A Report of Intestinal Sarcocystosis in the Bullsnake (Pituophis melanoleucus sayi) and a Re-evaluation of Sarcocystis sp. from Snakes of the Genus Pituophis

Authors: Peter Daszak, and Andrew Cunningham

Source: Journal of Wildlife Diseases, 31(3) : 400-403

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-31.3.400

Journal of Wildlife Diseases, 31(3), 1995, pp. 400–403

A Report of Intestinal Sarcocystosis in the Bullsnake (*Pituophis melanoleucus sayi*) and a Re-evaluation of *Sarcocystis* sp. from Snakes of the Genus *Pituophis*

Peter Daszak[1] **and Andrew Cunningham,**[2] [1] School of Life Sciences, Kingston University, Kingston-u-Thames, Surrey KT1 2EE, United Kingdom and [2] Veterinary Science Group, Institute of Zoology, Regent's Park, London NW1 4RY, United Kingdom

ABSTRACT: We report a severe enteric infection of *Sarcocystis* sp. from a wild-caught bullsnake (*Pituophis melanoleucus sayi*). The animal was collected in October 1988 by a commercial dealer, imported into the United Kingdom during November 1988 and purchased by the London Zoo, in December 1988. The animal was not fed after capture and was anorexic from the time of purchase to the time of death in January 1989. On necropsy, the animal was emaciated and the mucosa of the proximal intestine was markedly thickened. The lamina propria was packed with oocysts, and enterocytes were parasitized by an organism which closely resembled *Sarcocystis roudabushi* and *Sarcocystis idahoensis*, two bisporocystid coccidia described previously from *Pituophis melanoleucus*. We propose that *Sarcocystis idahoensis* and *Sarcocystis roudabushi* are synonymous since both occur in the same host species, both invade the intestinal lamina propria and enterocytes, and sporocyst measurement ranges of both species overlap. This is the first report of death believed to be due to sarcocystosis in a naturally-infected definitive host.

Key words: Pituophis melanoleucus, Sarcocystis, Isospora, sarcocystosis, pathology, taxonomy.

Sarcocystosis is an important disease of livestock caused by the asexual developmental stages of *Sarcocystis* spp. within non-intestinal tissues of the intermediate host (Dubey et al., 1989). In the definitive host, the parasite reproduces sexually within the intestinal mucosa. Reports of intestinal sarcocystosis causing clinical signs are rare, but occasionally symptoms of vomiting, diarrhea and anorexia have been reported in humans (Bunyaratvej et al., 1982). Here we report disease associated with an enteric infection of a *Sarcocystis* sp. in a wild-caught bullsnake (*Pituophis melanoleucus sayi*).

Identification of intestinal *Sarcocystis* spp. is complicated by the similarity of their oocysts and sporocysts to those of *Isospora* spp. and *Toxoplasma* spp. However *Sarcocystis* spp. undergo merogony in the intermediate host only (Dubey et al., 1989). The presence of fully sporulated oocysts within the host (endogenous sporulation) is another distinguishing characteristic (Upton et al., 1992). Only three coccidia with *Sarcocystis* sp. or *Isospora* sp. types of oocysts have been described in snakes of the genus *Pituophis*: *Sarcocystis roudabushi* Levine and Tadros, 1980, (Roudabush, 1937; Pellerdy, 1974) *S. idahoensis* Bledsoe, 1980 and an unnamed *Sarcocystis* sp. Upton et al. 1992 (Wacha and Christiansen, 1975). All are found in subspecies of *Pituophis melanoleucus*. In this paper we describe lesions associated with a *Sarcocystis* sp. in a wild-caught bullsnake, and propose that *S. roudabushi* and *S. idahoensis* are synonymous using the criteria by which they were originally described.

An adult male bullsnake (*Pituophis melanoleucus sayi*) was captured in northern Illinois within the area 39° to 42°N, 88° to 90°W, by a Tennessee dealer in October 1988. The snake was exported to the United Kingdom within 2 wk of capture, collected immediately upon arrival by a British dealer, who then sold the snake to London Zoo in mid-December 1988. Based on all available information, we believe that the snake was not fed in the USA between capture and export. The snake was not fed during or after import into the United Kingdom on 13 December 1988; indeed the snake refused to eat following purchase by the zoo until its death on 18 January 1989. The animal had no other signs of

disease. The snake was kept in quarantine from its arrival until its death.

The carcass was refrigerated at 3 C and necropsied 48 hr after death. Tissue samples were fixed in neutral buffered 10% formalin, embedded in paraffin, and 5 μm thick sections were cut and stained with hematoxylin and eosin. In addition, a sample of intestine was embedded in epoxy resin (Araldite CY212, Agar Scientific Ltd., Stansted, Essex, United Kingdom), and 1 μm sections were cut and stained with toluidine blue (Trump et al., 1961). The dimensions of the spaces left after collapse of sporocysts were measured from 1 μm resin sections. Samples of esophagus and intestine were streaked onto 5% horse blood agar and MacConkey's agar (both from Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). Media were incubated either aerobically or anaerobically at 25 C for 24 and 48 hr. Bacterial isolates were identified using API biochemical test strips (API-bio Merieux (UK) Limited, Basingstoke, Hampshire, United Kingdom).

The carcass weighed 200 g and was emaciated. The proximal gastro-intestinal tract was empty, but some digesta were present in the distal intestine. There was an area of necrosis in the esophagus and the mucosa of the proximal intestine was markedly thickened. Microscopically, the villous architecture of the proximal intestine was disrupted; the epithelium was necrotic with bacterial overgrowth and the lamina propria was greatly thickened (Fig. 1). The serosal vasculature was markedly congested and there was a slight infiltrate of granulated leukocytes within the serosa. The lamina propria was packed with bisporocystid oocysts; some remaining enterocytes also contained oocysts (Fig. 2). Sporocysts and oocysts had collapsed during fixation and processing. The collapsed sporocysts were not measured; however, the spaces left by collapsed oocysts measured 10.2 × 7.1 (9 to 16 × 5.5 to 12) μm. A pentastomid in the lung had provoked a mild infiltrate of mononuclear cells. No other lesions were seen. A mixed growth of *Proteus* spp. was cultured from the intestine. *Proteus* sp., *Pseudomonas* spp. and *Aeromonas hydrophila* were cultured from the esophagus.

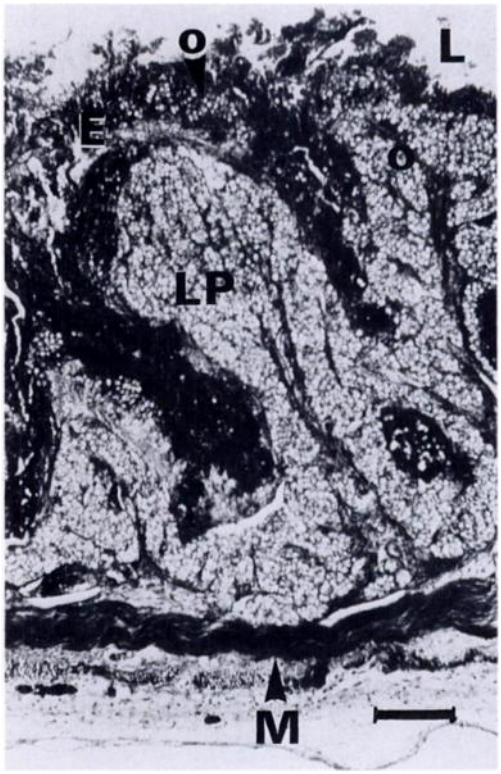

FIGURE 1. Histological section through the small intestine of a bullsnake infected with a *Sarcocystis* sp. There is extensive sloughing of the epithelium. Oocysts are visible within the remaining enterocytes (O, arrowhead) and throughout the lamina propria (O). E, epithelium; L, lumen; LP, lamina propria; M, layer of longitudinal muscle. Masson's trichrome stain. Bar = 200 μm.

Numerous *Sarcocystis* sp. use reptiles as definitive or intermediate hosts (Matuschka, 1987). Fantham and Porter (1950, 1952) described emaciation and destruction of enterocytes during intestinal sarcocystosis within definitive reptile hosts. Clinical signs and post mortem findings reported herein are consistent with those found by Bledsoe (1980) in a *Pituophis melanoleucus catenifer* experimentally infected with *Sarcocystis idahoensis*. Although the mucosal necrosis seen in this study may have been post mortem change,

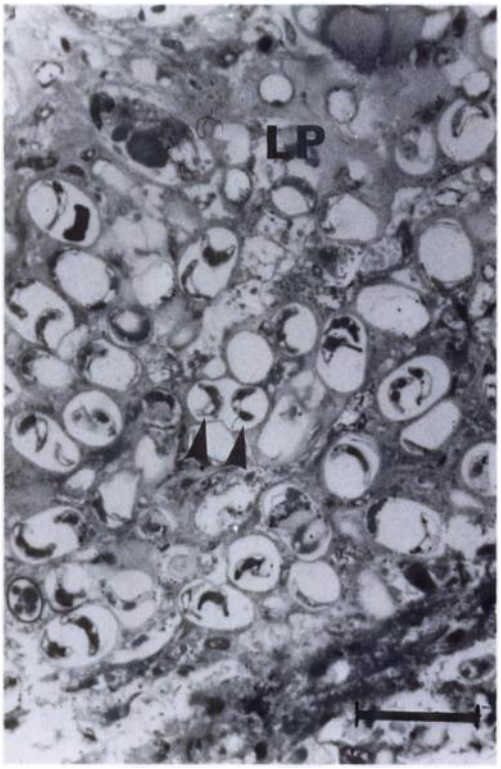

FIGURE 2. A 1 μm-thick plastic section through the small intesine of the infected bullsnake. Note the lamina propria, packed with bisporocystid (arrowheads) oocysts. LP, lamina propria. Toluidine blue stain. Bar = 20 μm.

the good preservation of other tissues and the presence of an inflammatory response is evidence that this was part of the disease process. This is the first report of death believed to be due to sarcocystosis in a naturally-infected definitive host. It is possible that the snake was infected with *Sarcocystis* sp. in the USA during the 2 wk between capture to export; however, the dealer asserted that the bullsnakes are not fed prior to export due to regurgitation problems, and only commercially bred, laboratory-strain white mice are used for feeding colubrid snakes.

Bisporocystid coccidia have been described previously from *Pituophis melanoleucus* and there has been some confusion over their classification (Levine and Tadros, 1980). At the time of the description of *S. idahoensis*, no other *Sarcocystis* sp. had been described from *Pituophis* spp. and Bledsoe (1980) separated *Isospora roudabushi* and *S. idahoensis* on the grounds of geographical separation of their respective hosts and location of oocyst development in the mucosa of the intestine. *Isospora roudabushi* recently has been termed *S. roudabushi* by Upton et al. (1992) and we put forward the following evidence that *S. idahoensis* and *S. roudabushi* are synonymous.

The geographical separation of hosts pertains only to subspecies of *Pituophis melanoleucus* and therefore cannot be used to distinguish the two parasite species (Ernst and Barbour, 1989). Furthermore, Bledsoe (1980) described *S. idahoensis* from three geographically separated subspecies of *P. melanoleucus*. The intermediate host for *S. idahoensis*, the deer mouse (*Peromyscus maniculatus*), occurs throughout the range of *P. melanoleucus* (Hall, 1981), and therefore within the range of the hosts of *S. roudabushi* and *S. idahoensis* (Ernst and Barbour, 1989).

Roudabush (1937) showed that *S. roudabushi* invades the lamina propria of the snake host intestine, and this was contrasted with the more superficial infection of the epithelial layer by *S. idahoensis* (Bledsoe, 1980). However, this cannot be used to distinguish species since other *Sarcocystis* sp. vary in their ability to invade the epithelium and lamina propria among different individuals of the same host species. For example, *Sarococystis* (syn. *Isospora*) *dirumpens* Matuschka and Hafner, 1984 (Hoare, 1933) has been recorded from both sites, with the lamina propria parasitized to varying degrees in different specimens of *Bitis arietans* (Hoare, 1933). Bledsoe (1980) reported invasion of the lamina propria following heavy experimental infection of *P. melanoleucus catenifer* with *S. idahoensis*, and published a photomicrograph to support this. Finally, *Sarcocystis idahoensis* cannot be distinguished from *S. roudabushi* using sporocyst dimensions, since the measurements given by Bledsoe

(1980) for *S. idahoensis* (11 to 12 × 13 to 14 μm) are within the range of those reported by Roudabush (1937) for *S. roudabushi* (8.8 to 11.0 × 9.6 to 13.2 μm). Thus, we propose that *Sarcocystis idahoensis* is a synonym of *Sarcocystis roudabushi* Levine and Tadros, 1980 (Roudabush, 1937; Pellerdy, 1974). Furthermore, an isosporan parasite described from *P. melanoleucus*, and renamed *Sarcocystis* sp. Upton et al., 1992 (Wacha and Christiansen, 1975) also may be synonymous with *S. roudabushi*, based on overlap of sporocyst dimensions. The measurements of sporocysts and oocysts of the parasite we report cannot be used in identification since the tissue had undergone shrinkage during fixation and treatment; however, the appearance and location of the endogenous developmental stages are evidence that the parasite we report may be *S. roudabushi*. Due to the lack of full life-cycle information and accurate measurements, it is proposed that this species be named *Sarcocystis* sp. *incertae sedis*.

We thank Professor S. J. Ball, University of East London, United Kingdom, for his advice and encouragement throughout this study. We also thank Mandy Gordon and Terry Dennett, Institute of Zoology, London, United Kingdom, for their assistance with the figures. Stewart Worth, Peter Foulsham, Peter Curry, and Jim Bryant of British Herpetological Supply, Hayes, Middlesex, United Kingdom kindly supplied us with information on the origins of the snake. We are grateful to Colin McCarthy of the Natural History Museum, London for providing information on host animals.

LITERATURE CITED

BLEDSOE, B. 1980. *Sarcocystis idahoensis* sp. n. in deer mice *Peromyscus maniculatus* (Wagner) and gopher snakes *Pituophis melanoleucus* (Daudin). The Journal of Protozoology 27: 93–102.

BUNYARATVEJ, S., P. BUNYAWONGWIROJ, AND P. NITIYANANT. 1982. Human intestinal sarcosporidiosis: Report of six cases. American Journal of Tropical Medicine and Hygiene 31: 36–41.

DUBEY, J. P., C. A. SPEER, AND R. FAYER. 1989. Sarcocystosis of animals and man. CRC Press, Inc., Boca Raton, Florida, 215 pp.

ERNST, C. H., AND R. W. BARBOUR. 1989. Snakes of eastern North America. George Mason University Press, Fairfax, Virginia, 289 pp.

FANTHAM, H. B., AND A. PORTER. 1950. The endoparasites of certain South African snakes, together with some remarks on their structure and effects on their hosts. Proceedings of the Zoological Society of London 120: 599–647.

———, AND ———. 1952. The endoparasites of some North American snakes and their effects on the Ophidia. Proceedings of the Zoological Society of London 123: 867–898.

HALL, E. R. 1981. The mammals of North America, Vol. 1 and 2. 2nd ed. John Wiley and Sons, New York, New York, 1,271 pp.

HOARE, C. A. 1933. Studies on some new ophidian and avian coccidia from Uganda, with a revision of the classification of the Eimeriidea. Parasitology 25: 359–388.

LEVINE, N. D., AND W. TADROS. 1980. Named species and hosts of *Sarcocystis* (Protozoa: Apicomplexa: Sarcocystidae). Systematic Parasitology 2: 41–59.

MATUSCHKA, F.-R. 1987. Reptiles as intermediate and/or final hosts of Sarcosporidia. Parasitology Research 73: 22–32.

PELLERDY, L. P. 1974. Coccidia and coccidiosis, 2nd ed. Paul Parey, Berlin, Germany, 959 pp.

ROUDABUSH, R. L. 1937. Some coccidia of reptiles found in North America. The Journal of Parasitology 23: 354–359.

TRUMP, B. F., E. A. SMUCKLER, AND E. P. BENDITT. 1961. A method for staining epoxy sections for light microscopy. The Journal of Ultrastructure Research 5: 343.

UPTON, S. J., C. T. MCALLISTER, S. E. TRAUTH, AND D. K. BIBB. 1992. Description of two new species of coccidia (Apicomplexa: Eimeriorina) from flat-headed snakes, *Tantilla gracilis* (Serpentes: Colubridae) and a reclassification of misnomer species within the genera *Isospora* and *Sarcocystis* from snakes. Transactions of the American Microscopical Society 111: 50–60.

WACHA, R. S., AND J. L. CHRISTIANSEN. 1975. Isosporan parasites from North American snakes. The Journal of Protozoology 22: 46A.

Received for publication 25 May 1994