Veterinary Integrative Sciences 2022; 20(1): 231-245. DOI; 10.12982/VIS.2022.018

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Molecular detection of *Chlamydia* spp. and risk factors in farmed siamese crocodile in the mid-northeastern provincial cluster of Thailand

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Abstract

This study surveyed the prevalence of chlamydial infection among farms in the mid-northeastern provincial cluster of Thailand by PCR and phylogenetic analyses of the sequences. Samples from 94 crocodiles were collected from 17 farms in five provinces together with farm management data. Chlamydiaceae was found in 48.94% of the samples (46/94). Of the 17 pooled samples analyzed using 16S rRNA sequencing, four samples exhibited 99.3 to 99.5% nucleotide identity with *Chlamydia psittaci*, three samples exhibited 99.1 to 99.3% nucleotide identity with *C. crocodili*, and one sample exhibited similarity to both species. The risk factors related to chlamydial infection included the source of young crocodiles and the frequency of water changes. Chlamydial infection was higher in nonclinical crocodiles than in clinical or dead crocodiles (P=0.003). Pharyngitis, fibrinous pharyngitis, hepatitis, pneumonia, and hydropericardium were commonly found in chlamydial-positive cases of ill or dead crocodiles. *C. psittaci* and *C. crocodili* were found in both clinical and nonclinical crocodiles. *C. psittaci* is a well-known zoonotic pathogen. *C. crocodili* is a new species found in Siamese crocodiles in Thailand. In conclusion, the source of young crocodiles and frequency of water changes were identified as risk factors for chlamydial infection in crocodile farms. *C. crocodili* should be further investigated to better understand its implications for crocodile heath.

Keywords: Chlamydia, *Chlamydia psittaci*, *Chlamydia crocodili*, Siamese crocodile

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Funding; The Department of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University, Thailand.

Article history; *received manuscript: 6 November 2021,*
revised manuscript: 10 December 2021,
accepted manuscript: 27 January 2022,
published online: 29 January 2022,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Chlamydia spp. are the cause of chlamydiosis, which is an important bacterial infectious disease in animals and humans. *Chlamydia* is a gram-negative coccus and obligate intracellular bacteria from the Chlamydiaceae family, which includes 15 *Chlamydia* species, namely, *C. abortus*, *C. avium*, *C. buteonis*, *C. caviae*, *C. felis*, *C. gallinacean*, *C. ibidis*, *C. muridarum*, *C. percorum*, *C. pneumonia*, *C. poikilothermis*, *C. psittaci*, *C. serpentis*, *C. suis*, *and C. trachomatis*, and 2 *Candidatus species*, namely, Ca. *C. corallus* and Ca. *C. sanzinia* (Sachse et al., 2015; Elwell et al. 2016; Staub et al. 2018). In 2021, *C. crocodili* was found for the first time in a Siamese crocodile carcass obtained from Trang Province, Thailand (Chaiwattanarungruengpaisan et al., 2021). *Chlamydia*ceae species are not included among the normal oral and gut flora (Lovely & Leslie, 2008; Charruau et al., 2019; Lin et al., 2019)

Chlamydia spp. have two morphologically distinct forms. First, an infectious form enters the host cell called the elementary body (EB). After that, the EB develops into a noninfectious form called the reticulate body (RB), which changes back to an EB and is released to infect other cells. Moreover, this form can persist in the host cell (Elwell et al. 2016; Panzetta et al. 2018). Some *Chlamydia* spp. are specific to certain host species; for example, *C. suis* causes respiratory disorders in pigs, *C. abortus* causes abortion in small ruminants, and *C. felis* causes conjunctivitis in dogs and cats. Some species are zoonotic pathogens, such as *C. pneumoniae*, which causes pneumonia and coronary disorder, and *C. psittaci*, which is transmitted to humans primarily from birds and causes psittacosis (Sakurai-Komada et al., 2014; Cheong et al. 2019). Both zoonotic pathogens are also the cause of anorexia, conjunctivitis, multifocal skin hemorrhage, fibrinous pharyngitis, pneumonia and granulomatous lesions on internal organs in reptiles (Vlahović et al., 2006; Jerrett et al., 2008; Timms, 2009).

C. psittaci, *C. pneumoniae*, and *C. caviae* have been found in crocodiles. The worldwide prevalence of chlamydiosis in crocodiles between 1990 and 2018 was 57.3% (Inchuai et al., 2021). In 2015, *C. psittaci* and *C. pneumoniae* were found in 8.1% and 5.8% of the reptiles in Japanese zoos, respectively (Kabeya et al., 2015). From 1994–2002, the mortality rate from Chlamydial infections in crocodile farms in South Africa and Papua New Guinea ranged between 11% and 26%, while from 2003–2004, the rate increased to 63% and 48%, respectively (Huchzermeyer et al., 2008). Chlamydiosis has been reported in saltwater crocodiles in Australia, with 31.1% in hatchlings, 83.3% in yearlings, 85.3% at necropsy, and 12.1% in wild crocodiles (Jerrett et al., 2008). In addition, 55% of 29 saltwater crocodiles with conjunctivitis and/or pharyngitis in Australia presented Chlamydial infection (Shilton, 2016). Sariya et al. (2015) reported that 74.2% of Siamese crocodiles in central Thailand were infected with *C. caviae* in 2012–2013. Moreover, 92% of juvenile Siamese crocodiles with conjunctivitis/pharyngitis lesions were infected with *Chlamydia* spp. (Paungpin et al., 2021).

Risk factors for Chlamydial infection have been investigated in some animal species. In 2014, the risk factor for *C. psittaci* in Psittaciformes was associated with the population density and cage hygiene of birds in pet markets

(Santos et al., 2014). In China, seasonal factors were reported as risk factors for *C. abortus* in white yaks (Qin et al., 2015). In eastern Saudi Arabia, new sheep and goats introduced into flocks, age of the animal, and type of the breeding system were risk factors for *C. abortus* infection while good farm hygiene was a protective factor (Fayez et al., 2021). However, information on the risk factors for Chlamydial infection in crocodile is still limited.

Crocodiles are important commercially farmed animals since their products have high export value. The export value nationwide of crocodile products in June 2021 was 1.68 hundred thousand (Suvarnabhumi Airport Fish Inspection Office, 2021). The Siamese crocodile (Crocodylus siamensis) is the most popular species for farming in the mid-northeastern region. Therefore, the aim of this study was to determine the prevalence and risk factors associated with Chlamydial infection in farmed Siamese crocodiles in the mid-northeastern provinces of Thailand.

MATERIALS AND METHODS

Animal ethics

This study was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University no. IACUC-KKU-155/62.

Sampling collection and location

From January to June 2020, 17 farms in Kalasin (3), Khon Kaen (3), Mahasarakham (1), Roi Et (9), and Udon Thani (1) provinces in northeastern Thailand participated in this study. All farms were registered with Thailand's Department of Fisheries. The following codes were assigned for each province: Kalasin, KSN; Khon Kaen, KKN; Mahasarakham, MKM; Roi Et, RET; and Udon Thani, UDN. The 94 samples were collected from cloacal swabs of crocodiles with nonclinical signs (n=55) and crocodiles with clinical signs (n=14) and organ tissue from dead crocodiles (n=25). In addition to the crocodile samples, All samples were transported at 4 °C to the laboratory for DNA extraction. Furthermore, the pond water was tested for its pH, chlorine (with a Tetra® test 6-in-1; Spectrum Brands Pet LLC., USA), and total ammonia (with an Aquacare 2000.4 Para ammonium test kit, Para test) (Paweenasakol et al., 2015). Risk factor data were obtained from interviews with the farm owners about their farm management.

DNA extraction

DNA extraction was performed using the Vivantis® GF-1 Tissue DNA Extraction Kit (Vivantis Technologies Sdn. Bhd., Malaysia). First, 500 µl of phosphate-buffered saline (PBS) at pH 7.2 was added to the sample tube and centrifuged at 800 × g for 5 min. The cotton swab was then removed, and the supernatant decanted. Two hundred microliters of PBS was then added to the tube and mixed completely by pipetting, and then 20 µL of proteinase K and 2 µL of lysis enhancer were added to the sample and immediately mixed. Two hundred microliters of tissue genomic DNA binding buffer (buffer TB) was then added and mixed thoroughly by pulsed vortexing. The mixture was incubated at 65 °C for 10 min, and 200 µL of absolute ethanol was added.

Then, the contents were mixed immediately and thoroughly by pulsed vortexing to obtain a homogeneous solution. A total of 650 µL of sample was then transferred into a column and centrifuged at 5000 × g for 1 min. The flow-through was discarded, and the process was repeated for the remaining sample. Then, the column was washed with 650 µL wash buffer and centrifuged at 5000 × g for 1 min. The flow-through was discarded, and the repeat column was washed again. The column was then dried by centrifugation at 10,000 × g for 1 min. The column was then placed into a clean microcentrifuge tube, and 50 µl of preheated elution buffer was finally added directly onto the column membrane and left to stand at room temperature for 2 min. Finally, it was centrifuged at 5,000 × g for 1 min to elute the DNA. The DNA yield was measured by a NanoDrop spectrophotometer and stored at 4 °C or -20 °C.

Polymerase chain reaction (PCR) test

The PCR process was modified from Robertson et al. (2010) and Suksai et al. (2019). Briefly, 2X PCR Master Mix solution (I-TaqTM, iNtRON Biotechnology, South *Korea* was used, with 10 µL of 2X PCR Master Mix Solution added to PCR tubes as the first step, followed by 2 µL of template DNA, 1 µL of 10 µM 16SG F primer (5′-GATGAGGCATGCAAGTC-3′), 1 µL of 10 µM 16SG R primer (5′-TTACCTGGTACGCTCAAAT-3′), and 6 µL of distilled, for a total volume of 20 µL. The mixture was mixed well by pipetting and spin down. After that, the tube was placed in a thermocycler. The predenaturation step was started with 5 min at 94 °C, followed by 35 cycles run at 94 °C for 20 sec, 52 °C for 10 sec, and 72 °C for 20 sec for denaturation, annealing, and extension, respectively, with a final extension at 72 °C for 5 min. The positive control was *Chlamydia felis* from Purevax® RCPch, and the negative control was distilled water. The PCR product was measured by a NanoDrop spectrophotometer stored at 4 °C or -20 °C.

Gel electrophoresis and sequencing

Using a 2% agarose gel with RedSafe™ (iNtRON Biotechnology, South *Korea*), the box was filled with 1X TBE (Tris-borate-EDTA) buffer until the gel was covered, and then 5 µL of PCR products was placed in each gel well and 2 µL Sizer™-100 DNA Marker solution (iNtRON Biotechnology, South *Korea*) was loaded into the first lane of the gel. The gel was run at 100 volts for 40 min to observe the fluorescence band under UV illumination (Haines et al., 2015; Pikulkaew and Potibut, 2021) The positive band was size 460 bp. Afterward, the pooled positive PCR products from each crocodile farm were sent to U2Bio (Thailand) Co., Ltd. for DNA sequencing by the barcode-tagged sequencing technique. The nucleotides were compared with the GenBank database by BioEdit (version 7.2) software. The sequences have been deposited in the National Center for Biotechnology Information (NCBI) database(https://www.ncbi.nlm.nih.gov/). Phylogenetic trees were built by the neighbor-joining model using MEGA X.10.2.6

Statistical analysis

For the risk factor analysis, a univariate logistic regression was performed to calculate the odds ratio and its 95% confidence interval (CI). The Chi-square test was used to determine the p values, with a P value <0.05 considered significant. All tests were conducted in the SPSS program (Statistical Package for the Social Sciences, IBM Corp. Released 2019, IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.).

RESULTS

Farm characteristics

The majority of farms were backyard operations for skin production. The ages of the crocodiles ranged from 5 months to 8 years. Their raising pens consisted of concrete blocks and polished concrete floors and were positioned in land and pond areas at a ratio of 1:1–2. The individual pen size was 1 m in width and 2 m in length, with a water depth of 0.25–0.6 m. Depending on the farm and area, the communal pen size was 4–20 m in width and 4–20 m in length, with a water depth of 0.6–1.5 m. Both pens were 1.20-1.50 m in height. The water sources were 65% groundwater and 35% ponds, while the water characteristics were pH 6.8–7.2, 0.5–2 mg/L ammonia, and 0–0.8 mg/L chlorine (levels that are safe for aquatic animals). The air temperature averaged 33.5 °C (30–37 °C), and the relative humidity averaged 71.43% RH (59.8–86.1% RH). Because of these conditions, many farms used nets to shade crocodiles.

All farmed crocodiles were fed frozen chicken heads, and Chlamydiaceae was not detected in the choana swabs of chicken heads on each farm by PCR. In addition, the owners treated the animals with unknown medicines in the case of illness. Diseased crocodiles exhibited anorexia, prolonged basking, and skin hemorrhage at the ventral scales (especially around the cloacal opening), and the gular fold and mucosa around the opening of the pharynx were red and swollen or covered by diphtheritic membrane in some crocodiles. Most crocodiles at postmortem did not show malnutrition, and their conditions included gross lesions, hydropericardium, lung edema with frothy exudate in the trachea, and hepatic enlargement (Figure 1). Two cases showed accumulated urate crystals in the internal organs and joints.

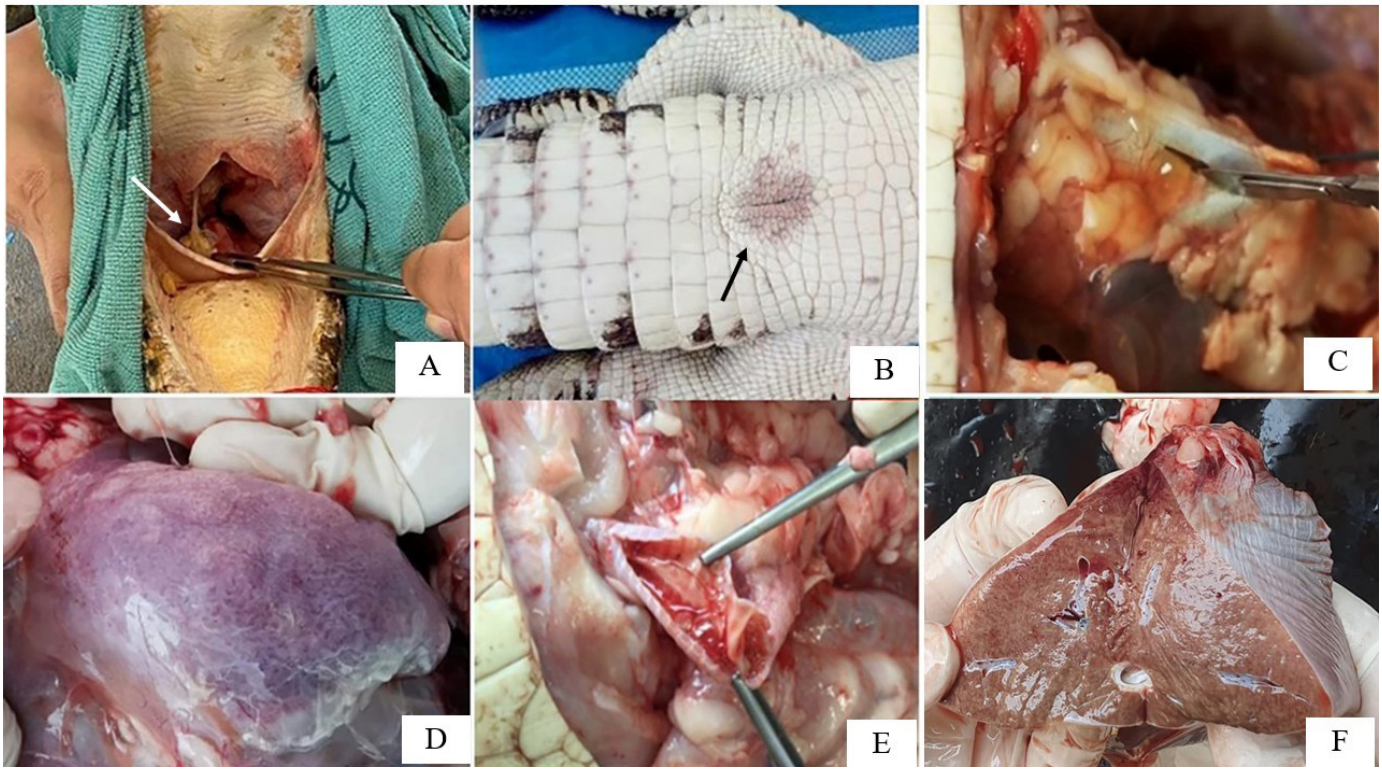

Figure 1 Lesions of affected crocodiles: (A) reddish oropharynx with fibrinous material; (B) skin hemorrhage at the ventral scales; (C) hydropericardium; (D) lung edema; (E) frothy exudate in the trachea; and (F) pale and bulge cut surface of liver.

Prevalence of chlamydial infection

Of the 94 samples examined, 46 (48.94%) were positive for Chlamydiaceae, with one for the clinical signs crocodiles, 35 for the nonclinical signs crocodiles, and 10 for the necropsy crocodiles (Table 1). Due to the low yield and small volume of PCR products, a pooled PCR product size of 460 bp was analyzed for nucleotide sequencing (Figure 2). The 16S rRNA gene of the bacteria detected in the crocodiles was separated into two groups. The first group, including GenBank accession nos. OM184273 (RET03.2), OM184274 (RET03.3), OM184276 (RET10), and OM184277 (KKN04), exhibited 99.1–99.3% nucleotide identity with *C. crocodili* and 98.9–99.1% nucleotide identity with the closely related species *C. poikilothermis*. A phylogenetic tree of the nucleotide sequences presented a 63% bootstrap value for grouping with *C. crocodili* and a 72% bootstrap value for separation from *C. poikilothermis* (Figure 3). The second group, including GenBank accession no. OM184271 (RET01), OM184272 (RET03.1), OM184275 (RET09), OM184278 (KSN02), OM184279 (KSN03), and OM184280 (UDN01), exhibited 99.5–99.7% nucleotide identity with *C. psittaci*. The phylogenetic tree of UDN01 showed a 99% bootstrap value for separation from *C. felis*. The nucleotide sequences of KSN02, KSN03, RET01, RET03.1 and RET09 presented a 62% bootstrap value for separation from *C. psittaci* (Figure 4). Both *Chlamydia* species were detected in farm RET03 (note that RET03.1, RET03.2, and RET03.3 were the same farm but included animals with different ages and sources).

Table 1 *Chlamydia* PCR test results

Provinces	PCR results (positive/total)[a]			
	Living crocodiles		Necropsy crocodile	Total
	Clinical signs	Nonclinical signs		
Kalasin	0/1	11/16	3/3	15/21
KhonKaen	1/6	2/2	1/2	3/7
Mahasarakham	0/1	0/1	0/0	0/2
Roi-Et	0/6	23/36	3/11	25/53
Udonthani	0/0	0/0	3/10	3/10
Total	1/14	35/55	10/25	46/94

[a] +/total, number of *Chlamydia*-positive PCR results per total number of samples

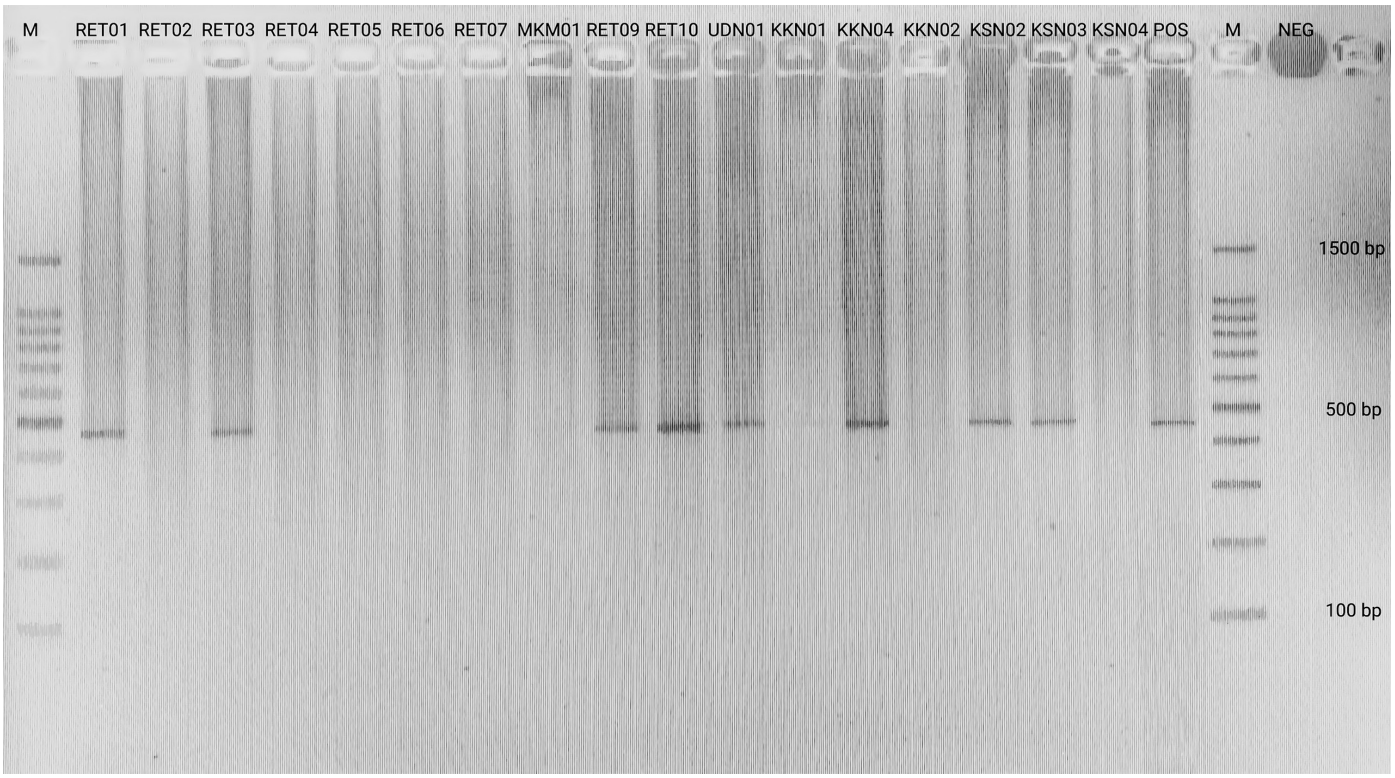

Figure 2 Pooled polymerase chain reaction (PCR) product samples from each crocodile farm tested for expected Chlamydia spp. and compared to a DNA ladder (Lanes 1, 20), positive control (Lane 19), and negative control (Lane 21). The PCR products of RET01, RET03, RET09, RET10, UDN01, KKN04, KSN02, and KSN03 have a single band at an almost similar target size (460 bp) in Lanes 2, 4, 10, 11, 12,14, 16, and 17, respectively.

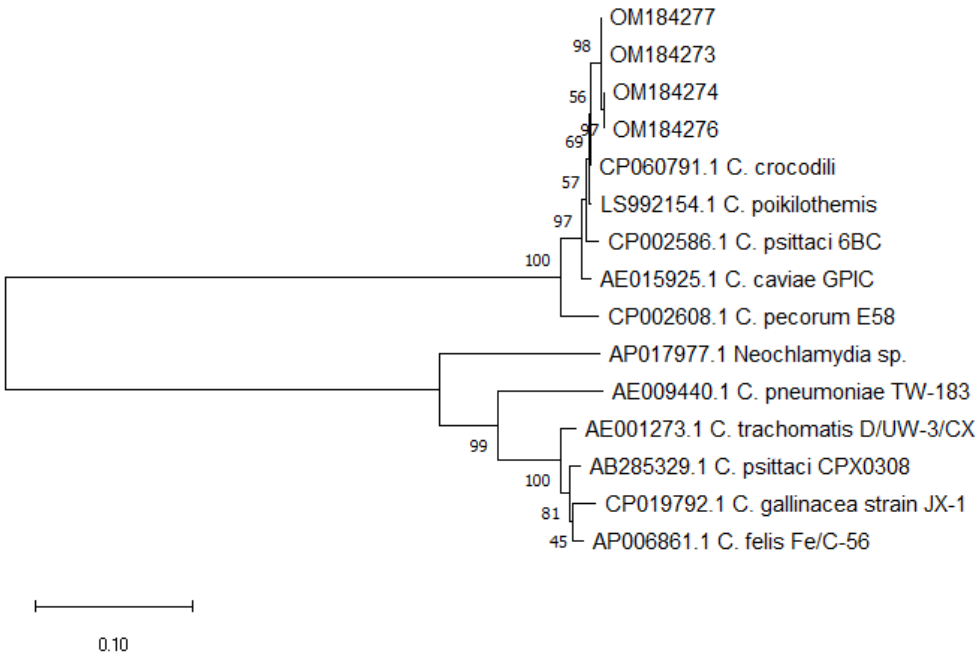

Figure 3 Phylogenetic tree based on the 16S rRNA sequence alignments showing the relationship of sequences OM184273 (RET03.2), OM184274 (RET03.3), OM184276 (RET10), and OM184277 (KKN04) to other *Chlamydia* spp. The tree was constructed using the neighbor-joining method implemented in MEGA 10.2.6.

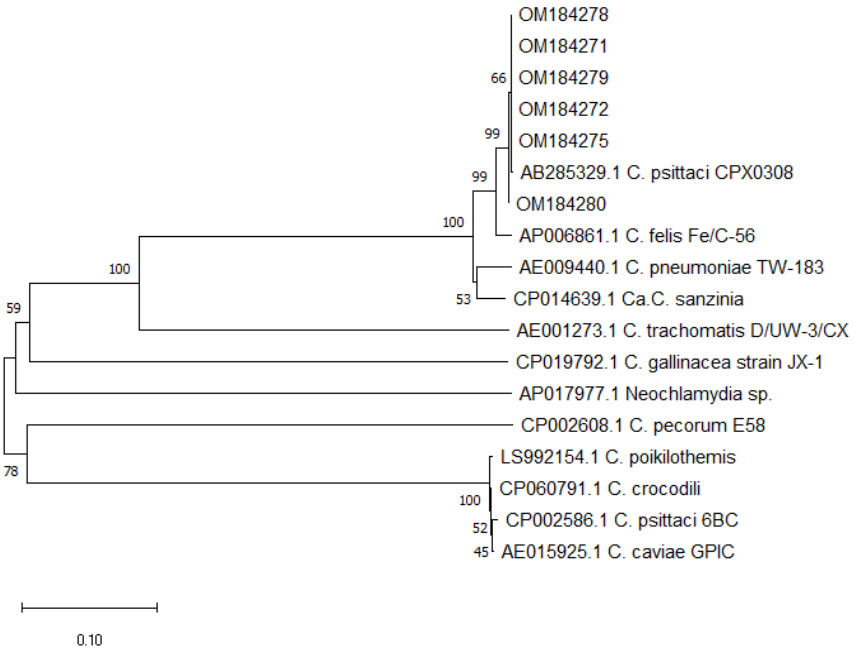

Figure 4 Phylogenetic tree based on the 16S rRNA sequence alignments showing the relationship of sequences OM184271 (RET01), OM184272 (RET03.1), OM184275 (RET09), OM184278 (KSN02), OM184279 (KSN03), and OM184280 (UDN01) to other *Chlamydia* spp. The tree was constructed using the neighbor-joining method implemented in MEGA 10.2.6.

Risk factors for chlamydial infection

A risk factor analysis for Chlamydial infection in crocodiles is presented in Table 2. The crocodile source, water change frequency, and clinical signs were identified as significant risk factors for Chlamydial infection in crocodiles. Crocodiles from company B had significantly higher odds (OR = 3.78; 95% CI: 1.35–10.54; P = 0.01) of Chlamydial infection than crocodiles from company A. The odds of Chlamydial infection were higher (OR = 8.22; 95% CI: 1.70–39.80) in individuals with a low frequency of water changes (more than 1 month/time) than in those with a high frequency of water changes (every 1-2 weeks). Crocodiles with nonclinical signs had a higher risk (OR = 0.28; 95% CI: 0.15–0.67) of Chlamydial infection than normal or subclinical crocodiles.

Table 2 Factors associated with chlamydial infection in Siamese crocodile farms (n=94)

Factors	Category	OR;	95% CI	P value
Owner's experience	0–5 years		1	
	5–10 years	1.74	0.37–8.12	0.482
	>10 years	1.10	0.47–2.57	0.829
Crocodile source	Company A		1	
	Company B	3.78	1.35–10.54	0.011*
	Others	0.52	0.14–1.91	0.326
Crocodile age interval	0–2 years		1	
	2–4 years	1.29	0.54-3.11	0.566
	>4 years	4.87	0.94-25.22	0.059
Pens	Individual pens		1	
	Communal pens	0.42	0.16–1.11	0.076
Water source	Pond		1	
	Ground water	0.92	0.41–2.06	0.833
Water change	Every 1–2 weeks		1	
	Every 2–4 weeks	1.76	0.58–5.32	0.315
	>1 month	8.22	1.70–39.80	0.009**
Depth of water area in pens	< 40 cm		1	
	40–80 cm	0.86	0.27–2.76	0.799
	>80 cm	0.7	0.52–3.29	0.552
Shade	Shade		1	
	No shade	0.61	0.16–2.31	0.459
Clinical signs	Clinical signs		1	
	Nonclinical signs	0.28	0.12–0.67	0.003**
Medication	Treated		1	
	Untreated	0.75	0.29–1.93	0.547
Sampling	Cloacal swabs		1	
	Organs	1.64	0.65–4.14	0.297

OR, Odds ratio; 95% CI, 95% confidence interval; *, significant at 0.05; **, significant at 0.01

Descriptive data of clinical signs and necropsy findings from Chlamydial positive cases of ill or dead crocodiles are presented in Table 3. The most common clinical signs and necropsy findings were anorexia, prolonged basking, skin ulcers, pharyngitis, hepatitis, and pneumonia. Fibrinous pharyngitis and hydropericardium were found in 6/6 and 5/5 crocodiles under examination, respectively.

Table 3 PCR results for Chlamydiaceae detection in clinical and necropsy crocodiles

Signs/lesions	PCR results (positive/total)[a]
Clinical crocodiles	
Anorexia	6/16
Fibrinous pharyngitis	6/6
Pharyngitis	12/24
Prolong basking	8/13
Skin hemorrhage	11/29
Necropsy crocodiles	
Coelomic inflammation	7/9
Gout	1/2
Hepatitis	11/24
Hydropericardium	5/5
Pneumonia	8/13

[a] +/total, number of *Chlamydia* positive PCR results per total number of crocodiles with each clinical signs

DISCUSSION

In this study, the prevalence of Chlamydial infection in crocodiles in the mid-northeastern region of Thailand was estimated at 48.9%, thus indicating a high prevalence of Chlamydial infection in crocodiles. Our result was similar to that from a previous study that estimated a 57.3% worldwide prevalence of Chlamydial infection in crocodiles (Inchuai et al., 2021). Our study identified *Chlamydia crocodile* and *Chlamydia psittaci* from crocodile samples, which was similar to a previous study in which *C. crocodili* was isolated from Siamese crocodiles in Thailand (Chaiwattanarungruengpaisan et al. 2021). *C. psittaci* was also identified from previous reports by Huchzermeyer (2002), Jerrett et al. (2015), and Thongkamkoon et al. (2018). However, the first report in Thailand showed that 74% of clinical Siamese crocodiles were positive for *C. caviae* (Sariya et al., 2015).

Our study found that the source of young crocodiles and the frequency of water changes were risk factors for Chlamydial infection in crocodiles. Therefore, farmers should be aware of the source of young crocodiles introduced into farms. The frequency of water changes has an influence on crocodile health because water changes reduce waste and ammonia levels (Food and Agriculture Organization of the United Nations, n.d.; Manolis and Webb, 2016). In this study, the low-frequency water changes (more than one month/time) increased the opportunity of *Chlamydia* infections in crocodiles. During the survey, sick and dead crocodiles were found. The owner treated the animals with unknown medication and increased the frequency of water changes according to the advice of the company and the Fisheries District Office, which recommended raising pens to be drained and scrubbed clean at least weekly to

maintain a low bacterial level. This practice is consistent with the recommendations of Ayensu et al. (1983). Brien et al. (2008) also reported that clean water is good for crocodiles and improves visibility for crocodile inspections. Nevertheless, changing the water too frequently affects the water temperature and stresses crocodiles; therefore, water should be changed as infrequently as every 2–3 months or before moss overgrowth. All farms in this study had normal water quality and used potassium permanganate and other (unknown) disinfectants to clean the pens. Therefore, the optimal cleaning and water changing frequencies are dependent on the pond volume, crocodile size, population density, food type, and environmental conditions. Water should be observed for physical and quality changes, such as color, odor, and turbidity. The most important point is that all disinfectants are thoroughly flushed out before the crocodiles return to the water. Aquaculture of animals similar to crocodiles also requires regular water quality checks and maintenance, such as the addition of 1 gram of salt per liter of water (3–5 ppt) for bacterial control (Inland Aquaculture Research and Development Regional Center 5, n.d.; Leslie and Spotila, 2000; Manolis and Webb, 2016; The DEW Fauna Permit Unit 2020).

Surprisingly, the percentage of Chlamydial infections was higher in crocodiles with nonclinical signs in our study. This could be explained by resistance to host defense mechanisms and entering a persistent state termed "*Chlamydia* persistence or *Chlamydia* stress response" when *Chlamydia* is exposed to stress caused by the deprivation of essential nutrients or exposure to interferon-gamma (IFN-γ) or beta-lactam antibiotics (Elwell et al., 2016; Panzetta et al., 2018). The owners used unknown medicines, resulting in bacterial resistance and relapse, changes in immune function and *Chlamydia* immune evasion. In addition, reptiles generally have very active immune mechanisms, and their immune systems respond more slowly than mammal immune systems (Ochsenbein and Zinkernagel, 2000; Ujvari and Madsen. 2006; Zimmerman et al., 2010; Dang et al., 2015; Rios and Zimmerman. 2015). The positive *Chlamydia* detection in both the clinical sign and nonclinical sign groups conformed to the results of Jerrett et al. (2008), who reported that 12% of nonclinical wild crocodile samples in Australia were chlamydiosis-positive. In 2015, the prevalence of *C. psittaci* and *C. pneumoniae* in crocodiles during routine reptile checkups from five zoos in Japan was 8.1% and 5.8%, respectively (Kabeya et al., 2015).

Chlamydia can be transmitted to animals or humans by direct contact from affected animals or humans, asymptomatic carriers, and contaminated environments (Sachse et al., 2015). Many countries, such as Belgium, China, and Italy, have reported *C. psittaci* and *C. gallinaceae* infecting poultry and transmitting to humans (Lagae et al., 2014; Guo et al., 2016; Donati et al., 2018). In the current study, the chickens fed to the alligators were expected to be infected and thus a source of *Chlamydia*; however, infection in chickens was not observed based on the PCR results.

Our study found that anorexia and prolonged basking were associated with Chlamydial infection in crocodiles. The symptoms of Chlamydial infection appeared a few days to several weeks after exposure (The Center of Food Security and Public Health, 2017; Borel et al., 2018). The general signs of affected crocodiles are abnormally prolonged basking behavior, flattened

tail, weight loss, anorexia, lethargy, and pale or red mucous membranes in the oral cavity and cloaca; on the other hand, sometimes no signs appear (Ebani, 2017; Gregory and Deidre, 2014). Acute hepatitis, conjunctivitis, and granulomatous inflammation in internal organs are common signs of chlamydiosis. Some cases present with pneumonia, enteritis, and myocarditis lesions as well (Bodetti et al., 2002; Huchzermeyer et al. 2008; Timms, 2009; Rohde et al., 2010; Cheong et al., 2019). According to this study, conjunctivitis was not observed. Nevertheless, pharyngitis, fibrinous pharyngitis, pneumonia, hydropericardium, and coelomitis lesions were associated with Chlamydial infection, and these findings were consistent with other studies on crocodiles with severe clinical signs (Jerrett et al., 2008; Paungpin et al., 2021; Sariya et al., 2015). Furthermore, coinfection of *Chlamydia* spp. with other pathogens, such as adenovirus, herpesvirus, and *Aeromonas* spp., would exacerbate the symptoms, especially septicemia (Paungpin et al., 2021, Pu et al., 2019; Thongkamkoon et al., 2018).

According to this study, *C. psittaci* and *C. crocodili* were detected crocodiles with/without clinical signs. Most of the infected animals showed significant signs, such as lesions, pharyngitis, fibrinous pharyngitis, coelomitis, hydropericardium, and pneumonia. The clinical signs and lesions indicate a primary or secondary Chlamydial infection but could also represent a persistent state or reinfection. Therefore, newly arriving crocodiles should be quarantined for disease control and surveillance and proper farm management practices and precautions should be exercised against diseases that could be transmitted to or from humans. In addition, the source of infection is still unknown, and new species with insufficient information should be further researched in the future.

ACKNOWLEDGEMENTS

The authors are grateful to the owners of the crocodile farms for providing information and facilitating the study, and they would also like to thank the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University, for providing laboratory operations.

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How to cite this article;

Rawikan Inchuai, Peerapol Sukon and Sompoth Weerakhun. Molecular detection of *Chlamydia* spp. and risk factors in farmed siamese crocodile in the mid-northeastern provincial cluster of Thailand. Veterinary Integrative Sciences. 2022; 20(1): 231-245.