A molecular survey of *Chlamydial* infection in pet and zoo captive reptiles

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Key words:
chlamydia, reptiles, real time PCR, zoonotic disease

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Received: 23 December 2016
Accepted: 12 Februart 2017

Abstract:

BACKGROUND: Chlamydiosis is a worldwide zoonotic disease caused by different microorganisms in the order Chlamydiales. **OBJECTIVES:** The aim of this study was to detect and determine the prevalence of Chlamydia infection in pet and zoo reptiles in Tehran, Iran. **METHODS:** In a period of 10 months from April 2015 to February 2016, swab samples were collected from cloaca and conjunctiva of 130 pet or zoo reptiles (18 snakes, 81 turtles, and 31 iguanas). A Real Time-PCR assay targeting 23s rRNA of chlamydial organisms was performed to detect chlamydial infection in clinical specimens. **RESULTS:** No positive sample could be detected in the investigated clinical specimens in the present study. **CONCLUSIONS:** Regarding the negative results which were achieved in this study, reptiles could not be important hosts of chlamydial organisms at least in the region of the present study, Tehran, Iran. Despite the present findings in reptiles, pet and aviary birds were previously shown to be remarkable hosts of *Chlamydia* spp. in Iran. Further studies particularly serologic surveys and other PCR methods are needed to thoroughly evaluate the significance of the chlamydial infection in reptiles. A rapid, accurate and cost-effective method was applied for Chlamydiaceae spp detection and discrimination of the most significant *Chlamydia* spp., causing disease complications in reptiles. The results indicated low zoonotic risk of *Chlamydia* spp in Iranian reptiles.

Introduction

Chlamydiosis is a worldwide zoonotic disease caused by microorganisms called Chlamydia. These bacteria are obligate intracellular parasites that belong to the family Chlamydiaceae (Suchland et al., 2003).

Several animal species can become infected by chlamydial organisms. According to a previous study by Krauss et al., 2003 the chlamydiosis was recognized in 32 species of mammals. Sheep, cats and goats are the most probable affected (Matsui et al., 2008, Stuen et al., 2011). Many infected animals do not show any clinical signs. The infection with *Chlamydia psittaci* in reptiles has broad spectrum clinical signs such as distress, purulent nasal discharge, diarrhea, frequent urination, lethargy, sinusitis and disorders of the central nervous system (Soldati et al., 2004, Yucesan et al., 2001). Commonly, reptiles such as chameleon and

snakes sporadically develop Chlamydiosis with diverse clinical signs (granulomatous inflammation and erythema), iguanas and giant turtles (inflammatory changes on various organic systems) and crocodiles (conjunctivitis) (Frutos et al.,2015, Gaydos et al., 1992). The severity of clinical sign depends on the species of *Chlamydia*, species of reptiles and age (Ebani and Fratini, 2005).

Human can occasionally get infected from animal contact (Andersen and Vanrompay., 2003). Meyers in 2009 indicated no transition of *C. pneumonia* from animal to humans (Myers et al., 2009). Immunodeficient and immunosuppressed human beings, pediatrics and geriatrics, are included as high risk population to chlamydiosis, which is transferred by reptiles (Pospisil et al., 2004, Mendall et al., 1995).

Identification of the infected reptile with bacterial agents is critical to control the disease in high risk people who are exposed to the mentioned animals (Jacobson et al.,2004). In general, the diagnosis of chlamydial infections by conventional methods is difficult and has a highly variable response (Black, 1997). Gold standardized methods (observing inclusion body by light or electron microscopy) for most Chlamydia spp. are not widely available (Bodetti et al., 2002). In order to speed up the analysis, the usage of PCR methods has been introduced to identify Chlamydia spp. in human and reptiles samples (Boman et al., 1999). Increasing demand for a quantitative, more sensitive and specific and rapid procedures are prompting the development of real-time quantitative PCR methods.

People who raise reptiles as pets have increased recently but microbiological information is limited. The purpose of the present study was to evaluate the presence of clinical and subclinical chlamydial infection in some pet and zoo captive reptiles in Tehran.

Material and Method

Clinical samples: All parts of sampling and DNA extraction were coordinated based on previous studies (Taylor-Brown et al., 2015, Madani and Peighambari, 2013, Di Ianni et al., 2015). For this purpose a single swab was used to take the samples from conjunctiva and cloaca, respectively. In a period of ten months from April 2015 to February 2016, 130 swab samples were collected from cloaca and upper respiratory tract or conjunctiva of different reptile species. The investigated animals were either admitted to Tehran small animal research and teaching hospital or were housed in Tehran zoo (24 zoo animals and 106 pet animals). The swabs were placed in sucrose-phosphate glutamine medium (SPG) and frozen for the future investigation. SPG contain 75 g of sucrose, 0.52 g of KH2PO4, 1.22 g of Na2HPO4 and 0.72 g of glutamic acid, distilled water to 1 liter (pH 7.4) (Merck Co., Germany), with 10% fetal calf serum (FCS) supplement, 500 mg vancomycin, 500 mg streptomycin, 200 mg of gentamicin and amphotericin 50 mg.

Real-time PCR assay: Total genomic DNA was extracted using High Pure PCR Template Preparation Kit (Roche, Germany) as instructed by the manufacturer. Extracted DNA samples were stored at −20 °C. All clinical samples were amplified on the Rotor Q machine (QIAGEN co., Germany) using the 23s rRNA-based Chlamydiaceae family-specific real-time PCR as described previously (Ehricht et al., 2006). The spe-

Table1. The scientific names and the number of pet and zoo reptiles which were sampled in the present study for molecular survey of chlamydia infection.

Common name	Scientific sample Name	Number
Python	*Pythonidae family*	5
Boa	*Boidae family*	6
Blind snakes	*Typhlopidae family*	1
Streaked snakes	*Oligodon taeniolatus*	2
Javelin sand boa	*Eryx jaculus*	3
Diadem snakes	*Spalerosophis diadema schiraziana*	1
Caspian turtle	*Mauremys caspica*	28
Pond slider	*Trachemys scripta*	41
Russian tortoise	*Testudo horsfieldii*	1
European pond turtle	*Emys orbicularis*	11
green iguana	*Iguana iguana*	22
bearded dragons	*Pogona*	4
Egyptian mastigure	*Uromastyx aegyptia*	1
desert iguana	*Dipsosaurus dorsalis*	4

cific primers were used for amplification of Chlamydia spp., including: Ch23S-F (5′-CTGAAACCAGTAGCTTATAAG-CGGT-3′), Ch23S-R (5′-ACCTCGC-CGTTTAA CTTAACTCC-3′), and probe Ch23S-p (FAM-CTCATCATGCAAAAG-GCACGCCG-TAMRA) (Ehricht et al.,2006). The primers targeted an amplicon in 23s rRNA gene with the size of 111 bp. In each reaction, 2.5 µl of extracted DNA was added to a mixture of reagents containing 12.5 µl of 2×TaqMan® Fast Universal PCR Master Mix (Jena Bioscience, Germany, Jena), with final concentration of 5 pmol/µl of each primer and the probe (Macrogen, South Korea) to yield a final volume of 25 µl. The cycling profile included an initial denaturation (95 °C, 10 min) followed by 45 cycles of denaturation at 94 °C for 15 s and 60 °C for 60 s. A cycle threshold (Ct value) of <38.00 was considered as positive, and all samples were tested at least in duplicate also all samples were compared to UT-NOIVBD (Veldhoven, the Netherland) as positive control and deionized water as negative control (Madani and Peighambari, 2013).

Results

A total 130 clinical samples were obtained from 16 snakes, 81 turtles, 33 iguanas. Ages vary from 1 month to 12 years. The frequency of conjunctivitis and respiratory lesions was 21% and 11%, respectively. According to positive control test, the results were interpreted as questionably positive if one threshold cycle (Ct) value was less than 38 while the other showed no Ct value. If one Ct value was above 38 and the other showed no Ct value, the result was interpreted as questionably negative. Thus, our results indicated no amplification products from 130 clinical samples by real time PCR. There were no positive samples with different strains of Chlamydia sp.

Discussion

Chlamydia spp. are a widespread group of obligatory intracellular bacteria in both warm-blooded and cold-blooded animals. Their infection was reported in different

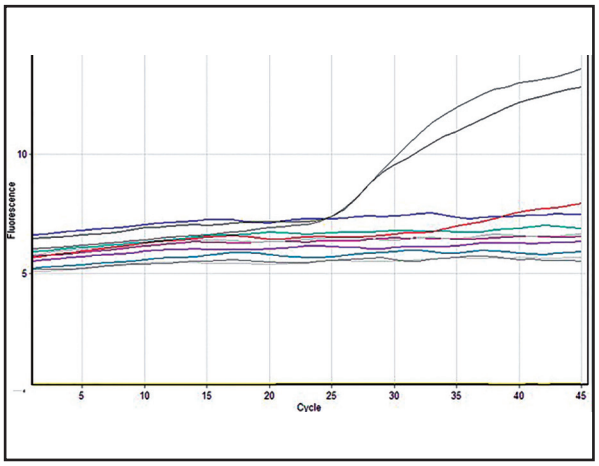

Figure1. As the graph illustrates, all of our samples were plateau, while control positive samples peaked in the 25th cycle and prove real time PCR efficiency.

hosts including human, cats, birds, sheep, dogs, pigs, koalas, and cattle (Suchland et al, 2003). Chlamydiosis was also described in various species of reptiles including turtles, chameleons, crocodiles, iguanas and snakes (Andreoletti et al., 2007, Corsaro and Venditti, 2004). In the present study 130 clinical specimens from 14 reptile species were subjected to Chlamydiaceae specific real-time PCR. None of them were positive for Chlamydia spp. 23s rRNA DNA using the highly sensitive molecular method.

Although it was relatively scarce in the literature, chlamydial infection was previously reported in reptiles. Using the conventional PCR, Jacobson et al., (1989) detected the bacterial DNA in a group of turtles with myocarditis, pneumonia, hepatitis, and splenomegaly. Kabeya et al., (2015) reported 8.1% of Chlamydia infection in reptiles. The incidence of *Chlamydia pneumoniae* DNA was significantly higher in reptiles (5.8%) than in mammals (0.3%) and birds (0.3%). Taylor-Brown et al., (2015) illustrated the variable occurrence of Chlamydia from 5% to 33% in captive snakes. In another report from Argentina, *C. pneumoniae* infection was diagnosed in a single collection of captive reptiles (Frutos et al., 2014). Hotzel et al., (2005) reported Chlamydia spp infection in 16 out of 155 (10.3%) nasal lavage specimens from tortoises. Di Ianni et al., (2015) found *Chlamydia* spp in a single animal with severe conjunctivitis using a PCR diagnosis. The Chlamydia sp. was isolated from the respiratory lesions of a Burmese python and also a *Corallus hortulanus* suffered from a chronic pulmonary thromboembolism, respectively in 2001 and 2002 (Jacobson et al., 2001, Bodetti et al., 2002). Soldati et al., (2004) detected *C. pneumonia*e DNA in nine out of 90 (10%) reptile samples. To the best of the authors' knowledge it was the first attempt in the molecular detection of chlamydiosis in captive and zoo reptiles in Iran.

The isolation of Chlamydia sp. is very time consuming and relatively difficult considering the obligatory intracellular nature of these organisms. Molecular methods are superior to the traditional isolation regarding the test sensitivity and the rapid one day results (Gaydos et al., 1992, Sachse et al., 2005). In the previous studies real time PCR was used for chlamydia sp. detection in birds and a koala (Madani and Peighambari, 2013, Krawiec et al., 2015, Mackie et al., 2016) and in reptiles Jacobson et al., (2004) used it in an emerald tree boa. The real-time PCR which was applied in the present study was very sensitive and a robust diagnostic method which has been repetitively employed in different studies since its invention by Ehricht et al., (2006).

Chlamydiosis is a zoonotic infection and it can cause a severe clinical condition in human (Andersen and Vanrompay, 2003, Bodetti et al., 2002, Jacobson et al., 2004, Huchzermeyer et al., 2008, Homer et al.,

1994). It was previously indicated that *C. pneumoniae* infection might be correlated with different pathologies such as atherosclerosis, coronary heart disease and Alzheimer in human (Roulis et al., 2013). The population of pet reptiles is growing in Iran (Darvish and Rastegar-Pouyani, 2012, Esmaeili et al., 2008). The captive reptiles can be a potential reservoir of different zoonotic organisms like Chlamydia infection. Their role in this specific infection could not be demonstrated in the present study. According to this survey, it can be concluded that the investigated captive reptiles were not a major threat for their owner regarding the chlamydial infections. The significance of chlamydial infection in farm animals (Esmaeili et al.,2015, Ebadi et al., 2014), birds (Madani et al., 2013, Ghorbanpoor et al., 2015, Tatari et al., 2016) and human (Hashemi et al., 2009, Ghazvini et al., 2012) have been previously shown in Iran.

Di Ianni et al., (2015) were unable to prove a cause-effect correlation between the presence of chlamydia and the disease situation in reptiles. Furthermore, the clinical manifestations of Chlamydia infection in the investigated cases were low. The necropsy was not performed in the current survey and consequently internal organs were not submitted for thorough investigation. While real time PCR has a promising sensitivity and specificity for the detection of bacterial infectious agents, Kabeya et al., (2015) indicated some limitations in the application of these molecular methods. It is recommended for future studies to apply two or more concurrent PCR methods to get a more reliable result.

A review on previous studies in which a high number of infections were reported revealed that almost all specimens in those studies were collected from a limited captive population or even a single population in a unique location. In contrast, the samples of the current study were mostly collected from pet reptiles which were owned by different owners and also were kept individually at home. The higher infection rate in other studies might be correlated with those specific captive populations. It is a common practice in pet markets of Iran to treat the animals with different antibiotics. Previous treatment with different antibiotics could decrease the infection rate. Unfortunately, there was no recorded history of antibiotic administration in our cases. However, larger sample size, different sampling methods especially collecting internal organs during necropsy, and applying various detection tests can increase our understanding about reptile chlamydiosis in Iran.

Acknowledgments

With best regard of our colleagues and staff of laboratory.

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مجله طب دامی ایران، ۱۳۹۶، دوره ۱۱، شماره ۳، ۲۳۳-۲۲۷

بررسی مولکولی عفونت‌های کلامیدیایی در خزندگان خانگی و باغ وحش شهر تهران

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(دریافت مقاله: ۵ آذر ماه ۱۳۹۵، پذیرش نهایی: ۲۳ بهمن ماه ۱۳۹۵)

چکیده

زمینه مطالعه: کلامیدیوز یک بیماری زئونوز با گسترش جهانی است که توسط میکرو ارگانیسم‌های مختلف از راسته Chlamydiales می باشد. **هدف:** این مطالعه در پی آن است که میزان شیوع عفونت‌های کلامیدیایی در خزندگان خانگی شهر تهران و همچنین باغ وحش تهران را با روش Real time PCR تعیین نماید. **روش کار:** در یک دوره زمانی ۱۰ ماهه از فروردین تا بهمن۹۴ نمونه‌ها با استفاده از سوآب استریل از کلوآک و ملتحمه ی ۱۳۰ خزنده خانگی و نیز خزندگان نگهداری شده در باغ وحش تهران (شامل ۱۸ مار، ۸۱ لاکپشت و ۳۱ ایگوآنا) گرفته شد. برای ارزیابی عفونت کلامیدیایی از روش Real time PCR بر روی قطعه ۲۳s rRNA استفاده شد. **نتایج:** درمطالعه حاضر هیچ نمونه مثبتی یافت نشد. **نتیجه گیری نهایی:** با توجه به نتایج منفی این مطالعه می‌توان نتیجه گرفت که این ارگانیسم حداقل در شهر تهران میزبان مهم و عامل انتقال بیماری به انسان نمی‌باشد. گرچه مطالعات گذشته در ایران حاکی از آلودگی پرندگان و دیگر حیوانات خانگی بوده است اما گزارشی در مورد آلودگی خزندگان در کشور وجود ندارد. بررسی‌های سرولوژی یا روش‌های PCR دیگر برای ارزیابی بیشتر لازم است. در مطالعه حاضر از یک روش تشخیصی دقیق و به نسبت مقرون به صرفه برای ارزیابی استفاده شد. این مطالعه نشان داد خطر زئونوتیک خزندگان از نظر کلامیدیوز در شهر تهران بسیار کم است.

واژه های کلیدی: کلامیدیا، خزندگان، Real time PCR، بیماری‌های مشترک با انسان

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