World Journal of Zoology 10 (2): 83-88, 2015
ISSN 1817-3098

DOI: 10.5829/idosi.wjz.2015.10.2.92164

Ultrastructural Charachteristic of a New *Sarcocystis* sp. Infecting the *Lizard scincusscincus* in Saudi Arabia

[1]Abdullah D. Alanazi, [2]Mohamed S. Alyousif and [3]Hesham M. Sharaf

[1]Department of Biological Sciences, Faculty of Science and Humanities,
Shaqra University, P.O. Box 1040 Ad-Dawadimi 11911, Saudi Arabia
[2]Department of Zoology, College of Science, King Saud University,
P.O. Box 26213, Riyadh 11486, Saudi Arabia
[3]Zoology Department, Faculty of Science, Zagazig University, Egypt

Abstract: In the present study 35 male and female *Scincusscincus*lizard were collected from different localities around Riyadh city, Saudi Arabia and investigated for Sarcocystis infection. Light microscopic examination of muscle cryosections and fecal samples indicate the infection of skeletal muscles only with sarcocysts of the genus Sarcocystis. No indication for the shedding of any coccidian oocysts and/or sporocysts were proved. This natural infection were concentrated in the tail muscles followed by the hind limb. The infection rate of such infection was about 11%(4/35). Light microscopic examination of semithin sections showed microscopically visible sarcocysts limited by thin cyst wall. Clear septa extending from the ground substance into the cyst interior dividing it into several compartments were observed. Cyst zoites were differentiated into cyst merozoites and metrocystes.Transmission electron microscopic examination showed the architecture and ornaments of the primary cyst wall and its protrusions. The cyst merozoitesand metrocytes have all the apicomplexan characteristics of motile stages peculiar structure of the primary cyst wall and its protrusions indicates new species of the genus *Sarcocystis*.

Ke words: SarcocytisSpp · Oocyst-reptiles · Electron microscopy · Saudi Arabia.

INTRODUCTION

In the year 1843 Miescher[1] observed, for the first time, cysts containing large numbers of banana-shaped Protozoan zoites in the skeletal muscles of a mice. These were termed Miescher's tubule until 1882. Because of the typical localization of the cysts within the muscles, Balbiani[2] proposed the name Sarcosporidia.

Today such cysts have been recorded in several reptiles, birds and many groups of mammals including man [3,4]. Among the different animals examined in this respect, reptiles are of special interest because they are very ancient group in the evolutionary scale and their parasites seem to become stabilized allover the world [5]. Moreover, this group of animals are very important in the natural balance of the ecosystem and they are considered as the stem of animal kingdom.

Regarding the obligatory heteroxenous life cycle of Sarcosporidia, reptiles were found to be the intermediate and/or the final host of such parasites [6-16]

The host-parasite relationship and the pathogenecity of such parasite are of great importance. Sarcocystis was found to be highly pathogenic to its host causing emuciation, fever, myocarditis, weight loss…etc and may lead to their death [17-19]

In spite of the intensive efforts oriented to identify and elucidate the parasitic fauna infecting Saudi animals, information and surveys on coccidian parasites specially Sarcosporidia infecting reptiles still scarce, inadequate and incomplete. To the best of our knowledge, the only published data in this respect are restricted to that of Abdel- Ghaffar*et al*.[18] and Abdel-Ghaffar and Al-Johany [20].

Corresponding Author: Mohamed S. Alyousif, Department of Zoology, College of Science, King Saud University, P.O. Box 26213, Riyadh 11486, Saudi Arabia. E-mail: sharaf_hesham@yahoo.com.

Therefore, this study aims to investigate the prevalence of natural *Sarcocystis* infection and the role of the examined hosts in the life cycle of such parasite and the ultrastructural features of the stages developed in one of the abundant skink *Scincusscincus*in Saudi Arabia fauna.

MATERIALS AND METHODS

A total of 35 male and female skinks *Scincusscincus* (Scincidae, Lacertilia, Reptilia) were collected from the central provinceofSaudi Arabia around Riyadh city. Animals were brought to the Parasitology Research Laboratory, Faculty of Science and Humanities, Shaqra University, where they were identified according to Arnold [21] and examined for the prevalence of*Sarcocystis* species infecting the freshskeletal muscle samples were snipped from the end of the tail region to detect Sarcocysts infections.

Heavily infected animals were isolated, dissected and skeletal muscle samples of different regions including tail, hind and fore limbs and abdominal muscles were examined by naked eye for macroscopic cysts and applying the cryosections technique [21] to detect the microscopic form. To identify the natural infection as final host of Sarcocystis fecal samples of the collected animals were daily examined for coccidian oocysts and/or sporocysts for 2 weeks applying the flotation technique[21].

For histological examination, heavily infected tissues were immediately fixed in 3% glutaraldehydebuffered in 0.1 M sodium cacodylate buffer (pH 7.3) at 4°C for at least 4h. The specimens were post fixed with 2% OsO4 in the same buffer. Fixed materials were processed in the usual technique for light and transmission electron microscopic examination. The Specimens were dehydrated in graded ethanol, transferred to propylene oxide and finally embedded in Araldite embedding medium. Semi-thin and ultra-thin sections were cut on a Reichert Ultracut. Semi-thin sections were stained with methylene blue and azureA and examined by Zeiss photo-research microscope. At the same time ultra-thin sections were contrasted with uranyl acetate and lead citrate before examination in Joel transmission electron microscope [18].

RESULTS

Prevalence of Natural Infection: Examination of the different muscle tissues of the collected skinks revealed the infection of 4animals only (4/35) where microscopic cysts were detected. In such study the infection rate was (11%). Regarding the site of infection, the tail muscles followed by the hind limb were the heavily infected sites. In contrast, feacal examination of collected skinks showed that they were devoid of any coccidian oocystsand/or sporocysts which proved that these animals are acting as intermediate host only of such Sarcocystis parasite.

Histological Examination

Light Microscopicexamination: Light microscopic investigation showed the presence of mature cyst from sarcocysts which appeared elongated in form. Each cyst were enclosed in thick and hairy cyst wall. The interior of the cyst seemed to be divided into several compartments by a number of septa extending from the ground substance just beneath the primary cyst wall to the core of the cyst including banana shaped merozoites and oval to rounded metrocytes (Figs. 1,2).

Transmission Electron Microscopic Examination: Transmission electron microscopic study proved that all sarcocysts examined had a single thin primary cyst wall only. Secondary cyst wall was not detected in the present study. Examination of the architecture and ornaments of this cyst wall showed that the primary cyst wall gave rise to hairy, leaf like stalked non branched protrusions which are broader at their bases and gradually get narrow to their blunt end. The protrusions were mostly vertically arranged to the primary cyst wall in palaside arrangement. The outer surface of the primary cyst wall and all its protrusions were not smooth in their appearance. This surface appeared in serrated form composed of successive knob-like elevations separated from each other by thin-wall small invaginations. The interior of each protrusion was filled with homogenous dense granulesevently distributed in the entire core of each protrusion.

A thick, relatively homogenous ground substance was observed just underlying the primary cyst wall. This ground substance was extended into the interior of the cyst forming numerous thick septa dividing the cyst core into several compartments. These compartments enclosed the different zoites which were compact to each other at some sites and scattered in other one (Figs. 3-5). The majority of recorded zoites are mainly mature merozoites. Little metrocytes were observed which indicate the maturity of the cysts examined (Fig. 5).

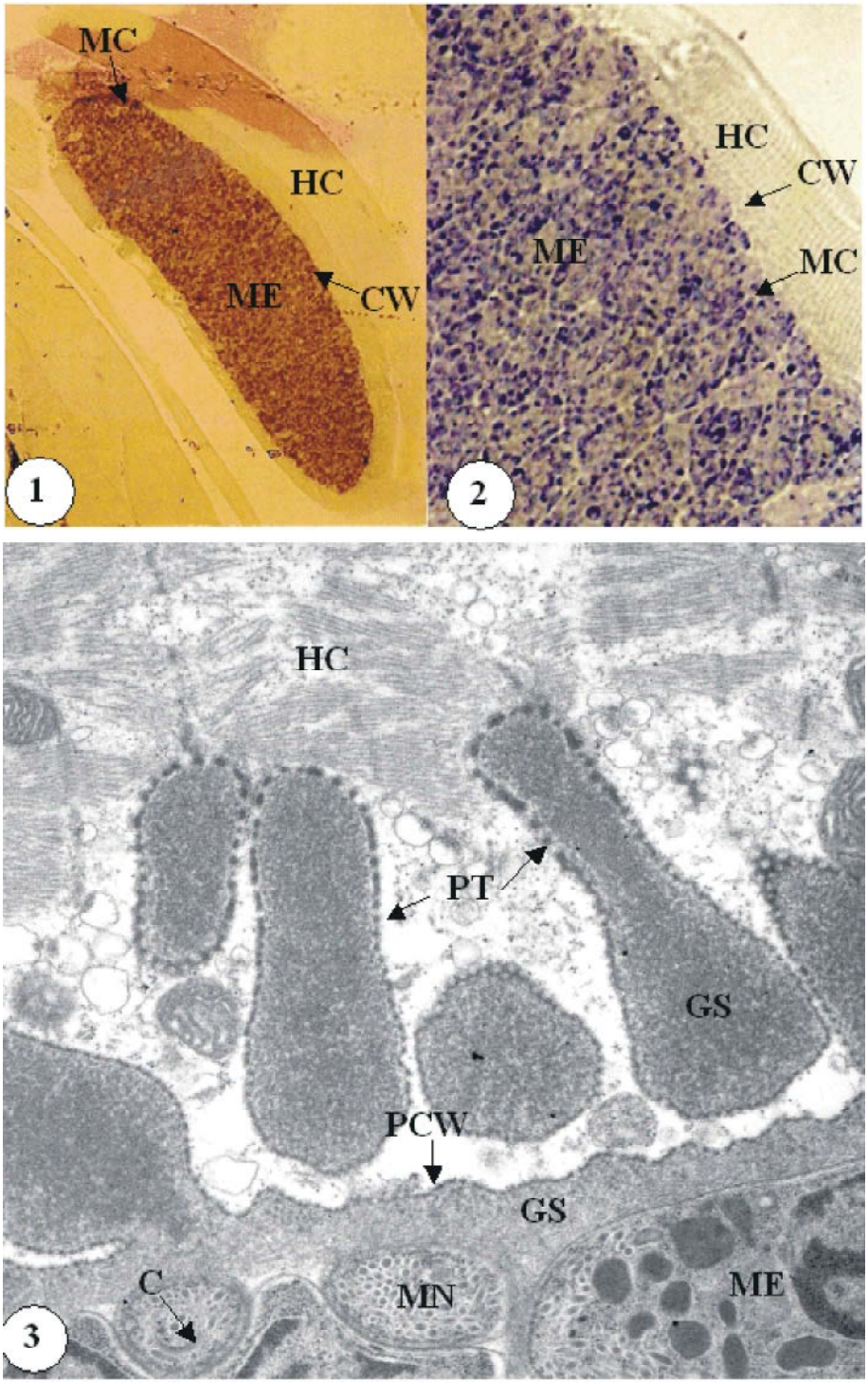

Fig. 1: Light micrograph of Sarcocyst infecting the skeletal muscle of the skink *Scincusscincus* in semithin sections (X 400). HC: Host cell, CW: Cyst wall, MC: Metrocyte, ME: Merozoite

Fig. 2: Enlarged sector of Fig.1 showing the cyst wall and contents of cyst interior (X 1000).

Fig. 3: Transmission electron micrograph showing enlarged sector of the sarcocyst through the primary cyst wall and its protrusions (X 40000).PCW:Primary cyst wall, PT:Protrusions, GS: Ground substance, MN:micronemes, C:Conoid,,

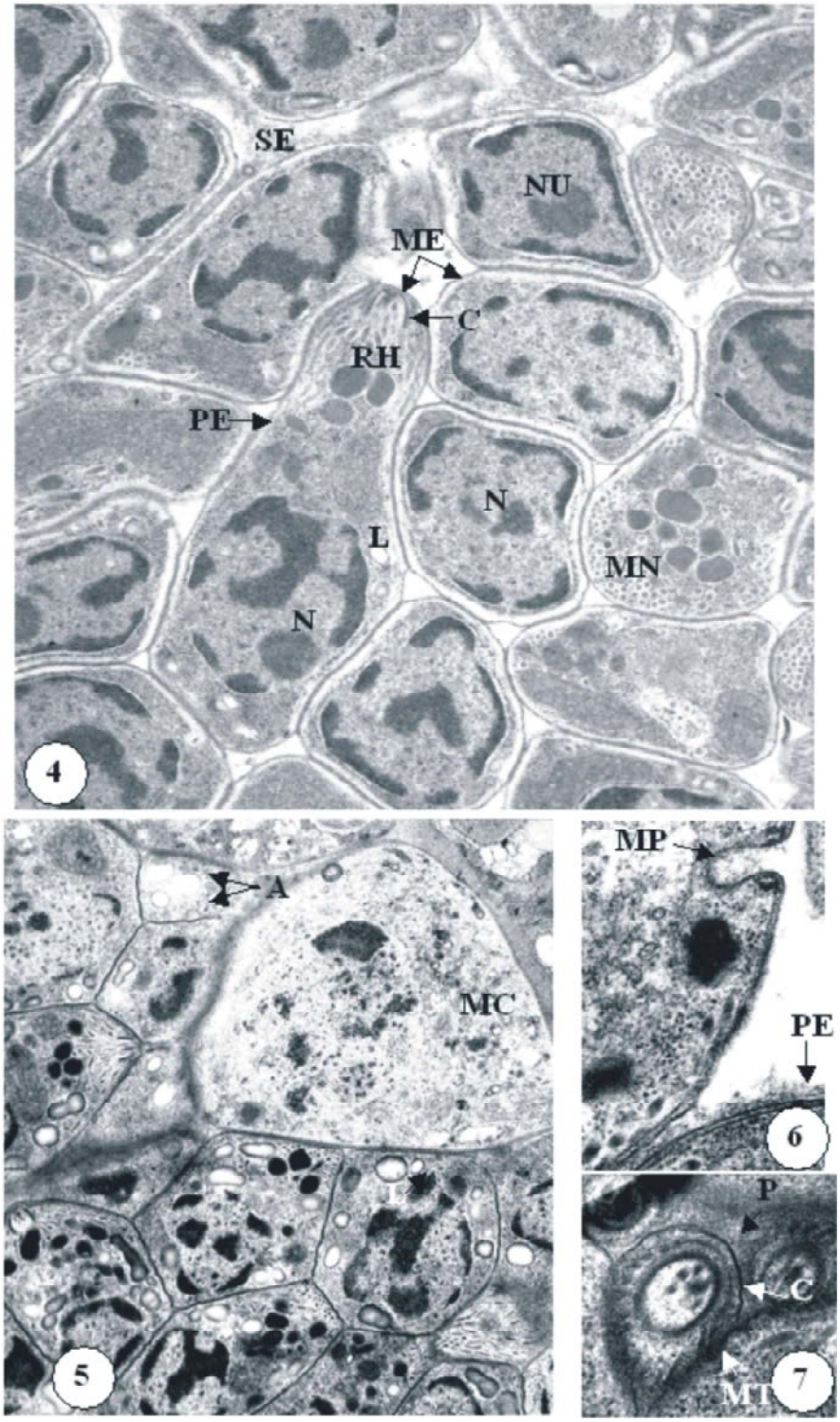

Fig. 4,5: Electron micrograph showing the interior of sarcocyst contain longitudinal and transverse sections of merozoites (ME) with apical complex characteristics and metrocystes (MC) and septa (SE) (X45000). RH:Rhoptries,NU:Nucleolus, SE:Septa, L:LipidMP:micropore, PE:Pellicle

Fig. 6: Electron micrograph showing the micropore and pellicle with an outer and inner membranes (X50000).

Fig. 7: Electron micrograph showing the lip of the apical complex, polorating (P), conoid (C) and microtubules (MT).

Merozoites examined were short and thick zoites showing all the characteristics of Apicomplexa. Each of them were enclosed in bilayered pellicle surrounding the whole body length (Fig4). The apical part was provided with clear conoidand anterior polar ring (Fig 7). Elongated rhoptries and micronemes were concentrated in the anterior third of the body length only. Characteristic feeding organ, the micropore consisting of perforation of the pellicle inner membrane and invagination of the outer one was also observed at the pellicle surface (Fig. 6). 22subpellicular microtubules arranged around the anterior polar ring were also detected (Fig 7). Few reserve food materials in form of lipid inclusions and amylopectin were demonstrated. Regarding the host-parasite relationships, striations of the muscle fibers surrounding the sarcocystwere mostly lost leading to clusters of vacuoles between the successive cyst wall protrusions.

DISCUSSION

The genus *Sarcocystis* of the family Sarcocystidae belonging to the phylum Apicomplexa contain about 200 spp. described in fishes, reptiles, birds and mammals [22]. However, many of these species are poorly described with no details about the ultrastructure and the host spectrum. Several reviews dealing with the distribution and systematics of Sarcocystis have been published since the establishment of the sarcosporidian obligatory prey predator heteroxenous life cycle [4, 16, 23]. Most of these described species of Sarcosporidia are reported from mammals while reptiles are poorly studied.

Regarding the host specificity and the species characterization (Particularly for the final host) among Sarcosporidia, contradictory and confusable close similarity in the fine structure of the sexual stages and the overlapping of sporocysts dimensions of *Sarcocystis* infecting different hosts has been recorded [3,10,24]. At the same time the characteristics of sarcocysts of different species of the genus*Sarcocystis* infecting the muscles of these intermediate host are more or less species specific

The ultrastructure of the primary cyst wall and its protrusions indicate the clear and definite specificity and variability of these structures among the different *Sarcocystis* species and their infected hosts [3,10,25,26]. Therefore, the ultrastructural features of the primary cyst wall and itsarised protrusions are the most important criteria for the identification of specific *Sarcocystis spp*.

In the present study, the fine structural characteristics of the cyst wall and its protrusions is quite different from those described before even within the parasite infecting the same family hosts. Examination of the skink *Scinusscinus* for the prevalence of *Sarcocystis*infection and whether these animals acts as intermediate and/or final host for such infection revealed that this reptiles acts as intermediate host only. Muscle sarcocysts were only detected and no shedding of any coccidian oocystsand/or sporocysts were found. Regarding the host specificity and sarcosporidian life cycle, it is clearly stated that all the mumbers of family Scincidae (Including: *Scinusscinus, Scinusmitranus, Chalcides ocellatus* and *Mabuyaaurata*) are acting as an intermediate host only for such infection. Non of them was proved to be the final host in such life cycle of Sarcosporidia.

Matuschka [7]in his work dealing with reptiles as intermediate and/or final hosts of Sarcosporidia stated that more than 10 of the lizards are intermediate hosts of Sarcocystis infection. The same was true for Abdel-Ghaffar *et al.* [3],Bannert[13],Abdel-Ghaffar*et al*. [18]andAbdel-Ghaffar and Al-Johany[20].The low infection rate recorded in the present study may be due to the small number of examined animals.

ACKNOWLEDGEMENTS

Authors would like to thank the Deanship of Scientific Research Shaqra University for financial supportof this project.

REFERENCES

1. Miescher, F., 1843. Ubereigentumliche Schlauche in den Muskelneiner Hausmaus. Ber. Verh. Naturf. Ges. Basel, 5: 198-202.
2. Balbiani, G., 1882.Les sporozoaires. J. Micrograph. Paris, 6: 80-89.
3. Abdel-Ghaffar, F., M. Hilali and K.E. Scholtysec, 1979.Ultrastructural study of *Sarcocystis fusiformis* (Railliet, 1897) infecting the Indian water buffalo (*Bubalus bubalis*) of Egypt. Tropenmedizin und Parasitologie, 29: 289-294.
4. Mehlhorn, H. and AO. Heydorn, 1978. *Sarcosporidia* (Protozoa, Sporozoa): Life cycle and fine structure. Adv. Parasitol, 16: 43-91.
5. Saleh, A., 1997. Amphibians and reptiles of Egypt. Ist Ed. Pub. Nat. Biodivers. Unit, 6: 87-91, 100-101.

6. Matuschka, F.R., 1981. Life cycle of *Sarcocystis* between poikilothermic hosts. Lizards are intermediate hosts of *Sarcocystispodarcicolubris* sp. n., snakes function as definitive hosts. Z. Naturforsch, 36: 1093-1095.
7. Matuschka, F.R., 1987a. Reptiles as intermediate and/or final hosts of Sarcosporidia. Parasitol. Res., 73: 22-32.
8. Matuschka, F.R., 1987b. *Sarcocystischalcidicolubris* n. sp.: Recognition of the life cycle in skinks of the genus *Chalcides* and skinks of genus Coluber. J. Parasitol. Res., 73(5): 1014-1018.
9. Matuschka, F.R. and H. Mehlhorn, 1984.Sarcocysts of *Sarcocystispodarcicolubris* from experimentally infected Tyrrhenian wall lizards (Podarcistiliguerta). S. gallotiae from naturally infected Canarian lizards (*Gallotiagalloti*) and S. dugesii from Madeiran lizards (*Lacertadugesii*). Protistologica, 20:133-139.
10. Abdel- Ghaffar, F., A.R. Bashtar, M.B. Ashour and T.H. Sakran, 1990. Life cycle of *Sarcocystis gongyli* Trinci 1911 in the skink *Chalcides ocellatusocellatus* and the snake *Spalerosophis diadema*. A light and electron microscopic study. Parasitol. Res, 76: 444-450.
11. Abdel-Ghaffar, F., M. Shazly, A. Ahmed and M. Fayed, 1994. Ultrastructural study of muscle cysts of *Sarcocystis* sp. Infecting the Egyptian gecko, Tarentolaannularis with special reference to endodyogeny. J. Union Arab Biol., 2(A): 371-389.
12. Bashtar, A.R., F.A. Abdel Ghaffar and A.K. Ahmed, 1991. Electron microscopic study of *Eimeria labbeana* infecting the Egyptian wild Doves and host parasite relationship. J. Egypt. Soc. Parasitol., 21(1): 263-272.
13. Bannert, B., 1992. *Sarcocystissimonyi*sp.nov. (Apicomplexa: Sarcocystidae) from the endangered Hierro giant *Gallotiasimonyi* (Reptilia: Lacertidae). Parasitol. Res., 78: 142-145.
14. Sakran, T.H., 1993. *Sarcocystis gongyli* (Sarcocystidae-Sarcospora) Predator-prey monoxeneous life cycle J. Egypt. Ger. Soc. Zool., 11: 91-105.
15. Lainson, R. and I. Paperna, 2000. The life cycle and ultrastructure of *Sarcocystis ameiva mastigodryas* in. sp. in the lizard Ameivaameiva (Teiidae) and the snake Mastigodryasbifossatus (Colubridae). Parasite, 7: 263-274.
16. Slapeta, J.R., D. Modry, J. Votypka, M. Jirku, J. Lukes and B. Koudela, 2003. Evolutionary relationships among cyst forming coccidian *Sarcocystis* sp. (Coccidia: Apicomplexa:Alveolata) in endemic African tree vipers and perspective for evolution of heteroxeneous life cycle. Mol. Phylogenet. Evol, 27(3): 464-475.
17. Dubey, J.P., 1976. A review of *Sarcocystis* and other coccidian of cats and dogs. Am. J. Vet. Med. Assoc., 169: 1061-1078.
18. Abdel- Ghaffar, F., A.R. Bashtar, S. Aquraishy., I.Al Nasrand and H. Mehlhorn, 2009. Sarcocystis infecting reptiles in Saudi Arabia 1- Light and electron microscopic study on Sarcocysts of *Sarcocystisturcicii* sp. nov. infecting the gecko *Hemidactylus tuncicus* Linnaeus. Parasitol. Res., 104: 503-508.
19. Abdel-Ghaffar, F., R. Entzeroth, B. Chobotar and K.E. Scholtysec, 1978. Ultrastructural studies of *Sarcocystis* sp. from the camel (*Camelus dromedarius*) in Egypt. Tropenmed. Parasit, 30: 434-438.
20. Abdel-Ghaffar, F. and A.M. Al-Johany, 2002. A light and electron microscope study of *Sarcocystis mitrani* (sp. Nov.) infecting the skink *Scincusmitranus* in the central region of Saudi Arabia. Parasitol. Res, 88: 102-106.
21. Arnold, EN., 1986. A key and annotated checklist to the lizards and amphisbaenians of Arabia. Fauna Saudi Arabia, 8: 335-435.
22. Odening, K., 1998. The present state of species systematic in *Sarcocystis*Lankester, 1882 (Protista, Sporozoa,Coccidia). Syst.Parasitol, 41: 209-233.
23. Levine, N.D., 1986. The taxonomy of Sarcocystis (Protozoa, Apicomplexa) species. J. Parasitol., 72: 372-382.
24. Tadros, W. and J.J. Laarman, 1978. A comparative study of the light and electron microscopic structure of the walls of the muscle cysts of several species of sarcocystideimeriidCoccidia (I and II). Verh K. Ned Akad Wet., 81: 469-491.
25. Mehlhorn, H. and F.R. Matuschka, 1986. Ultrastructural studies on the development of *Sarcocystisclethrionomyelaphis* within its final and intermediate hosts. Protistologica, 22: 97-104.
26. Lindsay, D.S., RD. McKown and J.P. Dubey, 2000. *Sarcocystis campestris* from naturally infected 13-lined ground squirrels, *Spermophilustridecemlineatustridecemlineatus*, from Nebraska. J. Parasitol., 86: 1159-1161.