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Coccidia, *Giardia* sp., and a Physalopteran Nematode Parasite from Black-footed Ferrets (*Mustela nigripes*) in Wyoming

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ABSTRACT: Oocysts of 3 species of *Eimeria* were found in feces and intestinal contents of free-ranging and captive black-footed ferrets (*Mustela nigripes*) from Wyoming. Oocysts, meronts, and gamonts of 2 of the intestinal coccidia; meronts and oocysts of an unidentified coccidian in the respiratory tract; and merozoites of an unidentified coccidian from the wall of the urinary bladder were seen histologically or in impression smears. Based on oocyst morphometry, 2 of the intestinal coccidia were identified as *E. ictidea* and *E. furonis*. The third intestinal coccidian and the respiratory and urinary bladder forms were not identified. Other parasites observed included *Giardia* sp. and *Physaloptera* sp. All parasites constitute new host records. The coccidia also represent new distribution records and the developmental stage descriptions are previously unreported in black-footed ferrets.

KEY WORDS: Black-footed ferret, *Mustela nigripes*, coccidia, *Eimeria ictidea*, *Eimeria furonis*, *Giardia* sp., *Physaloptera* sp.

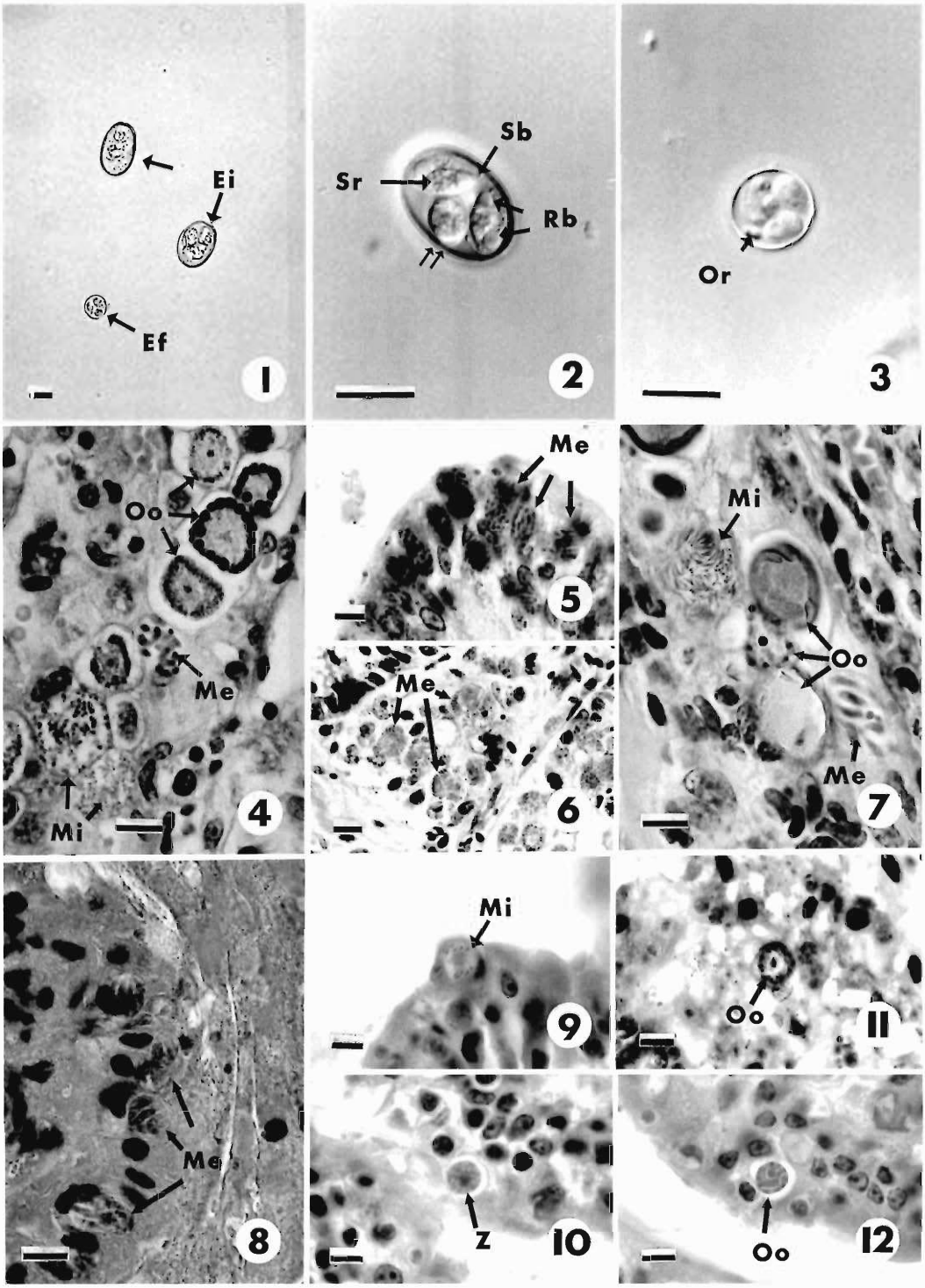
The black-footed ferret (*Mustela nigripes*), once thought to be extinct, was rediscovered near Meeteetse, Wyoming in 1981. The colony occupied a white-tailed prairie dog (*Cynomys leucurus*) town and was intensively studied over the next few years (Forrest et al., 1988). In the autumn of 1985, 6 animals (2 adult and 1 juvenile males, 1 adult and 2 juvenile females) were trapped to establish a captive breeding colony (Thorne and Williams, 1988). When captured, 2 of 6 animals were incubating canine distemper, a viral disease invariably fatal in this species. Subsequently, all of these animals died of canine distemper (Williams et al., 1988). Canine distemper, later found to be widespread in the free-ranging black-footed ferret population, was a major factor in making carcasses and excretory products available for examination and prompted a trapping operation that was completed in the spring of 1987.

Parasites have not been studied extensively in black-footed ferrets. Most attention has been on the ectoparasites: *Ixodes kingi* Bishopp, 1911; *I. sculptus* Neumann, 1904; and *Otodectes* sp. (Boddicker, 1968; Carpenter and Hillman, 1979; Schroeder, 1983). Other ectoparasites reported include: *Oropsylla* (*Opisocrostis*) *hirsuta* Baker, 1895 and *O. idahoensis* Baker, 1904; *Rhadinopsylla* (=*Rectofrontia*) *fraterna* Baker, 1895 and *Nearctopsylla brooksi* Rothschild, 1904; and unidentified ticks and fleas. *Molineus mustelae* Schmidt, 1965 and *Taenia* sp. have also been reported (Boddicker, 1968; Carpenter and Hillman, 1979; Schroeder, 1983). The cestode has since been identified through life history and structure as *T. mustelae* Gmelin, 1790 (Rockett et al., 1990). Unidentified coccidia were reported from 2 groups of captive black-footed ferrets (Carpenter and Hillman, 1979; Williams et al., 1988). Sarcocysts were found in skeletal muscle of several free-ranging black-footed ferrets (Schroeder, 1983; Williams et al., 1988).

The present report documents the occurrence of coccidia, a flagellate protozoan parasite (*Giardia* sp.) and a nematode (*Physaloptera* sp.), recovered from free-ranging and/or captive black-footed ferrets.

Materials and Methods

Feces from free-ranging black-footed ferrets were collected from holding cages following capture in 1982, 1984, and 1985 and from captive ferrets when cages were cleaned. Feces were macerated and held in 2% potassium dichromate solution at room temperature (22°C) and aerated to promote sporulation of oocysts. Samples were examined initially on arrival and every 24 hr thereafter by brightfield and/or phase-contrast microscopy of direct wet preparations or after flotation with a saturated sucrose solution (specific gravity 1.12). Sporulation was considered complete when at least 75% of oocysts contained sporocysts and sporozoites by microscopic examination. Complete necropsies were conducted on black-footed ferrets found dead in the field or those that died in captivity. Tissues were fixed in 10% buffered formalin and processed for histologic examination. Gastrointestinal tracts of free-ranging animals were examined for metazoan parasites. Measurements of coccidia and *Giardia* cysts were made with a calibrated filar micrometer.



Figures 1-12. Photomicrographs of sporulated oocysts and endogenous developmental stages of *Eimeria ictidea* and *E. furonis* from black-footed ferrets. 1. Two oocysts of *E. ictidea* (Ei) and 1 of *E. furonis* (Ef) showing relative sizes. 2. Oocyst of *E. ictidea* with double layer wall (double arrows), sporocysts showing residuum (Sr) and Stieda body (Sb), and sporozoites with refractile bodies (Rb). Oocyst polar body is not shown. 3. *Eimeria furonis* oocyst

Table 1. Measurements in micrometers of coccidial oocysts recovered from the feces of black-footed ferrets.

Form	Length (range)	Width (range)	L/W ratio
Large oval ($N = 5$)	37.0* ± 1.3 (35.0–38.6)	22.3* ± 2.3 (21.2–23.2)	1.66:1
Small oval ($N = 64$)	23.2* ± 2.3 (18.2–27.4)	15.5* ± 1.0 (13.0–16.2)	1.50:1
Spherical-subspherical ($N = 60$)	12.6* ± 1.2 (10.8–15.2)	11.9* ± 0.9 (10.1–12.9)	1.06:1

* ±1 SD.

Results

Eimeria spp. were encountered from field-collected black-footed ferret feces and from feces from all 6 captive black-footed ferrets with canine distemper. All passed medium-sized oval oocysts, 3 also passed smaller, spherical or subspherical oocysts, and a larger oval oocyst was occasionally seen. These large oocysts were seen again in a different black-footed ferret in 1991. Measurements of coccidial oocysts are listed for comparison in Table 1.

The medium oval form (Figs. 1, 2) sporulated in no less than 48, nor more than 72 hr, forming the 4 dizoic sporocysts typical of eimerian oocysts. Oocyst walls were bilaminate, without a micropyle; a polar body/granule was formed, but no residuum was seen. The elongate sporocysts possessed a Stieda body and a fine granular sporocyst residuum. The sporozoites lay in opposing directions, anterior-to-posterior, in the sporocysts, with a prominent refractile body near each posterior end. Often, sporocysts lay in pairs, with one at a right angle to the other pair. The morphometric and structural features are compatible with the species *Eimeria ictidea* Hoare, 1927 described from the domestic ferret (*M. putorius furo*) from England (Hoare, 1927).

The small spherical to subspherical form (Figs. 1, 3) sporulated in no less than 48, nor more than 72 hr, producing typical eimerian oocysts, with a pink double-layered wall and a coarse, sparse granular residuum; no micropyle or polar granule were seen. Elongate sporocysts with a Stieda body contained sporozoites with refractile bodies. Dimensions and internal structure of sporulated oocysts are consistent with the description of *Eimeria furonis* Hoare, 1927 reported from the domestic ferret in England and mink (*M. vison*) in Kazakhstan (Nukerbaeva and Svanbaev, 1973, cited by Levine and Ivens, 1981).

Merogony and gamogony of *E. ictidea* and *E. furonis* were seen in villar epithelial cells throughout the small intestine, but were most prevalent in the jejunum. Two morphologic types of meronts were seen in intestinal sections infected predominantly with *E. ictidea*; one was commonly seen near the bases of the villi and rarely in the crypts, whereas the other occurred at or near the tips. Merozoites formed by ectopolygenic merogony (Levine, 1985) were visible in the meronts near the bases of the villi (Fig. 6). The meronts seen in more apical regions of the villi contained larger merozoites and lacked an undifferentiated mass (Figs. 4, 5, 7). Sequential specimens were not available to allow determination of merogonic generations.

Eimeria ictidea gamogony also occurred throughout the small intestine, mainly in epithelial cells in the apical half of villi. Microga-

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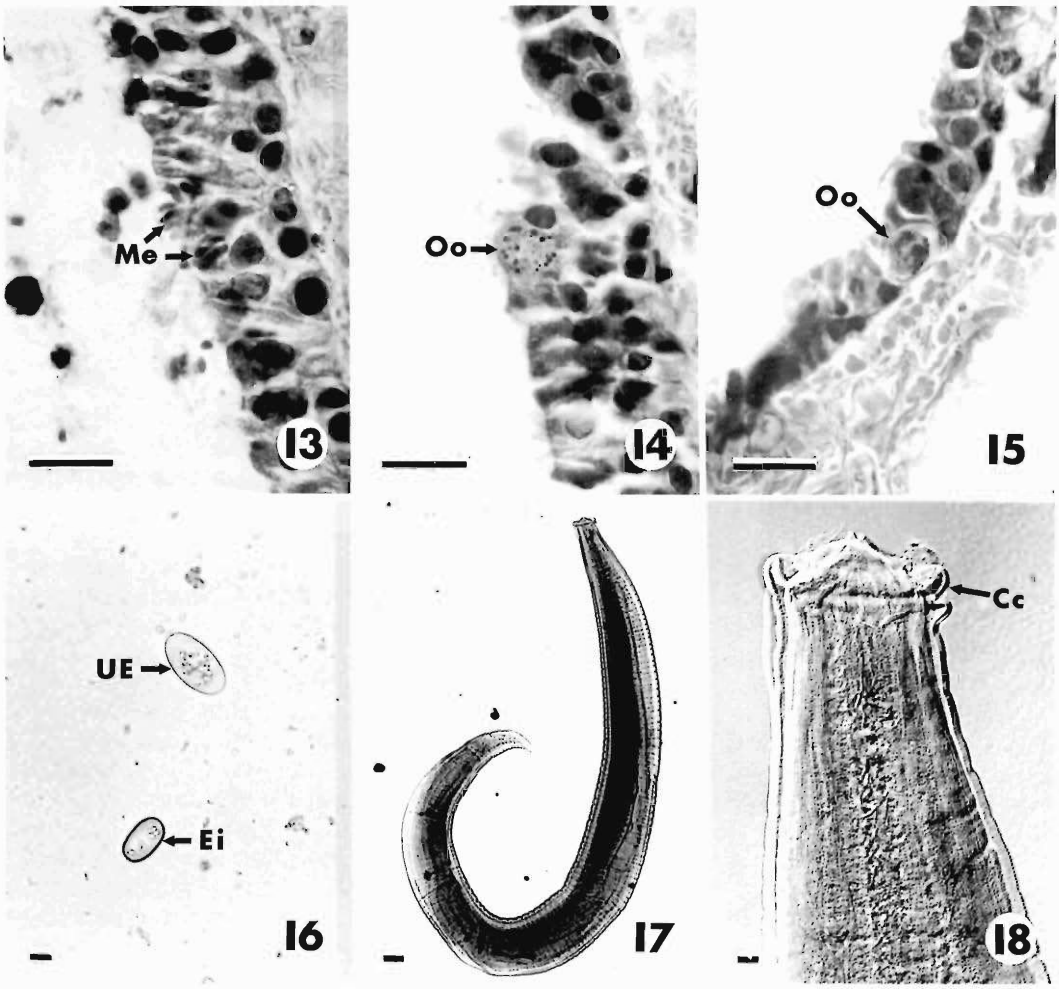
with indistinct sporocysts and oocyst residual granules (Or). Figures 4–7. Endogenous stages of *E. ictidea*. 4. Oocysts in various stages of development with wall-forming bodies (Oo), a meront with fully formed merozoites (Me), and microgamonts in development (Mi). 5. Meronts in cells at the tip of jejunal villus, with merozoites formed by fission (Me). 6. Meronts in cells at the base of jejunal villus, with merozoites formed by ectopolygeny (Me). 7. Oocysts, 2 fully formed, 1 of which shows finger-like interdigitations resulting from fusion of wall-forming bodies; central oocyst, still developing, shows wall-forming bodies (Oo). Meront (Me) shows fully formed merozoites. Microgamont (Mi) shows fully formed microgametes. Figures 8–12. Endogenous stages of *E. furonis*. 8. Meronts with fully formed merozoites (Me). 9. A microgamont with forming microgametes (Mi). 10. A macrogamont/zygote prior to formation of wall-forming bodies (Z). 11. A developing oocyst with wall-forming bodies (Oo). 12. A developing oocyst with wall-forming bodies undergoing fusion into interdigitations (Oo). Scale bars = 10 μm.



Figures 13–18. Photomicrographs of tracheal coccidia, oocysts of intestinal coccidia, and juvenile nematodes from black-footed ferrets. Figures 13–15. Endogenous stages of tracheal coccidia. 13. Meronts with fully formed merozoites (Me). 14. The surface of a developing oocyst with wall-forming bodies (Oo). 15. An oocyst with sporont and wall fully formed (Oo). 16. Oocysts of *E. ictidea* (Ei) and unidentified eimerian (UE), showing relative sizes. Scale bars Figures 13–16, 10 μm. Figures 17–18. *Physaloptera* sp. 3rd-stage larvae. 17. Whole mount, entire worm. Scale bar = 100 μm. 18. Anterior end showing cephalic collarette (Cc). Scale bar = 10 μm.

monts with developing or fully formed microgametes were common near macrogametes/zygotes and oocysts in various stages of development (Figs. 4, 7). As wall-forming bodies began to fuse, interdigitations (Fig. 7) commonly formed prior to the completion of oocyst wall development.

Endogenous stages of *E. furonis* were seen commonly in the top third but not in crypts or low basal regions of villi. Meronts were small, with 16 or fewer merozoites, and ectopolygeny was not seen (Fig. 8). Microgamonts and macrogametes/zygotes usually were seen in clusters (Figs. 9, 10) and occurred most commonly in cells of the apical one-third of villi, as were oocysts with wall-forming bodies or the oocyst wall interdigitations (Figs. 11, 12).

Sporulated oocysts of the large oval form (Fig. 16) were rarely seen, and no endogenous stages were seen that could be attributed to it, thus precluding its description and identification. Attempts to sporulate this species were variably successful under the conditions described, often with little or no development seen after a 10-day

incubation period in the potassium dichromate solution. Oocysts taken from the intestinal lumen failed to sporulate, whereas some of those passed in feces successfully did so. Measurements are given in Table 1. No coccidial oocysts with these measurements were reported by Levine and Ivens (1981) from mustelids or other carnivores, nor were any reported by Levine and Ivens (1990) or Thomas and Stanton (1994) from prairie dogs, rock squirrels, thirteen-lined, or other ground squirrels.

Meronts and oocysts of a small unidentified coccidian were found in the cells lining the trachea, a bronchus, and in associated bronchial glands in 1 black-footed ferret with canine distemper (Figs. 13–15). Identification awaits finding sporulated oocysts. Merozoites of another unidentified coccidian species were found in an impression smear of the epithelium of the urinary bladder of the same ferret with the respiratory coccidia (Fig. 19).

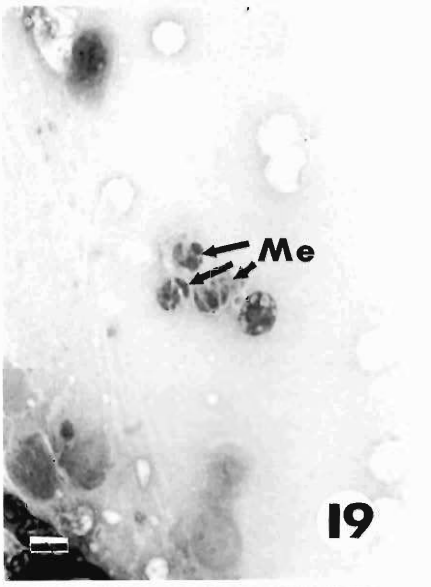


Figure 19. Photomicrograph of coccidian merozoites (Me) in impression smear from the wall of the urinary bladder from a black-footed ferret. Scale bar = 10 μm.

Cysts and trophozoites of a *Giardia* sp. were found in feces and in intestinal luminal contents in histologic sections from a black-footed ferret with canine distemper. The species was probably *G. lamblia,* based on cyst size (mean, 11 × 8 μm) and trophozoite morphological features.

Ten nematodes were recovered from the half-consumed carcass of a free-ranging black-footed ferret found in July 1983. Eight worms were found in the intact stomach and 2 in the remaining portion of the small intestine. These were identified as 3rd stage larval (L_3) *Physaloptera* sp. by virtue of the head collar and other morphological features (Figs. 17, 18).

Discussion

Coccidial oocysts, predominantly *E. ictidea,* were noted in relatively small numbers in feces from free-ranging black-footed ferrets. Oocyst production in ferrets with canine distemper was markedly higher, apparently due to immunosuppression associated with the viral disease (Kauffman et al., 1982). This clinical condition resembles that described by Hoare (1927) in domestic ferrets with canine distemper. In spite of the massive intestinal involvement noted by both Hoare (1927, 1935) and Williams et al. (1988), intestinal inflammation attributable to the coccidial infections was mild, though intestinal function was probably compromised. Mortality of black-footed ferrets due to coccidiosis has recently been reported (Williams et al., 1992).

Although mixed infections occurred, *E. furonis* was much less prevalent than *E. ictidea* both in healthy ferrets and those immunocompromised by canine distemper, and the large oval species was rare. The difficulty in getting the large oocyst to sporulate during incubation, and its scarcity in both normal and ill black-footed ferrets suggests that it may be a species poorly adapted to the black-footed ferret as a host. Those seen probably developed in the black-footed ferret rather than a prey species for several reasons; first, because it was found in feces of animals with canine distemper that were in isolation, anorexic, and fed only laboratory mice, skinned pieces of prairie dog, and nutritional supplements, and second, because none of the eimerians described from prairie dogs, ground squirrels, or mice are as large.

Neither the identity nor the clinical importance of the tracheal or the urinary bladder coccidian species is clear, as no lesions could be attributed to their presence. Identifying criteria were lacking but they are likely different species, based on morphometric and structural differences. Oocyst morphology, immunohistologic tests, and/or life cycle details would aid in their identification. The bladder form would need differentiation from *Toxoplasma, Neospora,* and *Hepatozoan* species, which it resembles in size,

ability to invade a variety of tissue types, and merogonic development. Unfortunately, those seen in the bladder were the only stages seen of coccidia outside of the intestinal and bronchotracheal linings, and immunohistologic tests were not done. *Hepatozoon mustelis* from the Siberian polecat (Novilla et al., 1978) and *Hepatozoon* sp. from mink (Presidente and Karstad, 1975) also resembled the bladder form in the ferret in this report. We are not aware of any reports of *Neospora* in mustelids, although its host range capability presently includes canidae and felidae in which natural infections have been reported in dogs and experimental infections established in cats (Dubey and Lindsay, 1989a, b). The clinical significance of the *Giardia* sp. infection is unknown, but it may have contributed to fluid and electrolyte loss.

The *Physaloptera* larvae could not be identified to species because mature worms are required for such identification. Any clinical effect on the ferrets by the nematodes was probably minor due to the relatively small number of worms and their stage of development.

This report constitutes new host records for *E. ictidea, E. furonis, Giardia* sp., and *Physaloptera* sp. and new distribution records for the coccidia. The intestinal, respiratory, and urinary bladder coccidians require additional study to determine their identities and significance in the black-footed ferret.

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Literature Cited

Boddicker, M. L. 1968. Parasites of the black-footed ferret. Proceedings of the South Dakota Academy of Science 48:141–148.

Carpenter, J. W., and C. N. Hillman. 1979. Husbandry, reproduction, and veterinary care of captive ferrets. 1978 Proceedings of the Annual Meeting of the American Association of Zoo Veterinarians, American Association of Zoo Veterinarians, Washington, D.C. Pages 36–47.

Dubey, J. P., and D. S. Lindsay. 1989a. Transplacental *Neospora caninum* infection in cats. Journal of Parasitology 75:765–771.

———, **and** ———. 1989b. Transplacental *Neospora caninum* infection in dogs. American Journal of Veterinary Research 50:1578–1579.

Forrest, S. C., D. E. Biggins, L. Richardson, T. W. Clark, T. M. Campbell III, K. A. Faggerstone, and E. T. Thorne. 1988. Population attributes for the black-footed ferret (*Mustela nigripes*) at Meeteetse, Wyoming, 1981–1985. Journal of Mammalogy 69:261–273.

Hoare, C. A. 1927. On the coccidia of the ferret. Annals of Tropical Medicine and Parasitology 21: 313–319.

———. 1935. On the endogenous development of the coccidia of the ferret, and the histopathological reaction of the infected intestinal villi. Annals of Tropical Medicine and Parasitology 29:111–122.

Kauffman, C. A., A. G. Bergman, and R. P. O'Connor. 1982. Distemper virus infection in ferrets: an animal model of measles-induced immunosuppression. Clinical and Experimental Immunology 47: 617–625.

Levine, N. D. 1985. Veterinary Protozoology. Iowa State University Press. 414 pp.

———, **and V. Ivens.** 1981. The coccidian parasites (Protozoa, Apicomplexa) of carnivores. Illinois Biological Monographs 51. University of Illinois Press. 248 pp.

———, **and** ———. 1990. The coccidian parasites of rodents. CRC Press. 228 pp.

Novilla, M. N., J. W. Carpenter, and R. P. Kwaplen. 1978. Dual infection of Siberian polecats with *Encephalitozoon cuniculi* and *Hepatozoon mustelis* n. sp. Proceedings of the Symposium on Comparative Pathology in Zoo Animals. Pages 353–363.

Nukerbaeva, K. K., and S. K. Svanbaev. 1973. [Coccidia of fur bearing mammals in Kazakhstan]. Vestnik Sel'skokh Nauki Kazakh 1973 (12):50–54 (cited by Levine and Ivens, 1981).

Presidente, P. J. A., and L. H. Karstad. 1975. *Hepatozoon* sp. infection in mink from southwestern Ontario. Journal of Wildlife Diseases 11:479–481.

Rockett, J., R. S. Seville, N. Kingston, E. S. Williams, and E. T. Thorne. 1990. A cestode, *Taenia mustelae,* in the black-footed ferret (*Mustela nigripes*) and the white-tailed prairie dog (*Cynomys leucurus*) in Wyoming. Journal of the Helminthological Society of Washington 57:160–162.

Schroeder, M. H. 1983. Post-mortem examination of two black-footed ferrets from NW Wyoming. US Fish and Wildlife Service Report. Mimeo. 17 pp.

Thomas, D. M., and N. L. Stanton. 1994. Eimerian species (Apicomplexa: Eimeriina) in Gunnison's prairie dogs (*Cynomys gunnisoni-zuniensis*) and rock squirrels (*Spermophilus variegatus grammurus*) from southeastern Utah. Journal of the Helminthological Society of Washington. 61(1): 17–21.

Thorne, E. T., and E. S. Williams. 1988. Diseases and endangered species: the black-footed ferret as a recent example. Conservation Biology 2:66–74.

Williams, E. S., E. T. Thorne, M. J. G. Appel, and D. W. Belitsky. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases 24:385–398.

———, ———, **D. R. Kwiatkowski, and B. Oalcleaf.** 1992. Overcoming disease problems in the black-footed ferret recovery program. Transactions of the North American Wildlife and Natural Resources Conference 57:474–485.