 5'-CAAGATTTTCTAGAYTTCATYTTGTT-3'	

Electrophoresis and sequencing: PCR products were resolved on a 2% agarose gel in 0.5X TBE buffer at 100V for 35 min and visualized under UV light. Amplicons with expected sizes 460 bp (16SG), 221 bp (16Cpsi), and 1058 bp (OmpCTU) were submitted to U2bio (Thailand) Co., Ltd. for Sanger sequencing.

Phylogenetic analysis: The obtained sequences were first evaluated for similarity against reference Chlamydiales sequences in the GenBank database

using the BLASTn algorithm (NCBI). Sequence editing and multiple sequence alignments were subsequently performed using MEGA X software. Phylogenetic trees were reconstructed using the Neighbor-Joining method based on the Tamura–Nei model. The reliability of the tree topologies was assessed by bootstrap analysis with 1,000 replicates.

Results

A total of 92 cloacal and anal swab samples were collected from captive animals at Songkhla Zoo. All animals were clinically healthy and showed no signs of infection at the time of sampling. PCR screening identified one positive sample [1.09%; 95% confidence interval (CI): 0.03–5.91%], designated as isolate R13SG-16SG, obtained from a giant Asian pond turtle (*Heosemys grandis*). The isolate produced a 460-bp amplicon targeting the Chlamydiales 16S rRNA gene. All samples tested negative for the ompA gene, indicating the absence of *Chlamydia psittaci* in the study population.

Molecular characterization based on the 16S rRNA gene sequence demonstrated that the detected strain,

designated as R13SG-16SG (*H. grandis*), belongs to the order Chlamydiales. Phylogenetic tree construction revealed that this sequence formed a distinct, well-supported monophyletic clade with Chlamydiales bacterium V4346-00 (GenBank accession no. AY845420.1) with a maximum bootstrap value of 100% (Fig. 1). This clade was phylogenetically divergent from established pathogenic lineages within the family Chlamydiaceae (e.g., *Chlamydia psittaci* and *C. trachomatis*) as well as other Chlamydia-like organisms (CLOs), including *Simkania negevensis* and *Waddlia chondrophila*.

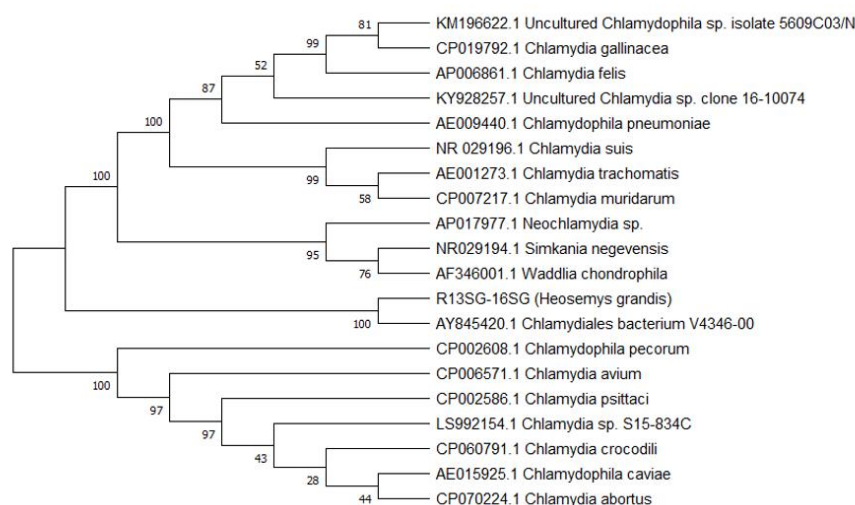


Figure 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of the strain newR13SG-16SG (*Heosemys grandis*) within the order Chlamydiales. Numbers at nodes indicate bootstrap percentages based on 1,000 replicates.

Discussion

This study presents the first detection of a Chlamydiales bacterium in an asymptomatic giant Asian pond turtle (*Heosemys grandis*) at Songkhla Zoo. Notably, the observed prevalence rate was 1.09% (95% CI: 0.03–5.91%), which is considerably lower than values previously reported in the literature. Global meta-analyses indicate varying but higher prevalence rates of *Chlamydia* infection across different animal taxa: approximately 23.5% in reptiles, predominantly involving *C. psittaci* and *C. pneumoniae* (Inchuai et al., 2021); 19.5% in birds, primarily driven by *C. psittaci* (Sukon et al., 2021); and 22–70% in mammals (Collingro et al., 2020). Region-specific zoo studies also show higher numbers; for instance, Chiang Mai Zoo in Thailand reported a 9.57% prevalence of *C. psittaci* among captive reptiles (Boonsri et al., 2022), while a survey of Japanese zoos detected *C. psittaci* in 3.9% of mammals, 7.2% of birds, and 8.1% of reptiles, alongside a 5.8% prevalence of *C. pneumoniae* in reptiles (Kabeya et al., 2015). Molecular analysis in this study classified the isolate within the order Chlamydiales (family Chlamydiaceae), aligning with the high genetic diversity supported by 16S rRNA data in prior taxonomic frameworks (Everett et al., 1999; Unemo and

Papp, 2010; Lienard et al., 2011; Pillonel et al., 2018; Pace et al., 2022).

The remarkably low incidence (only one positive sample) in this cohort could stem from several epidemiological and methodological factors. Firstly, it may reflect a genuinely low pathogen circulation within the sampled population during the study period. Secondly, chlamydial shedding in animals is well-documented to be intermittent and often triggered by stress; thus, sampling asymptomatic individuals during a non-shedding phase could lead to under detection. Lastly, low bacterial loads in the initial swabs or the potential presence of PCR inhibitors in the clinical samples cannot be entirely ruled out.

Despite this low incidence, monitoring these microorganisms is crucial. Chlamydiales, or CLOs, have been isolated from an expanding host range—including fish, reptiles, birds, livestock, and humans—as well as various environmental sources (Taylor-Brown et al., 2015; Pace et al., 2022). These organisms are implicated in diverse pathologies, such as bovine abortion (Lamoth et al., 2015), respiratory distress in captive snakes (Staub et al., 2018), and granulomatous inflammation in up to 54.4% of examined reptile tissues (Soldati et al., 2004). Taxonomical

advancements continue to reveal novel chelonian Chlamydiales taxa. For example, Bellinati *et al.* (2022) identified a novel strain in *Trachemys scripta* (pond slider) that was closely related to *C. pneumoniae* (75.4% identity) and clustered with the *C. pecorum* clade previously found in Chelonians (Mitura *et al.*, 2017). Furthermore, the detection of CLOs in 30% of ticks from a Swiss zoological garden highlights the potential role of arthropod vectors in transmission (Vanat *et al.*, 2023), while in human medicine, CLOs have been linked to trachoma, affecting 21.5% of adults and 4.2% of children in endemic regions like Sudan (Ghasemian *et al.*, 2018). Consequently, continuous surveillance remains critical to strengthening biosecurity and improving animal health management in zoological collections. Future research should incorporate larger sample sizes, a broader host range, and environmental sampling to fully elucidate the epidemiology and transmission dynamics of these cryptic pathogens.

Acknowledgements

This work was supported by the Faculty of Veterinary Science and the Research and Development Institute, Rajamangala University of Technology Srivijaya (Grant No. 4940). The authors also thank The Zoological Park Organization of Thailand under the Royal Patronage of H.M. The King: Songkhla Zoo for their invaluable cooperation and support.

Statement of author contributions: **Rawikan Inchuai:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Resources, Writing – original draft, Writing – review & editing. **Sunisa Khongthong:** Conceptualization, Methodology, Validation, Project administration, Supervision, Writing – review & editing. **Boontarikaan Wititkornkul:** Investigation, Data curation, Validation, Resources, Writing – original draft, Writing – review & editing. **Naruepan Wongpluksin:** Investigation, Data curation, Resources.

Declaration of artificial intelligence use: During the preparation of this work, the authors used ChatGPT (OpenAI) to improve English language, grammar, readability, and manuscript organization. All scientific content, interpretation of results, conclusions, and final editing were performed and verified by the authors. The authors take full responsibility for the integrity, accuracy, and originality of the manuscript.

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