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SHORT COMMUNICATION

Parasites in feces of the endemic rattlesnake, *Crotalus triseriatus* (Serpentes: Viperidae), from Mexican highlands

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ABSTRACT. There are few studies about parasitic infections in *Crotalus triseriatus* (Wagler, 1830), an endemic rattlesnake from the highlands of central Mexico. This species occupies several habitats, from conserved forested regions to heavily impacted landscapes. To increase the parasitological knowledge of this reptile species without impacting populations, we obtained fecal samples of 16 rattlesnakes between 2012 and 2014 from Toluca Valley, Mexico. By using flotation technique, we found oocysts of *Eimeria* sp. and eggs of Capillariidae sp. The most frequent parasite was *Eimeria* sp. (62.5%). This study provides the first records of occurrence of parasites in reptilian feces from Mexico. Our results may be important for wildlife conservation purposes, for example, they could indicate of the population health of this species during processes of translocation.

KEY WORDS. Coccidia, Mexico, Nematoda, reptile, urban wildlife.

Reptiles are hosts of a wide variety of protozoan and metazoan parasites (Wilson and Carpenter 1996). Most of these parasites have been recorded through necropsies of specimens, although the diagnosis could be achieved by revealing parasitic elements in feces (e.g., eggs, larvae, oocysts). Even though, the latter non-invasive method only allows detecting intestinal parasites, may be useful for conservation projects, which involve knowing the health status of a reptile population in wildlife (Jorge et al. 2013).

In Mexico, 864 reptile species have been recorded (i.e., 8.7% of the reptiles of the world), of which 57% are endemic (Flores-Villela and García-Vazquez 2014). However, the inventory of their parasites is far from complete, since only a few more than 200 Mexican reptile species have been recorded as hosts of helminths, acari and crustaceans (Paredes-León et al. 2008).

Mexico holds the highest species richness of rattlesnakes, with 42 species (Heimes 2016), but wild populations have declined due to direct hunting and habitat loss caused by the expansion of croplands and urban areas. Particularly, the Mexican dusky rattlesnake, *Crotalus triseriatus* (Wagler, 1830), is restricted to the Trans-Mexican Volcanic Belt, where occupies forested regions heavily impacted (Bryson et al. 2014). This species is considered in the category of "least concern" in the IUCN and it has not received any level of protection by Mexican government due probably to a lack of knowledge in demographic and ecological aspects (Bryson et al. 2014, Sunny et al. 2015).

Translocations have increased for wildlife conservation, including the herpetofauna. One concern associated with conservation translocations is that the released individuals may suffer from parasitic diseases resulting in establishment failure. However, infectious hazards may be mitigated when diseases and/or parasites of translocated species or species at the destination are known (Bobadilla Suarez et al. 2017). Unfortunately, for *C. triseriatus* little is known about its parasites (Paredes-León et al. 2008), even though this information is essential to know the population health of this species and potentially useful in

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future translocation processes of species from one population to another. Our objective was to investigate the occurrence of parasites in feces of *C. triseriatus* to increase parasitological knowledge without affecting wild populations.

This study was conducted in Toluca Valley located in the State of Mexico, a highly anthropized area and considered the fifth largest metropolis in Mexico, with 2.1 million inhabitants (SEDESOL, CONAPO, INEGI 2004). The region has a humid temperate climate, with an annual precipitation of 500–600 mm and a range of average annual temperature between 12 and 15 °C (García 2004, Gobierno del Estado de México 2012). The sampling sites were El Cerrillo located at 5.1 km north of metropolitan area of Toluca City (19°24′27″N; 99°41′40″W; 2615 masl) and the Sierra Morelos Park located on the edge of the metropolitan area of Toluca City (19°18′39″N; 99°41′33″W; 2630 masl).

To capture individuals of *C. triseriatus* and collect individual fecal samples, during 2012 to 2014 we made 24 monthly visits at each study site, where five observers simultaneously walked five linear transects of 800 m long and separated by 300 m in a schedule of 09:00 am–13:00 pm. We capture the rattlesnakes with herpetological hooks and once subjected, we recorded total length (snout-vent length) in centimeters and weight in grams only with descriptive purposes. After we obtained fecal samples of each specimen place them in Eppendorf tubes and we released the rattlesnakes at their capture sites.

Each fecal sample was conserved in 70% alcohol and examined in the laboratory using the flotation technique (Bowman et al. 2004). Approximately 0.5 g of each sample was macerated and mixed with 9.0 ml of 0.8% saline solution. Each homogenized sample was filtered and placed in a vial, which was centrifuged for three minutes at 1500 rpm. After centrifugation, the supernatant was discarded, and a saturated glucose solution was used to re-suspend the pellet. The suspension was centrifuged for five minutes at 1000 rpm and three subsamples of top part of the flotation were obtained and examined by light microscopy. Parasitic elements (i.e., eggs and oocysts) were counted in each subsample to obtain an average per sample and to determine the levels of infection according to Rodríguez-Vivas and Cob-Galera (2005). Eggs and oocysts were photographed and measured using a light microscope at 40 and 100X magnifications (Leica DFC 490) and identified in accordance with Mehlhorn et al. (1993) and Wolf et al. (2014).

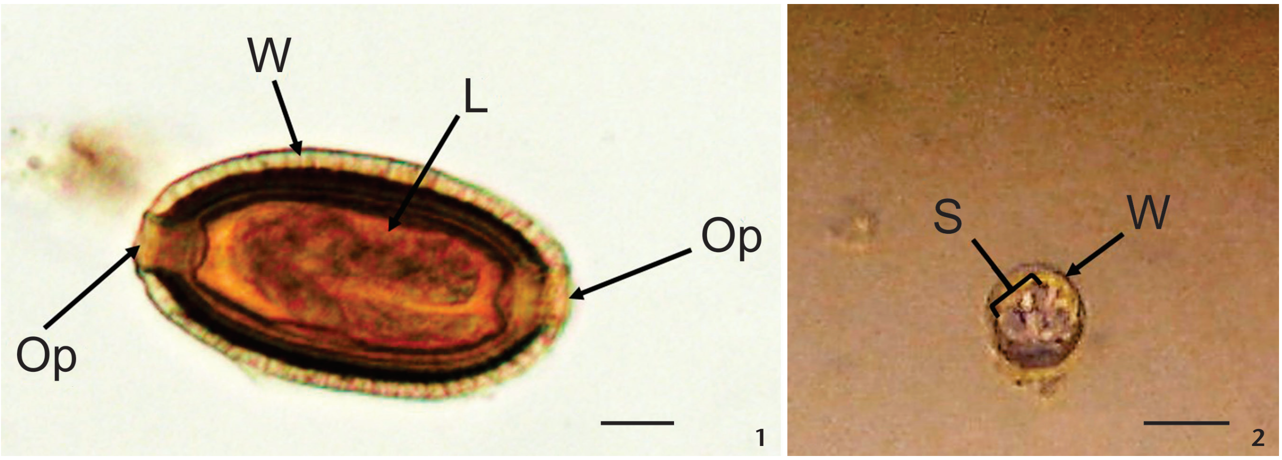
A total 16 Mexican dusky rattlesnakes were caught, 6 in El Cerrillo with 46–66 cm (56.42 ± 6.56) and 71–270 g (190.17 ± 73.7), and 10 in Sierra Morelos Park with 43–67 cm (55.03 ± 7.47) and 70–350 g (202.78 ± 78.19). Ten of the 16 fecal samples showed the presence of parasitic elements: nematode eggs of Capillariidae sp. and coccidian oocysts of *Eimeria* sp., with total prevalence of 62.5% y 6.25%, respectively (Table 1, Figs 1–2). The most frequent parasite was *Eimeria* sp. with high infection levels in both localities. Only one double parasitic infection was found in El Cerrillo.

Knowledge about the parasites of Mexican rattlesnakes is still far from complete. For example, in *Crotalus* spp. only 21 taxa of metazoan parasites (1 Acanthocephala, 3 Cestoda, 9 Nematoda, 3 Acari, and 5 Crustacea) have been recorded through necropsies of bred or wild specimens (Table 2). Of them, *Ophiotaenia perspicua* La Rue, 1911, *Kalicephalus inermis coronellae* (Ortlepp, 1923), *Ixobioides* sp., and *Ophionyssus natricis* (Gervais, 1844) were recorded in *C. triseriatus*. Thus, findings of Capillariidae sp. and *Eimeria* sp. represent new host records and this study is the first on the parasites of wild reptilians in Mexico using fecal samples.

The eggs of Capillariidae sp. found in *C. triseriatus* were not identified at specific level, since capillariid eggs are morphologically almost identical even among species of different genera (see Moravec 1994). Therefore, we cannot know if the nematode is a parasite or a pseudoparasite for Mexican dusky rattlesnakes, i.e., a parasite of the rodents on which hosts feed (personal observation of hair follicles in all fecal samples). In this context, Fuehrer (2014) recognized that endo- and ectoparasites of potential prey animals can be recorded as transiting parasites in the intestinal tract of reptiles, for example eggs of *Capillaria hepaticum* (Bancroft, 1893), a parasite species in mammals, have been documented in fecal samples from reptiles fed with infected rodents. On the other, Šlapeta et al. (2018) pointed out that the nematodes of *Capillaria* (Zeder, 1800) are the only known trichurids affecting reptiles, infecting primarily the intestine and secondarily other organs as the liver and gonads. It is known that their life cycle is direct, unfortunately the life cycles of snakes capillarids have not been investigated (Anderson 2000).

An examination of fecal samples of captive reptiles, including specimens of *Crotalus* Linnaeus, 1758, from Campania region in southern Italy revealed the most frequent parasites were species of groups of Oxyurida, *Rhabdias* Stiles & Hassall, 1905, *Kalicephalus* Molin, 1861, *Capillaria* and Eimeriidae Minchin, 1903 (Rinaldi et al. 2012). Analysis of the fecal samples in the present work confirmed that capillariids and eimeriids are the most frequent parasites of the reptiles, but they did not show the presence of any of the helminths recorded of rattlesnakes in Mexico (Table 2).

The oocysts of *Eimeria* sp. were not identified at specific level, because we did not observe some taxonomic characteristics. But it is known that *Eimeria* Schneider, 1875 is the most numerous group of snake coccidian with more than 80 species, which differ in both the morphology of exogenous stages (oocysts) and in endogenous development (Modrý 2004). Most species of *Eimeria* inhabit the intestine of reptiles, which become infected after ingesting oocysts. Schizogony occurs in the epithelial lining of intestine, depending on parasite species. Oocysts are shed with the feces following gametogony. The pathologic effects of coccidian appear mild for most reptiles; however, more severe cases resulting in epithelial ulceration and fibrosis, and septicemia from the loss of epithelial integrity, may occur (Mitchell 2007).



Figures 1–2. Parasitic elements in feces of *Crotalus triseriatus* from Mexico: (1) Egg of Capillariidae sp. with one larva (L), brown color, thick and rough wall (W), one operculum at each pole (Op), which are asymmetrical. (2) Sporulated oocyst of *Eimeria* sp., spheroidal type, rough outer wall (W), absence of micropyle, ovoid sporocysts (S). Scale bars: 10 µm.

Table 1. Infection levels of parasitic elements in feces of *Crotalus triseriatus* from two localities in the State of Mexico, Mexico. Range of oocysts/eggs per gram of feces and infection levels slightly modified of Rodríguez-Vivas and Cob-Galera (2005): 2–9 very low (+), 8–18 Low (++), 20–30/10–15 Medium (+++), 32–50 High (++++).

Localities	Host			Collection date	*Eimeria* sp.	Capillariidae sp.
	ID	Length (cm)	Weight (g)			
Sierra Morelos Park	SMP1	43.0	70	May 26, 2012	+	Negative
	SMP2	57.0	233	July 12, 2012	Negative	Negative
	SMP3	63.0	250	September 15, 2012	Negative	Negative
	SMP4	59.0	212	September 15, 2012	+++	Negative
	SMP5	53.0	Undetermined	April 27, 2014	++	Negative
	SMP6	53.0	185	September 20, 2014	Negative	Negative
	SMP7	51.8	200	October 18, 2014	++++	Negative
	SMP8	45.0	125	October 18, 2014	++++	Negative
	SMP9	67.0	350	November 01, 2014	Negative	Negative
	SMP10	58.5	200	November 01, 2014	Negative	Negative
El Cerrillo	CE1	66.0	250	October 20, 2012	++++	Negative
	CE2	54.5	140	December 08, 2012	+++	+++
	CE3	46.0	71	December 08, 2012	++	Negative
	CE4	55.0	200	August 30, 2014	+	Negative
	CE5	58.0	210	November 08, 2014	+++	Negative
	CE6	59.0	270	November 08, 2014	Negative	Negative

Jorge et al. (2013) found the detectability of nematodes was significantly lower in feces than in the intestine of reptiles, suggesting a large number of fecal samples and temporal replication to increase the likelihood of detection of intestinal parasites. Undoubtedly, parasitological studies based solely on feces most probably underestimate the true species richness present in a given host population and/or locality. However, such studies may be important for wildlife conservation purposes mainly when the translocations are employed, since the released individuals or individuals within the destination ecosystem may suffer from parasitic disease linked to the translocation process (Bobadilla Suarez et al. 2017). Specifically, it has been shown the vulnerability of populations of *C. triseriatus* in the Toluca Valley, due to the proximity to urban areas (Sunny et al. 2015), therefore, a complete parasitological record would aid wellbeing of snakes by receiving appropriate treatment plan and/or quarantine procedures. That allow to move the rattlesnakes outside the urban area, without the danger of infecting other populations with any diseased.

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Table 2. Records of metazoan parasites for *Crotalus* spp. in Mexico.

Parasite	Stage	Habitat	Host	State	Reference
Cestoda					
Mesocestoides sp.	Tetratiridium	Body cavity	*C. enyo* (Cope, 1861)	Baja California Sur	Goldberg et al. (2003)
			C. molossus Baird & Girard, 1853	Undetermined	Goldberg and Bursey (1999)
			C. pricei Van Denburgh, 1895		
			C. willardi Meek, 1905	Chihuahua, Sonora	Goldberg and Bursey (2000)
Oochoristica sp.	Adult	Intestine	*C. basiliscus* (Cope, 1864)	Colima	Paredes-León et al. (2008)
Ophiotaenia perspicua	Adult	Intestine	*C. cinerens*	Hidalgo	Flores-Barroeta et al. (1961)
Acanthocephala					
Oligacanthorhynchidae sp.	Cystacanth	Body cavity	*C. basiliscus*	Michoacán	Goldberg et al. (2006)
			C. enyo	Baja California Sur	Goldberg et al. (2003)
			C. lepidus (Kennicott, 1861)	Undetermined	Goldberg and Bursey (1999)
			C. tigris Kennicott, 1859		
			C. willardi	Chihuahua, Sonora	Goldberg and Bursey (2000)
Nematoda					
Hastospiculum onchocercum Chitwood, 1932	Adult	Stomach	*C. tzabcan* Klauber, 1952	Quintana Roo	Carbajal-Márquez et al. (2018)
Hexametra boddaertii (Baird, 1860)	Adult	Body cavity	*C. tzabcan*	Yucatán	Carbajal-Márquez et al. (2018)
			C. basiliscus	Sinaloa	Goldberg et al. (2006)
Kalicephalus inermis coronellae	Adult	Intestine	*C. pusillus* Klauber, 1952	Michoacán	Comroe (1948), Schad (1962)
			C. triseriatus		
Kalicephalus inermis macrovulvus (Caballero, 1954)	Adult	Intestine	*C. molossus*	Ciudad de México	Goldberg and Bursey (1999)
Macdonaldius oschei Chabaud & Frank, 1961	Adult	Post vena cava	*C. basiliscus*	Colima	Telford (1965)
Ophidascaris labiatopapillosa Walton, 1927	Adult		*C. molossus*	Durango	Klauber (1972)
Ozolaimus ctenosauri Caballero, 1938	Adult	Intestine	*C. polystictus* (Cope, 1865)	Ciudad de México	Caballero y Caballero (1939)
Physocephalus sp.	Larva	Mesentery	*C. mitchellii* (Cope, 1861)	Baja California	Goldberg et al. (2013)
Travassosascaris araujoi (Schneider, 1866)	Adult	Liver	*C. tzabcan*	Quintana Roo	Carbajal-Márquez et al. (2018)
Acari					
Amblyomma dissimile Koch, 1844	Adult	Body surface	*C. durissus* Linnaeus, 1758	Guerrero	Paredes-León et al. (2008)
Argasidae sp.		Body surface	*C. viridis viridis* (Rafinesque, 1818)	Chihuahua	Gatica-Colima et al. (2014)
Ixobioides sp.	Adult	Body surface	*C. triseriatus*	Ciudad de México	Paredes-León et al. (2008)
Ophionyssus natricis	Adult	Body surface	*C. durissus*	Ciudad de México	Paredes-León et al. (2008)
			C. triseriatus		
			C. cerastes cercobombus Savage & Cliff, 1953	Nuevo León	Rodríguez and Lazcano (1992)
			C. lepidus lepidus (Kennicott, 1861)		
			C. atrox Baird & Girard, 1853	Puebla	Paredes-León et al. (2008)
			C. ravus Cope, 1865		
Ornithodoros turicata (Dugès, 1876)	Larva		*C. mitchellii*	Baja California	Gutsche and Mutschmann (2011)
			C. ruber Cope, 1892	Baja California Sur	
Crustacea					
Porocephalus basiliscus Riley & Self, 1979	Adult		*C. basiliscus*	Colima	Riley and Self (1979)
Porocephalus crotali Humboldt, 1812	Adult		*C. atrox*	Nuevo León	Peláez and Julia (1983)
			C. basiliscus	Colima	Peláez and Julia (1983)
			C. catalinensis Cliff, 1954	Santa Catalina Island, Baja California Sur	Goldberg et al. (2003)
			C. culminatus Klauber, 1952	Guerrero	Pelaéz and Julia (1983)
			C. durissus	Tamaulipas	Peláez and Julia (1983)
			C. tzabcan	Campeche, Quintana Roo	Carbajal-Márquez et al. (2018)
Porocephalus tortugensis Riley and Self, 1979	Adult		*C. tortugensis* Van Denburgh & Slevin, 1921	Tortuga Island-Baja California Sur	Riley and Self (1979)
Raillietiella crotali Ali, Riley and Self, 1984	Adult	Lung	*C. ruber*	Pond Island, Baja California	Ali et al. (1984)
Raillietiella furcocercum (Diesing, 1836)	Adult	Lung	*C. atrox tortugensis* Baird & Girard, 1853	Tortuga Island-Baja California Sur	Ali et al. (1984), Klauber (1972)

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