

# Asian Pacific Journal of Tropical Disease

journal homepage: <http://www.apjtdm.com>



Original article <https://doi.org/10.12980/apjtd.7.2017D7-48>

©2017 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

## A study on endoparasitic and ectoparasitic fauna of snakes in Mizoram, India

Gautam Patra<sup>1\*</sup>, Sonjoy Kumar Borthakur<sup>1</sup>, Sheikh Sahanawaz Alam<sup>2</sup>, Subhamoy Ghosh<sup>1</sup>, Hmar Lalliankimi<sup>3</sup>, Hniang Lalrinkima<sup>1</sup>

<sup>1</sup>Department of Veterinary Parasitology, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, India

<sup>2</sup>Department of Veterinary Microbiology, West Bengal University of Animal & Fishery Sciences, K.B. Sarani, Kolkata, W.B., India

<sup>3</sup>Department of Livestock Production & Management, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, India

### ARTICLE INFO

#### Article history:

Received 7 Mar 2017

Received in revised form 9 May 2017

Accepted 18 Jun 2017

Available online 4 Aug 2017

#### Keywords:

Endoparasites

Ectoparasites

Snakes

SEM

Mizoram

India

### ABSTRACT

**Objective:** To record the prevalence of parasitic fauna of snakes from different parts of Mizoram, India.

**Methods:** Collected fecal samples of different snakes were examined by sedimentation and floatation techniques. Similarly, blood samples were examined for presence of any haemoprotozoa following Giemsa staining technique. Ectoparasites were identified on the basis of morphological keys. Scanning electron microscopy (SEM) was performed for detailed surface structure studies of few parasites.

**Results:** Sixty one percent (40/65) snakes examined were found positive for parasitic infection. The predominant endoparasites included five species of nematodes, one species of cestodes, one species of tissue protozoa, and two species of haemoprotozoa. Ectoparasites recorded were ticks of the genera *Aponomma* and *Amblyomma*. The most abundant nematode recorded was *Kalicephalus* species.

**Conclusions:** The study indicates that parasitic infection of snakes is quite common in this part of India and deserves attention for zoological studies.

## 1. Introduction

Reptile plays a significant role in the ecosystem. They become one of the common pet animals throughout the world. Like other species, they can also harbour various parasites[1-7]. In zoos, reptilian species including snakes are often closely confined together and this causes stress resulting in alteration of host-parasite relationship. Sometimes reptile can also be responsible for transmitting disease to animals and humans. The reptiles harbor parasites for a considerable period of time without showing any observable symptoms unless other factors undermine the host immunity and allow the disease to develop[8].

For veterinarians or zookeepers, accurate identification of parasites and their stages is crucial for health of reptiles. Apart from sporadic reports on parasitic infections of reptiles from different parts of India[5-7], there is limited information on prevalence of parasites in snakes.

In view of the above mentioned, the present study was conducted

to record endoparasites and ectoparasites of snakes by various methods. Scanning electron microscopy (SEM) was also applied for detailed surface structures of few parasites.

## 2. Materials and methods

### 2.1. Place of study

Study area was confined to different parts of Aizawl District of Mizoram and Aizawl Zoo, Mizoram, where snakes are kept for public view. The survey was carried out from October, 2016 to January 2017. Further, few snakes run over by vehicles or killed by people were also included in this survey. A total of 65 snakes belonging to the families Pythonidae, Viperidae, Elapidae, and Colubridae were examined.

### 2.2. Collection and examination of fecal samples

Fresh feces ( $n = 235$ ) were collected from captive snakes as practicable as possible. Direct smear, flotation and sedimentation techniques were used for fecal examination as per standard procedure[9-12]. Fecal samples of dead snakes were examined during post mortem examination.

### 2.3. Examination of blood samples

Blood samples were collected from few live snakes at captivity as well as from the recently killed snakes and stained with Giemsa stain

\*Corresponding author: Gautam Patra, Department of Veterinary Parasitology, College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl, India.

Tel: +91 8582859415

E-mail: [dr.gautampatra@yahoo.co.in](mailto:dr.gautampatra@yahoo.co.in)

All experimental procedures were conducted according to Institutional Animal Ethic Committee with reference No. CVSc/CAU/IAEC/no. 6641, dtd, Selesih, the 25th, April, 2016.

Foundation Project: Supported by Central Agricultural University, Imphal, Manipur, India by granting college research funding support (sanction order No. IRP/1921/NE/2016, dtd, the May, 31, 2016).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

as per procedure and examined under oil immersion microscope.

#### 2.4. Postmortem examination

At least 30 dead snakes were brought to the Department of Parasitology during the whole study period and postmortem examinations were conducted. Snakes were cut open in the laboratory, and viscera were examined and any parasites recovered were kept in 70% alcohol for further study.

#### 2.5. Ectoparasite survey

Skin of live or dead snakes was carefully examined for presence of any ectoparasites. Ectoparasites found were also stored in 70% alcohol for further study. Bodies of the ticks were perforated with entomological pin before boiling with 10% potassium hydroxide for 5–10 min. The treated samples were then placed in 70% alcohol for 5 min and 90% alcohol for another 5 min for dehydration. Finally, the specimens were kept in carbolic acid for 10 min before mounting in DPX.

#### 2.6. Preparation of sample for SEM

Nematodes were fixed in 3% glutaraldehyde. Before dehydration, any unwanted materials like fecal debris, mucus, blood or other body fluids were carefully removed by washing several times with nuclease free water (NFW) with the help of a camel brush. The samples were then fixed at 4 °C for 24 h. The fixed nematodes were washed in phosphate buffered saline (pH 7.2) for three times and then in double distilled water followed by acetone dehydration. After acetone dehydration, the specimens were dried with liquid CO<sub>2</sub> at its crucial point *i.e.* 31.5 °C at 1100 psi. The specimen was then dipped in tetra methyl saline (TMS) for 5–10 min with two changes at 4 °C. They were then dried at room temperature (25–26 °C). The samples were then mounted on aluminium stubs. Finally the samples were coated with gold sputter and observed under SEM (JSL 6300– JEOL) at sophisticated analytical instrument facilities laboratory at North Eastern Hill Univeristy (NEHU), Shillong, Meghalaya, India.

#### 2.7. Ethical statement

All experimental procedures were conducted according to Institutional Animal Ethic Committee with reference No. CVSc/CAU/IAEC/no. 6641, dtd, Selesih, the 25th, April, 2016.

### 3. Results

A total of 65 snakes belonging to 5 families (Pythonidae,

Colubridae, Elapidae and Viperidae) were examined and the results of the fecal samples and blood samples are shown in Table 1. Thirty snakes (46.15%) were found to be infected with six different species of nematodes and one species of cestode. Two snake fecal samples were positive for *Isospora* sp. and seven snakes were found infected with two species of haemoprotozoans, namely, *Hepatozoon* sp. and *Trypanosoma* sp.

**Table 1**

Endoparasites of snakes.

Endoparasites		Venomous (n = 35)	Non-venomous (n = 30)
Nematodes	<i>Kalicephalus</i> sp.	10	3
	<i>Ophidascaris</i> sp.	5	2
	<i>Polydelphis</i> sp.	0	2
	<i>Gnathostoma</i> sp.	1	0
	<i>Tanga tiara</i>	1	0
	<i>Strongylus</i> sp.	2	2
Cestodes	<i>Ophiotaenia</i> sp.	1	1
Tissue protozoa	<i>Isospora</i> sp.	1	1
Haemoprotozoa	<i>Hepatozoon</i> sp.	2	3
	<i>Trypanosoma</i> sp.	2	0

Results of the ectoparasitic fauna of different snakes are shown in Table 2. Nine snakes (13.84%) were positive for ticks *Aponomma varanense* and 5 (7.69%) positive for *Amblyomma gervaisi*.

**Table 2**

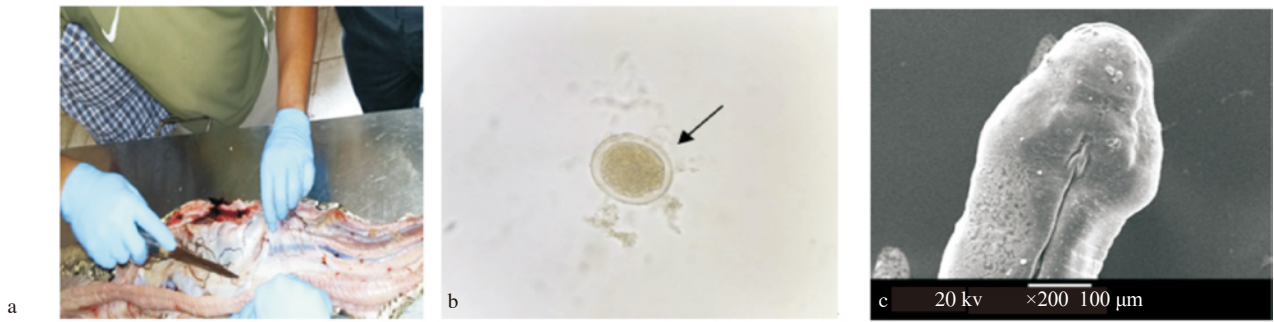
Ectoparasites of snakes.

Ectoparasites	Species of snakes			
	<i>P. molurus</i> (n = 20)	King cobra (n = 20)	Green viper (n = 15)	<i>Ptyas mucosa</i> (n = 10)
<i>Aponomma varanense</i>	4	3	1	1
<i>Amblyomma gervaisi</i>	1	2	1	1

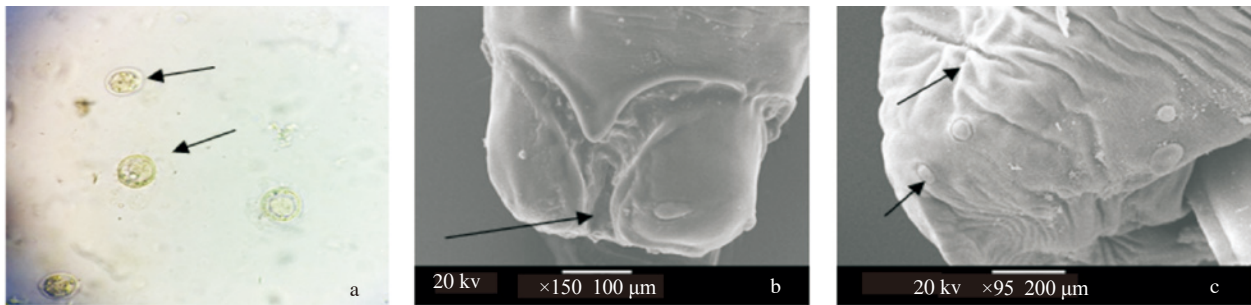
Based on egg and morphology under light and electron microscopy, five species of nematodes were identified as *Kalicephalus* sp. (Figure 1), *Ophidascaris* sp. (Figure 2), *Polydelphis* sp. (Figure 3), *Strongylus* sp. (Figure 4) and *Gnathostoma* sp. (Figures 5 and 6). Further, tapeworm ova (Figure 7) were detected only in rat snakes (*Ptyas mucosa*) and isosporan oocysts detected in two fecal samples (Figure 8). Five snakes (one python, two green vipers and two king cobras) were found positive for *Hepatozoon* sp.; on the other hand, two venomous snakes were found positive for *Trypanosoma* sp. (Figure 9). Light microscopical (LM) and SEM pictures of *Aponomma varanense* and *Amblyomma gervaisi* are shown in Figure 10.



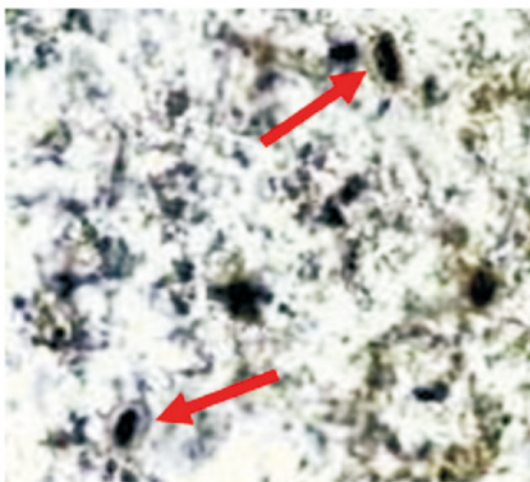
**Figure 1.** Egg of *Kalicephalus* sp. (40×) (a), anterior part of *Kalicephalus* sp. (b) and posterior part of *Kalicephalus* sp. (c).



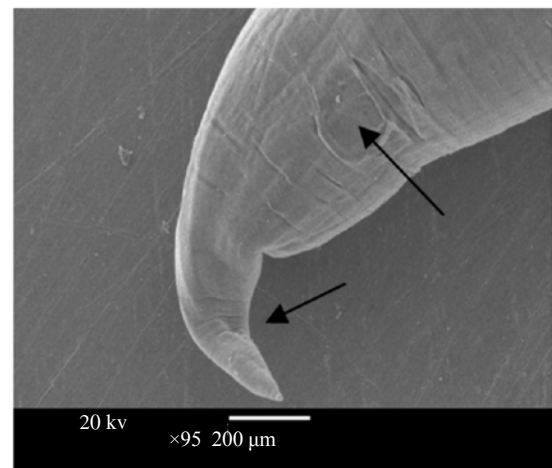
**Figure 2.** Adult *Ophidascaris* sp. from a dead python (a), egg of *Ophidascaris* sp. (b) and anterior part of *Ophidascaris* sp. (c) under SEM.



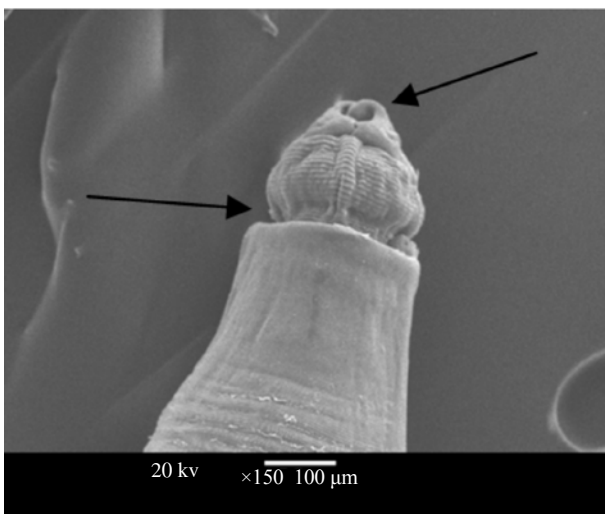
**Figure 3.** Egg of *Polydelphis* sp. (a), anterior end of *Polydelphis* sp. (b) and posterior end of male *Polydelphis* sp. (c).



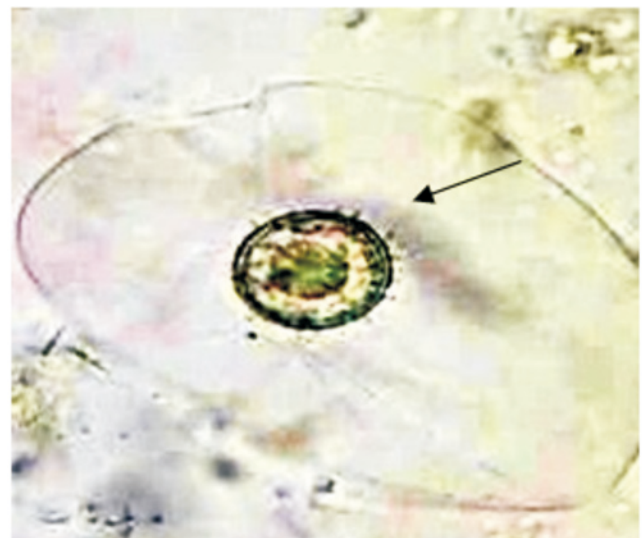
**Figure 4.** Egg of *Strongylus*.



**Figure 6.** Posterior end of female *Gnathostoma* sp.



**Figure 5.** Anterior end of *Gnathostoma* sp.



**Figure 7.** Egg of tapeworm (10×).



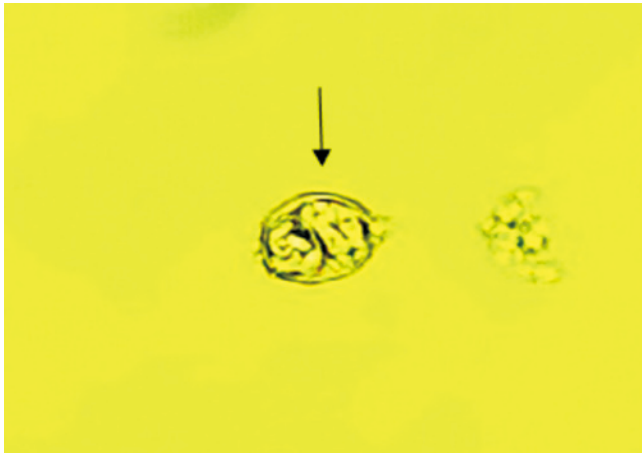


Figure 8. Sporulated oocyst of *Isospora* sp.

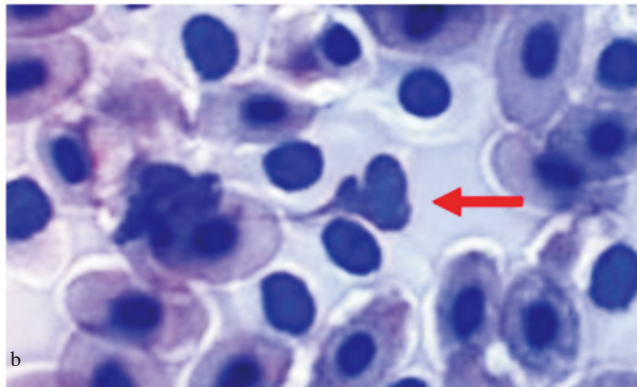
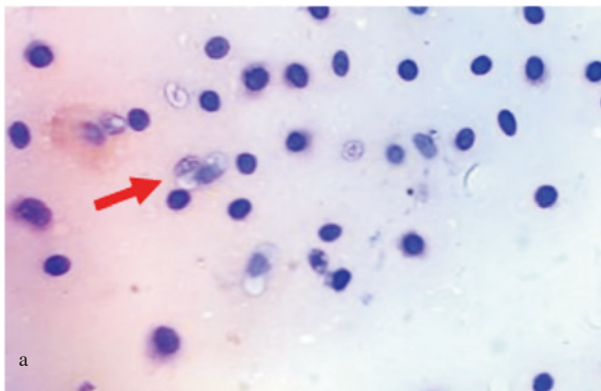


Figure 9. *Hepatozoon* sp. (a) and *Trypanosoma* sp. (b) in the blood of snake.

#### 4. Discussion

The present study was undertaken to record the baseline data of ectoparasitic and endoparasitic fauna of snakes of different parts of Mizoram, India. Parasites may have detrimental effects on the well-being of reptiles kept in zoos, farms or as domestic pets[4,5]. The different techniques were used for detection of parasitic ova in feces of snakes as per previous researchers[9-12]. For the identification of nematode eggs and protozoan oocysts, fecal floatation was found superior to other methods. Eggs and oocysts were identified based on morphometry. The result indicated high prevalence of *Kalicephalus* sp. (20%) which agreed with the finding of previous reports[13,14]. The species of *Kalicephalus* is characterized by a strongly developed buccal capsule with three teeth like papillae and a well developed oesophagus with bulbous posterior end. The high prevalence of *Kalicephalus* sp. may be due to sub-optimal conditions of the captivity, lack of deworming practices in zoo as well as different feeding habits of snakes. At postmortem examinations, adult worms were identified on the basis of morphology and ova were detected from the gut contents. Ascarid eggs and coccidian oocysts as well as tapeworm were also detected. Ascarid eggs were spherical to sub-spherical in shape with brownish yellow shell, striated and were about 5–6  $\mu$ m in thickness. The prevalence rates of ascarid in poisonous and non-poisonous snakes were found similar. *Ophidascaris* was more common in venomous snakes, while *Polydelphis* was more abundant in non-poisonous snakes. Several species of the family Ascarididae including *Ophidascaris* and *Polydelphis* have been reported[15-17]. De Souza *et al.*[18] and Bino Sundar *et al.*[19] also reported *Ophidascaris* in snakes. Eggs of *Ophidascaris* and *Polydelphis* were found in the fecal samples from pythons, cobras, green vipers and rat snakes. Out of 26 venomous snakes, 10 were found to have mixed infections. On the other hand, out of 14 non-venomous snakes, 4 had mixed infections.

Only one rat snake was found positive for cestode infection. This is in contrast to the previous report by Rajesh *et al.*[11] who failed to record any tapeworm infection.

In the present investigation, *Gnathostoma* infection in one Indian cobra was recorded. The parasite was found morphologically similar to the species *Tangra tiara* observed by Chanhome and Chaiyabutr[13]. Two haemoprotozoan parasites namely *Hepatozoon* sp. and *Trypanosoma* sp. were also observed in this present study. Smith[20] reported that *Hepatozoon* species are the most frequently

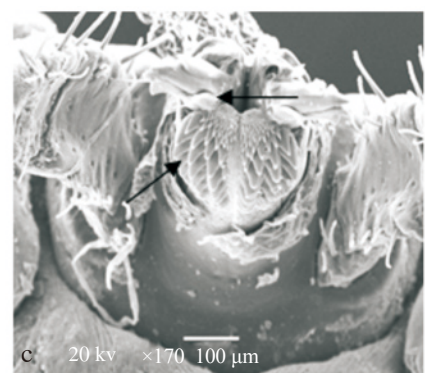
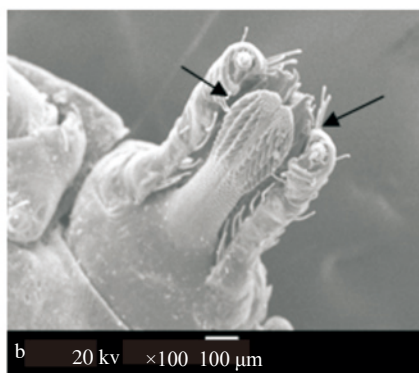
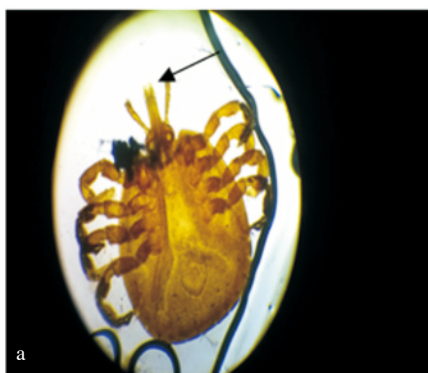


Figure 10. *Amblyomma gervaisi* (a), anterior part of *Amblyomma gervaisi* under SEM (b) and mouth parts of *Aponomma varanense* (c) under SEM.

occurred haemogregarins in different species of snakes. Two venomous snakes and three non-venomous snakes were found positive for *Hepatozoon* sp. Two venomous snakes were also found positive for *Trypanosoma* sp.

Lower level of coccidian infections was found in the present study. *Aponomma varanense* and *Amblyomma gervaisi* were the only two genera of ticks recovered from 14 snakes in the present study. The *Aponomma varanense* was identified on the basis of keys provided by Hoogstraal et al.[21]. BurrIDGE[22] reported that *Aponomma varanense* is one of the most widespread species of the genus *Aponomma*, occurring in snakes of many countries including India. Horak et al.[23] synonymized *Aponomma* and *Amblyomma* under the same genus *Amblyomma*; however, many scientists have not accepted this modification. *Amblyomma gervaisi* was found more abundant in poisonous snakes than in non-poisonous one. Ectoparasites, especially ticks, are frequently seen in wild reptiles but the level of infection and the effects of ticks on the snakes are poorly studied[24]. Although tick rarely occurs in large numbers in snakes to cause direct injuries, they can act as carriers of vector borne diseases to other animals and even to humans.

The findings indicated that many snakes harboured ectoparasites or endoparasites which may be due to several factors that are complementary to each other such as feeding habits, environmental conditions, management practices, immune deficiency, etc. Hence, it is worthy to examine clinical samples for health status of snakes under captivity. Furthermore, antiparasitic treatment and better management ensure the health and well being of snakes.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

The authors are thankful to the Dean, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, India for providing necessary facilities for conducting the experiment.

### References

- [1] Al-Moussawi AA. Incidence of *Telorchis assula* (Dujardin, 1845) (Digenea: Telorchidae) in two colubrid snakes in Baghdad city, Central Iraq. *J Entomol Zool Stud* 2015; **3**(2): 321-3.
- [2] Choe S, Lim J, Kim H, Kim Y, Kim H, Lee D, et al. Three nematode species recovered from terrestrial snakes in Republic of Korea. *Korean J Parasitol* 2016; **54**(2): 205-13.
- [3] Hedley J, Eatwell K, Shaw DJ. Gastrointestinal parasitic burdens in tortoises: a survey of tortoise owners and potential risk factors. *Vet Rec* 2013; **173**: 525.
- [4] Cervone M, Fichi G, Lami A, Lanza A, Damiani GM, Perrucci S. Internal and external parasitic infections of pet reptiles in Italy. *J Herpetol Med Surg* 2016; **26**(3-4): 122-30.
- [5] Pandit P, Bandivdekar R, Geevarghese G, Pande S, Mandke O. Tick infestation on wild snakes in northern part of Western Ghats of India. *J Med Entomol* 2011; **48**: 504-7.
- [6] Ghosh HS, Misra RK. Scanning electron microscope study of a snake tick, *Amblyomma gervaisi* (Acari: Ixodidae). *J Parasit Dis* 2012; **36**(2): 239-50.
- [7] Patra G, Borthakur SK, Devi RS, Lalrinkima H, Lalliankimi H. Surface ultra structural studies of *Aponomma varanense* of an Indian King Cobra (*Ophiophagus hannah*). *J Entomol Zool Stud* 2016; **4**(6): 433-7.
- [8] Griffin C, Reavill DR. Common pathology and diseases seen in pet store reptiles. In: Douglas RM, Stephen JD, editors. *Current Therapy in Reptile Medicine and Surgery*. United States: Saunders; 2014, p. 13-9.
- [9] Wolf D, Vrhovec M, Failing K, Rossier C, Hermosilla C, Pantchev N. Diagnosis of gastrointestinal parasites in reptiles: comparison of two coprological methods. *Acta Vet Scand* 2014; **56**: 44.
- [10] De Souza JL, Barbosa Ada S, Vazon AP, Uchoa CM, Nunes BC, Crtez MB, et al. Parasitological and immunological diagnoses from feces of captive bred snakes at Vital Brazil Institute. *Rev Bras Parasitol Vet* 2014; **23**(2): 123-8.
- [11] Rajesh NV, Rajesh KD, Jayathangaraj MG, Raman M, Sridhar R. Parasitic fauna of captive snakes in Tamilnadu, India. *Asian Pac J Trop Dis* 2015; **5**(7): 547-51.
- [12] Zajac AM, Conboy GA. Fecal examination for the diagnosis of parasitism. In: Zajac AM, Conboy GA, editors. *Veterinary Clinical Parasitology*. 8th ed. Ames: Wiley-Blackwell Publishing; 2012, p. 3-27, 164-9.
- [13] Chanhom L, Chaiyabutr N. Parasites in snakes of Thailand. *Bull Maryland Herpetol Soc* 2002; **38**(2): 50.
- [14] Rataj AV, Lindtner-Knific R, Vlahovic K, Mavri U, Dovc A. Parasites in pet reptiles. *Acta Vet Scand* 2011; **53**: 53-68.
- [15] Biswas D, Das S, Abdul AMD. The first record of *Ophidascaris* (Nematode: Ascarididae) in the Asian Rock Python (*Python molurus molurus*) in Bangladesh. *Sch J Agric Sci* 2014; **4**(1): 24-6.
- [16] Murvanidze L, Lomidze T, Nikolaishvili L. The endoparasites (Pentastomida, Nematoda) of African Rock Python (*Python sevae*; Gmmailn,1788) in Tbilise Zoological Park. *Bull Georgian National Acad Sci* 2015; **9**(3): 143-9.
- [17] Ganjali M, Keighobadi M, Khedri J. First record of *Ophidascaris filaria* (Nematode: Ascarididae) from the Indian Python in Sistan, Iran. *Comp Clin Pathol* 2015; **24**: 1285-8.
- [18] De Souza JL, Barbosa Ada S, Vazon AP, Uchoa CMA, Nunes BC, Cortez MBV, et al. Parasitological and immunological diagnoses from feces of captive-bred snakes at Vital Brazil Institute. *Rev Bras Parasitol Vet* 2014; **23**(2): 123-8.
- [19] Bino Sundar ST, Kavitha KT, Gomathinayagam S, Sangaran A, Latha BR, Harikrishnan TJ. A report on the incidence of *Ophidascaris* sp. in a reticulated python. *Int J Sci Environ* 2016; **5**(5): 3328-31.
- [20] Smith TG. The genus *Hepatozoon* (Apicomplexa: Adeleina). *J Parasit* 1996; **82**: 565-85.
- [21] Hoogstraal H, Wasseff HY, Buttiker W. Ticks (Acarina) of Saudi Arabia Fam. Argasidae, Ixodidae. *Fauna Saudi Arabia* 1981; **32**: 25-110.
- [22] BurrIDGE MJ. Ticks (Acari: Ixodidae) spread by the international trade in reptiles and their potential roles in dissemination of diseases. *Bull Entomol Res* 2001; **91**: 3-23.
- [23] Horak IG, Camicas JL, Kaeirans JE. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names. *Exp Appl Acarol* 2002; **28**: 27-54.
- [24] Zug GR, Vitt LJ, Caldwell JP. *Herpetology: an introductory biology of amphibians and reptiles*. 2nd ed. San Diego: Academic Press; 2001.