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Nosema parkeri sp. n., a Microsporidan from the Argasid Tick, *Ornithodoros parkeri* Cooley

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SYNOPSIS. **Nosema parkeri** sp. n. is described from nymphs and adults of the argasid tick, *Ornithodoros parkeri* Cooley, from a laboratory colony. Schizogonic and sporogonic stages are described from various tick tissues. Spores are binucleate, measuring 3.2 (3-4) × 1.9 (1.8-2.5) μm. Transmission is transovarial and transstadial. The parasite does not appear to affect adversely the development or reproduction of the tick. *Dermacentor andersoni* Stiles was experimentally infected. Attempts to infect Swiss mice by tick feeding or by injection of infected tick suspensions were unsuccessful. The microsporidan differs in structure from *Encephalitozoon ixodis* Weiser) and *Nosema slovaca* Weiser & Řeháček, the only other microsporidans known from ticks.

Index Key Words: *Nosema parkeri* sp. n.; natural and experimental infection in argasid tick (*Ornithodoros parkeri*); experimental infection in ixodid tick (*Dermacentor andersoni*); light microscopy.

HITHERTO, 2 species of microsporidan parasites have been described from ticks. The first was seen in a single nymph of *Ixodes ricinus* (Linnaeus) in Czechoslovakia (3). This protozoon was named *Nosema ixodis* Weiser but was subsequently renamed *Encephalitozoon ixodis* (Weiser) (4). The 2nd microsporidan was described from a single adult *Ixodes ricinus* in Czechoslovakia, and named *Nosema slovaca* Weiser & Řeháček (4).

During a study of transmission of *Rickettsia rickettsii* by the argasid tick, *Ornithodoros parkeri* Cooley, microsporidan spores were observed in hemolymph and hemocytes of adult and nymphal ticks from a laboratory colony. This microsporidan is described as a new species of *Nosema*.

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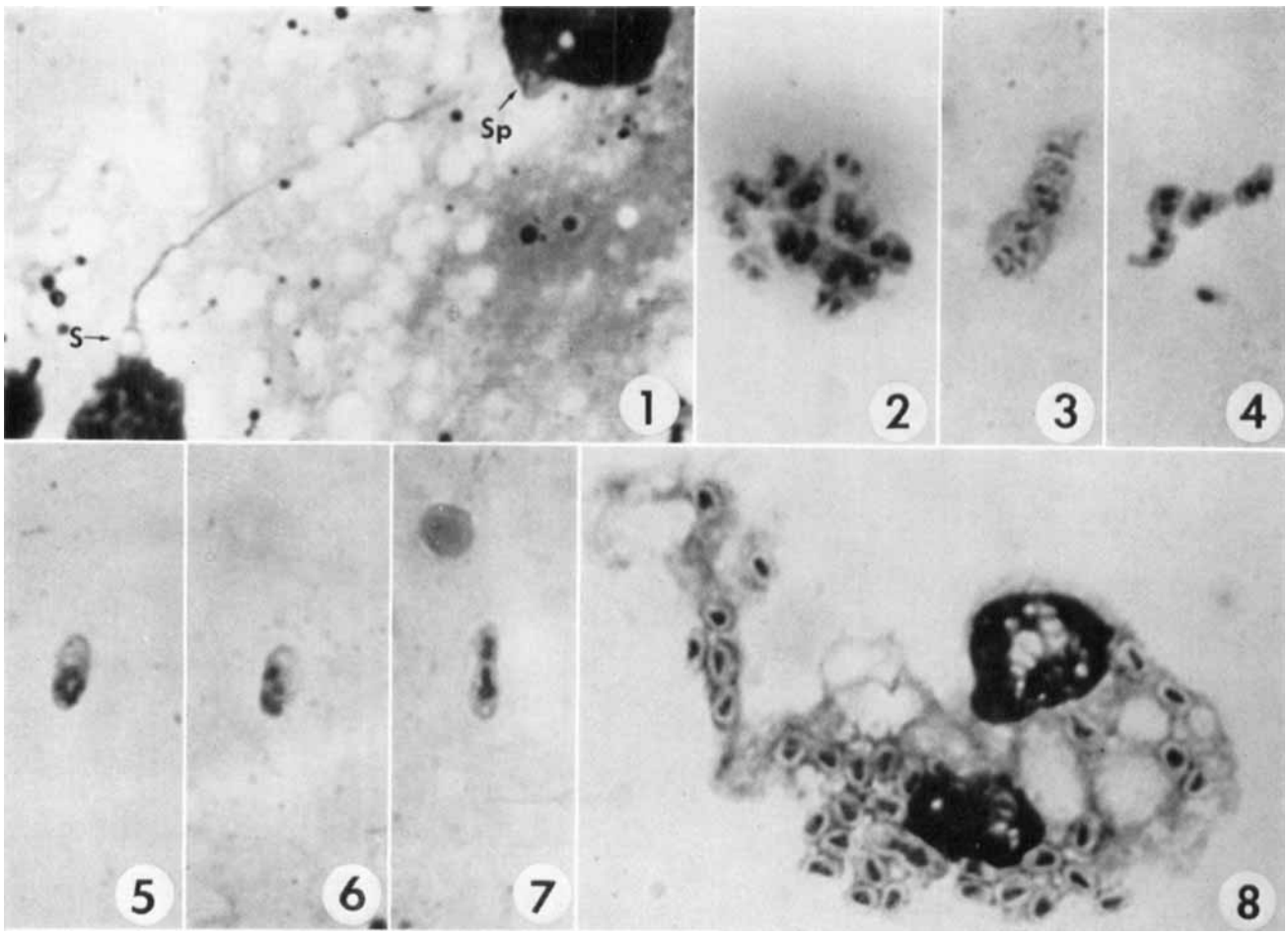
MATERIALS AND METHODS

The infected *O. parkeri* were found in a colony maintained at the Rocky Mountain Laboratory (RML) for more than 25 years. The original laboratory stock was collected from rodent burrows in California. Laboratory specimens have been routinely fed on RML Swiss mice. Ticks used in this study were maintained at 24-26 C and 79% relative humidity.

To screen ticks for microsporidan infections, a drop of hemolymph obtained from each tick (1) was stained with basic carbol fuchsin and malachite green (2) or Giemsa, and examined microscopically. Ticks in which the parasites could not be found were used as recipients for experimental inoculation. Uninfected ticks (laboratory-reared *O. parkeri* and *Dermacentor andersoni* Stiles) were inoculated intracoelomically with hemolymph from naturally infected *O. parkeri*. A glass capillary tube (35 × 1.7 mm drawn out to a diameter



Figs. **1-8**. [Figs. 3, 5-7: stages from experimentally infected *O. parkeri*; others from naturally infected *O. parkeri*. Giemsa's stain. × 2000.] **1**. Spore (S) with extended polar filament and sporoplasm (Sp) in midgut cells. **2**. Binucleate schizonts from salivary gland tissue. **3**. Multinucleate schizonts from central nerve mass. **4**. Binucleate sporonts from salivary gland tissue. **5**, **6**. Quadrinucleate sporonts from central nerve mass. **7**. Dividing sporont from ovarian tissue. **8**. Spores in hemocytes.

of 0.2-0.3 mm) attached to a rubber catheter (8 Fr.) topped by a rubber bulb was used to inoculate the ticks. Naturally and experimentally infected ticks were dissected, and stained tissue smears were examined for microsporidans. Tissue smears were fixed in methanol for 5 min and hydrolyzed in 0.01 M HCl at 60 C for 6-10 min, then stained with Giemsa's. Samples of spores were also stained with periodic acid Schiff's (PAS) reagent. Measurements of developmental stages of the microsporidan were made from camera lucida drawings and photomicrographs.

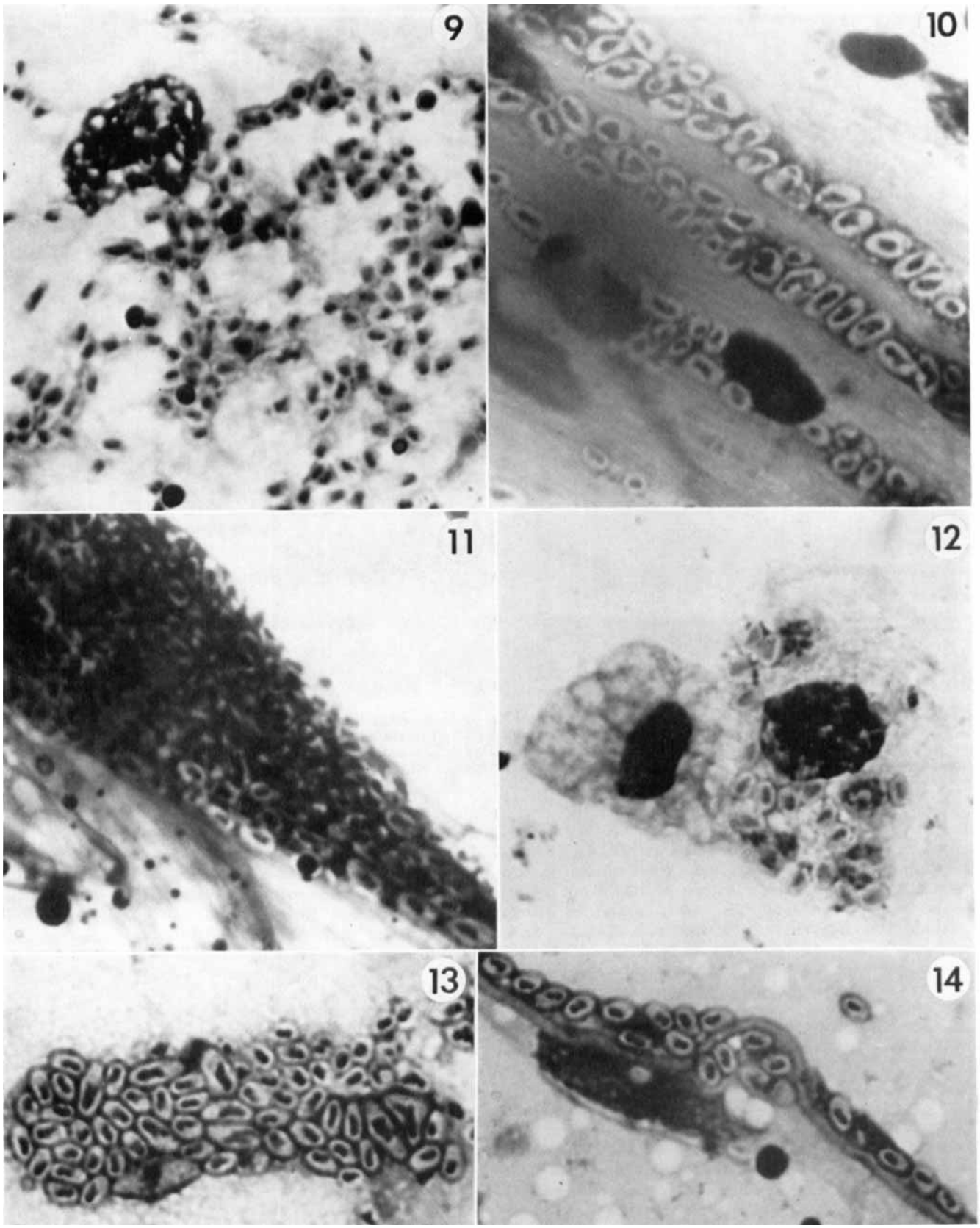
Infected and control female adult ticks, fed on Swiss mice, were exposed to male ticks for mating, and then allowed to oviposit. Eggs and progeny of naturally and experimentally infected females were examined for microsporidans. Eggs, larvae, and 1st-instar nymphs were squashed, stained, and examined microscopically.

Attempts were made to infect 21-day old Swiss mice with the tick microsporidan by feeding infected ticks on them or by inoculating them with suspensions of ground infected ticks [in brain heart infusion (BHI)]. All mice used (with the exception of those which were splenectomized) were injected subcutaneously with 2.5 mg cortisone acetate weekly. One week after the 1st dose of cortisone, naturally infected ticks were fed on 20 mice of which 12 were exposed to adult ticks (1-4/mouse) and 8 to 4th-instar nymphs (12-22/mouse). From 14 to 28 days after tick feeding, mice were killed, and smears of peritoneal scrapings and impression smears of liver, spleen, and brain were made in duplicate and subjected to Giménez's or Giemsa's stains. In addition, muscle strands of diaphragm and smears of white blood cells (buffy coat) were examined from 9 mice exposed to infected adult ticks.

TABLE 1. *Larvae produced by infected and control* O. parkeri *females.*

F_1 adults	F_2 larvae	Hatch rate*
Infected		
♀ 1 F_{1a}	67	25%
♀ 1 F_{1b}	60	49
♀ 3 F_{1a}	150	48
♀ 3 F_{1b}	164	46
♀ 3 F_{1c}	99	42
Control		
♀ A	42	47
♀ B	154	54
♀ C	123	75

* No. larvae/No. eggs laid.



Figs. **9-14**. [Figs. 13, 14: tissues from experimentally infected ticks; others from naturally infected ticks; Fig. 13, stages from *D. andersoni*; others from *O. parkeri*. Giemsa's stain, × 2000.] **9**. Sporogonic stages in Malpighian tubule cells. **10, 11**. Developmental stages in connective tissue underlying scutum. **12**. Sporogonic and schizogonic stages in hemocyte. **13**. Developmental stages in salivary gland tissue. **14**. Developmental stages surrounding tracheole.

TABLE 2. *Distribution of microsporidans in experimentally infected* D. andersoni (D.a.) *and* O. parkeri (O.p.)

Tissues examined	Day after inoculation							
	7		14		21		28	
	D.a.	O.p.	D.a.	O.p.	D.a.	O.p.	D.a.	O.p.
Hyp:	1/1*	1/1	2/2	2/2	2/2	2/2	4/4	3/3
MT:	1/1	1/1	1/2	1/2	2/2	2/2	3/4	2/3
SG:	1/1	0/1	2/2	1/2	2/2	2/2	4/4	3/3
MG:	0/1	1/1	2/2	2/2	2/2	2/2	4/4	3/3
O[T]:	0/1	0/1	2/2	2/2	2/2	1/1 [1/1]	3/3	1/1 [2/2]
CNM:	0/1	0/1	2/2	2/2	2/2	2/2	3/4	3/3

* No. of ticks infected/No. ticks examined. Abbreviations: Hyp, hypodermis (includes muscles and connective tissue); MT, Malpighian tubules; SG, salivary glands; MG, midgut; O, ovary, T, testes; CNM, central nerve mass.

Three 21-day old Swiss mice were each inoculated intraperitoneally (1 week after the 1st dose of cortisone) with 0.5 ml of a suspension of naturally infected ticks (eight 4th-instar nymphs in 3 ml BHI). The mice were killed 14, 18, and 19 days later. Smears of peritoneal scrapings and impression smears of liver, spleen, kidney and brain were stained with Giménez's and Giemsa's and examined for parasites.

Five 21-day old Swiss mice were splenectomized under ether anesthesia. One week later, naturally infected ticks were fed on them. Larvae were fed on 2 mice; 2nd-instar nymphs, on 2 mice; and adult ticks, on 1 mouse. Hematocrit determinations were made every other day, and blood smears were examined every few days for 1 month after tick feeding. Seven months after tick feeding, the animals were killed and smears of peritoneal scrapings and impression smears of brain tissue were stained with Giemsa's and examined for parasites.

RESULTS

Microsporidan spores were first seen in 4 of 30 ticks that were to be used in a rickettsial transmission study. Subsequently, 20 additional naturally infected ticks were found in the laboratory colony of ~ 100. Most of these were used as sources of microsporidans for animal inoculation.

Description of the Parasite.—Developmental stages of the microsporidan are illustrated in Figs. 1-8. The spores were pyriform to oval and binucleate. Two small nuclei, adjacent to each other, were situated laterally near one end of each spore. The sporoplasm stained deep red with basic carbol fuchsin and the polaroplast remained unstained. PAS-positive material was present at the anterior tip of each spore just below the spore capsule. Giemsa's-stained spores ($n = 50$) in hemolymph had a length of 3.2 (3-4) μm and a width of 1.9 (1.8-2.5) μm. The length to width ratio ranged from 1.5-2.0.

Extruded polar filaments (Fig. 1) seen in a few tissue smears were 30-60 μm long. The sporoplasm at the tip of each filament was binucleate.

Schizonts (Figs. 2, 3) varied in shape and had 2 or more nuclei. Schizonts with 2 nuclei had the nuclei adjacent to each other. Dividing nuclei were seen. Binucleate and quadrinucleate sporonts (Figs. 4-6) were observed. A mitotic spindle was visible in 1 sporont in which 2 nuclei were adjacent to a dividing nucleus. Some sporonts had an anterior polar granule (Fig. 5). Sporoblasts attached to each other in pairs were noted (Fig. 7). Therefore, it appears that the sporonts are disporous. The structure of developing and mature spores found in tissues of *O. parkeri* is typical of a *Nosema* species.

Infections in Ticks.—Schizogonic and sporogonic stages were found in naturally and experimentally infected *O. parkeri*. Developmental stages were observed in smears of hemolymph (Figs. 8, 12), hypodermis, Malpighian tubules (Fig. 9), salivary glands (Figs. 2, 4, 13), midgut (Fig. 1), ovary (Fig. 7), testes, and central nerve mass (Figs. 3, 5-6). Sporogonic stages were present in coxal fluid. Microsporidans were frequently found in connective tissue (Fig. 10) and muscle fibers lying just below the dorsal cuticle. Occasionally, massive aggregations of parasites appeared to be enclosed within a cyst (Fig. 11). Sporogonic stages were common in the tissues surrounding tracheoles (Fig. 14). No pathologic changes were noted in tissues examined *in situ* at ×30 magnification or on microscope slides at ×1000.

About 100 infected ticks of the F_1 progeny of 4 naturally infected *O. parkeri* (see modes of infection below) were raised in the laboratory. Infected ticks fed and molted normally. Infected female ticks were mated with normal males. The numbers of larvae produced from eggs of infected females and normal *O. parkeri* females reared and maintained under the same conditions are given in Table 1. The mean numbers of larvae produced by infected and control females were, respectively, 108 and 106.

The results of experimentally infecting paired samples of *O. parkeri* and *D. andersoni* adult ticks are given in Table 2. Experimentally infected ticks were dissected at weekly intervals after intracoelomic inoculation. All tissues examined were infected by 14 days postinoculation. The parasite had no apparent adverse effect on survival of the ticks soon after infection, as seen from a comparison of survival rates of experimentally infected ticks and sham-inoculated controls (Table 3).

Modes of Infection.—Microsporidans were transmitted transstadially and between tick generations through the eggs. Rates of infection in eggs and progeny of 4 naturally infected *O. parkeri* are given in Table 4. Schizogonic and sporogonic stages were seen in eggs, larvae, nymphs, and adult ticks. No morphologic differences were discerned between stages occuring

TABLE 3. *Percent of mean survival of experimentally infected and control (sham-inoculated)* O. parkeri (O.p.) *and* D. andersoni (D.a.).

Tick species		Day postinoculation	
		14	21
O.p.	I*	85	80
	C†	62	50
D.a.	I*	65	60
	C†	62	38

* Experimentally infected ($n = 20$); † Control ($n = 8$).

TABLE 4. *Percentages of infected eggs and progeny of female* O. parkeri *infected with* N. parkeri.

Progeny		Female No.			
Generation	Devel. stage	1	2	3	4
F_1	larvae	—*	—	95†	90†
	nymphs (1st instar)	100‡	100‡	100†	95†
	nymphs (4th instar)	—	—	100‡	—
F_2	eggs	93 ($n = 41$)	100 ($n = 28$)	—	100 ($n = 32$)
	larvae	90,† 90†	—	75,† 80,† 90†	75,† 95,† 95†

* Not determined; † $n = 20$; ‡ $n = 10$.

in eggs and the same stages found in larvae, nymphs, and adults.

Six weeks after experimental infection, 1 *D. andersoni* female was fed on a guinea pig. The tick was mated to a normal male and allowed to oviposit. A sample ($n = 50$) of F_1 larvae were examined for microsporidans; 6% were found infected with spores. The spent female was dissected and all tissues (the same as those listed in Table 2) were found infected with microsporidans. In addition, an egg removed from the ovary was infected.

Susceptibility of Vertebrates.—Microsporidan parasites were not seen in tissues of mice exposed to infected ticks by feeding or inoculation. Exposed mice did not develop ascites, and gross pathologic changes in tissues were not seen. Hematocrits of exposed mice did not differ from those of normal mice used as controls.

TAXONOMIC SUMMARY

Nosema parkeri sp. n.

Diagnosis.—**Spores**: binucleate, pyriform or oval; measuring 3.2 (3-4) × 1.9 (1.8-2.5) μm, length:width ratio 1.5-2.0; transmitted transstadially or transovarially. **Schizonts** and **sporogonic stages**: binucleate, with 2 small nuclei adjacent to each other, or multinucleate.

Type host.—Ornithodoros parkeri Cooley. California (rodent burrows); also laboratory-reared in Rocky Mountain Laboratory, Montana.

Additional host.—Dermacentor andersoni Stiles. Laboratory-reared in Rocky Mountain Laboratory, Montana.

Location in host.—Schizogonic and sporogonic stages in various tick tissues, including hemolymph, hypodermis, connective tissue, muscles, salivary glands, Malpighian tubules, midgut, central nerve mass, ovary, testes.

Remarks

Binucleate spores of *N. parkeri* sp. n. from *O. parkeri* distinguish this parasite from *E. ixodis* and from other species of *Encephalitozoon*. *Nosema slovaca*, the only other microsporidan known from ticks, has elongate-oval spores, measuring 4 ± 0.5 × 1.6 ± 0.1 μm, as compared with pyriform-to-oval, shorter, and wider spores of the species of *Nosema* from *O. parkeri*. On the basis of the foregoing, and because the host is a North American argasid tick, I propose to designate the parasite described in the present paper as a new species, *Nosema parkeri*.

DISCUSSION

The *Nosema* species from *O. parkeri* described in this paper is the only microsporidan that has been found in ticks in North America and the only microsporidan known to occur in an argasid tick.

Host-Specificity.—Ornithodoros parkeri and *O. turicata* (Dugès) were used in the initial study in which the species of *Nosema* from the former was discovered. Hemolymph samples from large numbers of both tick species were examined, but microsporidans were seen only in *O. parkeri*. The *O. turicata* were from a colony that was maintained at the RML under the same conditions as those used for *O. parkeri*. Therefore, it is likely that the *O. parkeri* used to establish the colony were naturally infected with microsporidans.

The experimental infection of *D. andersoni* with *N. parkeri* indicates that the conditions for development of the parasite are not species-specific and suggests that other ticks, including ixodid species, also may act as hosts.

Transmission.—Although spores from infected coxal fluid may be dispersed in the environment, the only known mechanisms of transmission are transstadial and transovarial. Unengorged *O. parkeri* were observed feeding on engorged ticks of the same species in the RML colony; therefore, cannibalism may be another potential means of transmission.

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