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Case Report

Sarcocystis Infection in Laboratory Rabbits

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Sarcocystosis, presumably caused by *Sarcocystis cuniculi*, was diagnosed in 2 purpose-bred, SPF Dutch belted laboratory rabbits from a class A breeder. The rabbits were purchased by a research facility and conventionally housed individually in stainless-steel suspended caging. At necropsy and tissue harvest, gross lesions were not observed in the muscles. Upon histologic examination, sarcocysts were found in the eyelid of one rabbit and the tongue of the other. To our knowledge, this report is the first description of infection by *Sarcocystis* spp. in laboratory rabbits.

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Sarcocystis is a genus of ubiquitous, apicomplexan protozoa with a worldwide distribution that occurs in many wild and domestic species. *Sarcocystis* spp. have an obligatory 2-host life cycle, with a carnivorous definitive host and an herbivorous intermediate host.[1,4] Sexual reproduction (gametogony and fertilization) occurs in the intestinal epithelium of the definitive host, and infective sporocysts or sporulated oocysts are shed in the feces. After ingestion by the intermediate host, infective sporozoites invade the intestinal mucosa and reproduce asexually in vascular endothelium, forming schizonts containing merozoites. Merozoites enter the blood and spread to muscle tissue, where they enter myofibers, form a sarcocyst, and divide and mature into bradyzoites. The formation of the sarcocysts in muscle occurs approximately 3 mo from ingestion of the oocysts by an intermediate host.[9] The cycle is completed when the definitive host eats muscle tissue containing sarcocysts.

Sarcocystosis is common in wild rabbits, uncommon in domestic rabbits, and has not previously been reported in laboratory rabbits.[8,9] Two species of *Sarcocystis* are generally recognized in the rabbit: *S. cuniculi* in domestic (*Oryctolagus* spp.) rabbits and *S. leporum* in wild cottontail (*Sylvilagus* spp.) rabbits.[4-8]

The definitive host for both *S. cuniculi* and *S. leporum* is the domestic cat (*Felis catus*).[2,3,6-9] Rabbits become infected by ingesting forages or feeds contaminated with cat feces, and sarcocysts form in skeletal and cardiac striated muscle. Commonly reported sites are tongue, esophagus, diaphragm, thigh, loin, and thoracic wall muscle.[2,4,8,9] Heavy infections can be seen grossly as thin pale streaks oriented parallel to the muscle fibers. Mild infections are usually grossly inapparent. Most infections in rabbits are subclinical but, on occasion, severe infections can cause lameness.[9] This report describes the incidental finding of sarcocysts in 2 Dutch belted purpose-bred, SPF laboratory rabbits.

Case Report

Two rabbits (4 and 6 mo of age on receipt) were purchased from a class A breeder. The rabbits were certified to be free of CAR bacillus, *Encephalitozoon cuniculi*, *Treponema cuniculi*, *Clostridium piliforme*, *Pasturella multocida*, *Bordetella bronchiseptica*, *Eimeria* spp., and *Coccidia* spp. and ectoparasites for IACUC-approved research projects. They were conventionally individually housed at a cat-free AAALAC-accredited, USDA-registered, and PHS-assured research facility in separate indoor rooms in suspended stainless-steel cages with stainless-steel grate flooring with no-contact bedding. Enrichment was provided in the form of nonedible toys that were sanitized by a commercial cage washer and stored in sealed plastic bins. Reverse-osmosis–purified water was provided without restriction via an automatic watering system, and the only source of food was certified PMI Rabbit Diet 5325 (PMI Nutrition International, St Louis, MO). The rabbits were used on a study and were euthanized in accordance with the protocol at study termination. They were not treated with any known infectious or immunomodulatory agents.

The rabbits were submitted for diagnostic necropsy at 8 and 13 mo of age, respectively. No gross observations were noted in the muscle tissue. A full complement of tissues was collected, preserved in 10% neutral buffered formalin, processed, sectioned, and stained with hematoxylin and eosin. Histologic evaluation of routine tissue sections revealed sarcocysts in both rabbits. In the first rabbit, a single sarcocyst was present in the muscle of the tongue. The sarcocyst was round and approximately 50 µm in diameter, with a thin hyalinized wall, and contained numerous crescent-shaped bradyzoites measuring approximately 4 × 10–15 µm. The adjacent myofibers were normal, without evidence of degeneration or inflammation. In the second rabbit, several sarcocyst profiles were present in the small muscles of the eyelid. These parasite cysts were elongate, measuring as large as 50 × 400 µm and containing similar bradyzoites as in the first animal (Figures 1 and 2). No degeneration or inflammation was observed in adjacent myofibers.

Discussion

This report is the first description of sarcocystosis in laboratory rabbits. The *Sarcocystis* infection in these rabbits was mild and incidental. Mild infections of *Sarcocystis* spp. in skeletal or cardiac muscle are unlikely to interfere with the majority of research projects because sarcocysts are typically innocuous and self-limiting.[8,9] Although individual sarcocysts can

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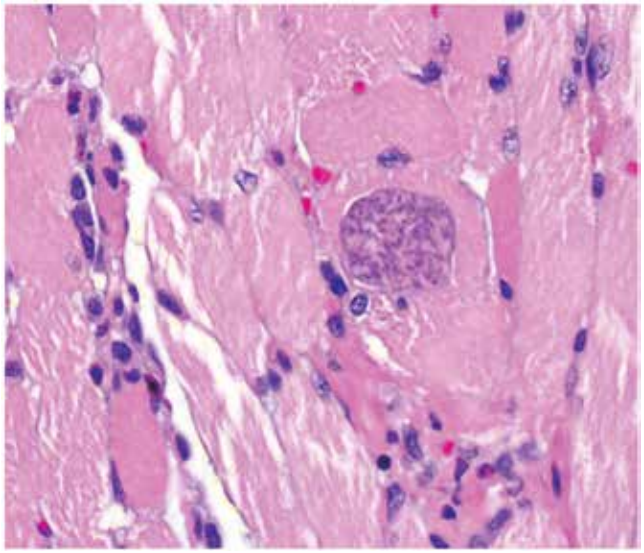

Figure 1. Sarcocystosis, skeletal muscle, tongue, Dutch belted rabbit. Sarcocyst within myofiber, with numerous internal crescent-shaped bradyzoites. Although scattered myofibers are degenerate (hypereosinophilic and/or fragmented, with rare inflammatory cells), the degeneration and inflammation do not involve the sarcocyst. Hematoxylin and eosin stain; magnification, 40×.

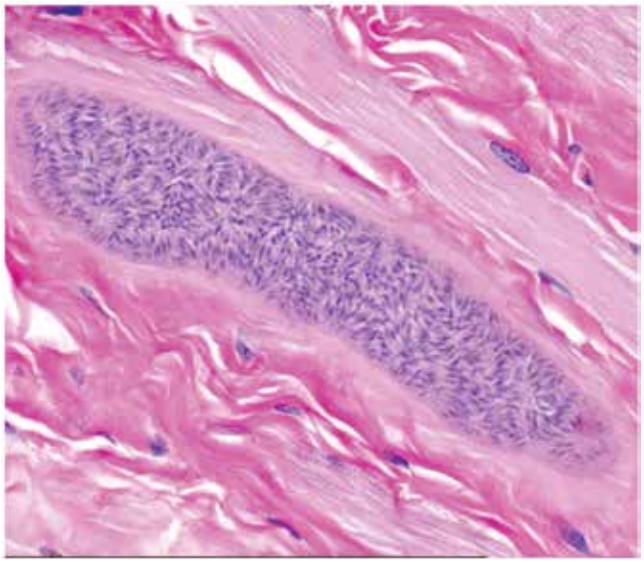

Figure 2. Sarcocystosis, skeletal muscle, eyelid, Dutch belted rabbit. Note the hyalinized wall, numerous internal crescent-shaped bradyzoites, and lack of degeneration or inflammation in associated muscle fibers. Hematoxylin and eosin stain; magnification, 40×.

rupture and trigger local inflammation, free bradyzoites have not been shown to form additional sarcocysts in any species of *Sarcocystis*.[11]

A presumptive diagnosis of *S. cuniculi* was made based on the microscopic appearance in the rabbits and the suggestion by Fayer that *S. leporum* is not infective in domestic rabbits.[5,8,9] Although molecular techniques have been used to differentiate and speciate sarcocysts,[10] no published studies have used molecular tools to differentiate *S. cuniculi* from *S. leporum;* we were not able to pursue such methods for these animals. Serologic diagnosis of sarcocystosis has been performed by using indirect immunofluorescent antibody testing. When antibody levels were evaluated after experimental transmission from cat to rabbit, the antibody response was first seen at 20 d after infection, peaked at 50 d, and was gone by 100 d.[2] Thus, the utility of indirect immunofluorescent antibody testing for sarcocystosis depends on the infective dose and the time since infection and can detect only recent or recurrent infections.[2] Because our rabbits were at the facility for 4 mo or longer, sarcocysts form approximately 3 mo after ingestion of infective oocysts, and investigations at the facility failed to reveal food contamination with cat feces, using an indirect immunofluorescent antibody test would be highly unlikely to aid in confirmation of a *Sarcocystis* infection and therefore was not performed.

To prevent infection, rabbits raised for biomedical research should be isolated from cats. Care should be taken to prevent cat feces from contaminating bedding, food, including fresh vegetables, and hay offered as enrichment.

References

1. **Bowman DD.** 2013. Protozoans. p 106–107. In: Bowman DD, editor. Georgi's parasitology for veterinarians. 10th ed. St Louis (MO): Elsevier.
2. **Cerná Z, Loucková M, Nedvědová H, Vávra J.** 1981. Spontaneous and experimental infection of domestic rabbits by *Sarcocystis cuniculi* brumpt, 1913. Folia Parasitol (Praha) **28:**313–318.
3. **Crum JM, Prestwood AK.** 1977. Transmission of *Sarcocystis leporum* from a cottontail rabbit to domestic cats. J Wildl Dis **13:**174–175. https://doi.org/10.7589/0090-3558-13.2.174.
4. **Duszynski DW, Couch L.** 2013. Sarcocystidae Poche, 1913, the predator-prey coccidia in rabbits: Besnoitia, Sarcocystis, Toxoplasma. p 216–227. In: The biology and identification of the coccidia (*Apicomplexa*) of rabbits of the world. Waltham (MA): Elsevier.
5. **Elwasila M, Entzeroth R, Chobotar B, Schpoltyseck E.** 1984. Comparison of the structure of *Sarcocystis cuniculi* of the European rabbit (*Oryctolagus cuniculus*) and *Sarcocystis leporum* of the cottontail rabbit (*Sylvilagus floridanus*) by light and electron microscopy. Acta Vet Hung **32:**71–78.
6. **Fayer R, Kradel D.** 1977. *Sarcocystis leporum* in cottontail rabbits and its transmission to carnivores. J Wildl Dis **13:**170–173. https://doi.org/10.7589/0090-3558-13.2.170.
7. **Munday BL, Smith DD, Frenkel JK.** 1980. Sarcocystis and related organisms in Australian Wildlife: IV. Studies on *Sarcocystis cuniculi* in European rabbits (*Oryctolagus cuniculus*). J Wildl Dis **16:**201–204. https://doi.org/10.7589/0090-3558-16.2.201.
8. **Pritt S, Cohen K, Sedlacek H.** 2012. Parasitic diseases. p 415–446. In: Suckow MA, Stevens KA, Wilson RP, editors. The laboratory rabbit, guinea pig, hamster, and other rodents. Waltham (MA): Elsevier. https://doi.org/10.1016/B978-0-12-380920-9.00015-8
9. **Schoeb TR, Cartner SC, Baker RA, Gerrity LW.** 2007. Parasites of rabbits. p 459–460. In: Baker DG, editor. Flynn's parasites of laboratory animals, 2nd ed. Ames (IA): Blackwell Publishing. https://doi.org/10.1002/9780470344552.ch15
10. **Stojecki K, Karamon J, Sroka J, Cencek T.** 2012. Molecular diagnostics of *Sarcocystis* spp. infections. Pol J Vet Sci **15:**589–596. https://doi.org/10.2478/v10181-012-0090-7.
11. **Sykes JE, Dubey JP, Lindsay LL, Prato P, Lappin MR, Guo LT, Mizisin AP, Shelton GD.** 2011. Severe myositis associated with *Sarcocystis* spp. Infection in 2 dogs. J Vet Intern Med **25:**1277–1283. https://doi.org/10.1111/j.1939-1676.2011.00828.x.