VERTEBRATE HOST SPECIFICITY AND EXPERIMENTAL VECTORS OF PLASMODIUM (NOVYELLA) KEMPI SP. N. FROM THE EASTERN WILD TURKEY IN IOWA

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VERTEBRATE HOST SPECIFICITY AND EXPERIMENTAL VECTORS OF *PLASMODIUM* (*NOVYELLA*) *KEMPI* SP. N. FROM THE EASTERN WILD TURKEY IN IOWA

Bruce M. Christensen,[1] H. John Barnes,[2,4] and Wayne A. Rowley[3]

ABSTRACT: Vertebrate host specificity, experimental laboratory vectors, and a description of *Plasmodium* (*Novyella*) *kempi* sp. n. from eastern wild turkeys (*Meleagris gallopavo silvestris* Vieillot) in Iowa are presented. *Plasmodium kempi* is infective for domestic turkeys, bobwhites (*Colinus virginianus*), chukars (*Alectoris graeca*), guinea fowl (*Numida meleagris*), peacocks (*Pavo cristatus*), and canaries (*Serinus canaria*), produces a transient infection in mallards (*Anas platyrhynchos*) and domestic geese (*Anser anser*), but will not infect ring-necked pheasants (*Phasianus colchicus*), pigeons (*Columba livia*), Japanese quail (*Coturnix coturnix*), leghorn white chickens (*Gallus gallus*), or starlings (*Sturnus vulgaris*). Oocysts and (or) sporozoites were recovered from 68% (84/124) and 98% (60/61) of the *Culex pipiens pipiens* and *C. tarsalis* examined, respectively. Oocysts developed faster and sporozoites invaded the salivary glands sooner in *C. tarsalis* (6 days) than in *C. p. pipiens* (7 days). *Culex tarsalis* transmitted *P. kempi* more effectively than *C. p. pipiens*, although both species were capable of transmitting the parasite by natural feeding. Oocysts developed and sporozoites also were produced in *C. restuans*, but its ability to transmit the parasite was not determined. *Aedes aegypti* (Rockefeller strain) and *A. triseriatus* were refractive to *P. kempi*. *Plasmodium kempi* produces trophozoites with large refractile globules and fine cytoplasmic extensions, mature schizonts in the form of a condensed fan containing four to eight nuclei (usually 5), and elongate gametocytes with irregular borders. All stages are confined almost exclusively to mature erythrocytes, with no effect on host cell size or position of host cell nucleus. *Plasmodium kempi* is most similar morphologically to *P.* (*Novyella*) *hexamerium* and *P.* (*Novyella*) *vaughani*. It differs from *P. hexamerium* in having large refractile globules in trophozoites and immature schizonts, an inability to infect starlings, an absence of phanerozoites in capillary endothelium of the brain, and the ability to develop in *C. pipiens* mosquitoes. *Plasmodium kempi* is more like *P. vaughani* morphologically, but differs by infecting turkeys and ducks (transient), by its inability to infect starlings, its lack of morphological variation even when in different hosts, and its ability to develop in *C. pipiens* and *C. tarsalis*.

INTRODUCTION

The first recovery of *Plasmodium* from wild turkeys in North America was made in Florida (Forrester et al., 1974), and this parasite was described subsequently as *P.* (*Huffia*) *hermani* by Telford and Forrester (1975). Although similar to *P. durae*, described from turkeys in Kenya (Herman, 1941), *P. hermani* showed distinct morphological and biological differences (Telford and Forrester, 1975). Several reports of *Plasmodium* infections in turkeys exist for the Old World (Garnham, 1966), but until this time, only *P. hermani* has been reported from turkeys in the New World.

In early 1976, a species of *Plasmodium*, in the subgenus *Novyella*, was recovered from one of four eastern wild turkeys which had been live-trapped, and three of 17 hunter-killed birds in Stephen State Forest, Lucas County, Iowa. Recoveries only were made by subinoculating collected blood into domestic turkey poults. All peripheral blood films from wild turkeys were negative for *Plasmodium*. This paper presents data on (1) vertebrate host specificity, (2) laboratory studies on the extrinsic development and transmission, and (3) gives a description and taxonomic designation for this *Plasmodium* in eastern wild turkeys.

MATERIALS AND METHODS

Isolation from wild turkeys

Heparinized blood samples were obtained from wild turkeys by two methods: (1) by cardiac puncture of hunter-killed birds, or (2) from the wing vein of live-trapped turkeys. Approximately 2–3 ml of blood from each wild turkey was subinoculated intramuscularly into 3- to 4-wk-old domestic turkey poults (broad-breasted-white; Lewis Rich Foods, Ellsworth, Iowa 50075, USA). All recipient birds were maintained in isolation facilities.

Blood films were made daily from recipient poults

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beginning 5 days after inoculation and continuing for 4 wk and were stained with Giemsa's stain (pH 7.2; 1:10 dilution for 1 hr). Approximately 20,000 erythrocytes were examined with oil immersion optics (1,000×) on each blood smear. Measurements and drawings were made with the aid of a calibrated ocular micrometer. All measurements are expressed in μm.

Vertebrate host susceptibility studies

Chukars, bobwhites, guinea fowl, peacocks, canaries, mallards, domestic geese, ring-necked pheasants, pigeons, Japanese quail, starlings, and leghorn white chickens were tested for their susceptibility to the malaria parasite. Ducks and geese were obtained from Schiltz Goose Hatchery, Bancroft, Iowa 50517, USA; pigeons from a colony at the Veterinary Medical Research Institute (VMRI), Ames, Iowa 50011, USA; chickens from the Specific Pathogen Free Laboratory, VMRI; Japanese quail, bobwhites, guinea fowl, peacocks, chukars, and ring-necked pheasants from the Poultry Science Department, Iowa State University, Ames, Iowa 50011, USA; canaries from a local breeder; and starlings were live-trapped at the swine farm, Iowa State University, Ames, Iowa 50011, USA.

Birds were exposed by subinoculation of 0.2–0.5 ml of heparinized blood (IM, IP, or IV) from domestic turkeys with an active parasitemia. Blood inoculated was from the third, fifth, or sixth passage from the original isolate. Recipient birds were maintained in isolation facilities and blood films were made twice each week beginning 5 days after inoculation and continuing for 8 wk. Blood films were stained and examined as described previously.

Laboratory vector studies

Three ornithophilic mosquito species (*Culex tarsalis*, *C. pipiens pipiens*, and *C. restuans*) and two *Aedes* species (*A. aegypti* (Rockefeller strain) and *A. triseriatus*) were used in this study. Field-collected larvae of *C. restuans* were reared to adults in the laboratory for use in these studies. *Culex tarsalis*, *A. aegypti*, and *A. triseriatus* were obtained from laboratory colonies that had been maintained for several years. The *C. p. pipiens* colony was newly established from field-collected egg rafts, and second generation adults were used in this study.

Larvae were reared in white enamel trays (25 × 42 × 7 cm) and fed a diet of tropical fish food (Tetramin®). Pupae were harvested and placed in crystalizing dishes in a screened cage (56 × 46 × 46 cm) for emergence. A cotton pad saturated with 0.3 M sucrose solution was placed on the screen and served as the energy source for adult mosquitoes. Sucrose pads were removed 24 hr before blood feeding. All mosquitoes were 4–6 days old when exposed to an infective blood meal. All mosquitoes were maintained at 26.5 ± 1 C and 80 ± 5% RH throughout the study.

Broad-breasted white turkey poults, approximately 14 days old, were blood infected from sporozoite-infected domestic turkeys (i.e., first passage). Parasitemia, percentage of gametocytes, and exposure index (=parasitemia × percentage of gametocytes) were determined for each poult on the day they were exposed to mosquitoes.

Poults were restrained and placed in mosquito cages overnight (approximately 10 hr). Blood-fed mosquitoes were removed and placed in lots of 50 in 0.473-liter ice cream cartons with a fine-mesh marquisette covering. Mosquitoes were dissected in *Aedes* saline (Hayes, 1953) with the aid of a stereomicroscope. Midguts and salivary glands were examined with phase contrast optics. Measurements of living oocysts were made with a calibrated ocular micrometer. Following examination, midguts were fixed in AFA and stained with hematoxylin, and salivary glands were smeared on a slide, methanol fixed, and stained with Giemsa's stain at pH 7.2 (1:10 dilution for 1 hr).

Fourteen-day-old turkey poults were used in transmission studies, and were maintained in a mosquito-proof environment. Individual poults were subjected to the bite of from one to 17 infective *Culex* mosquitoes, or to the bite of several hundred *Aedes* mosquitoes. *Culex* mosquitoes were dissected following feeding to determine if their salivary glands contained sporozoites. Thin blood smears were prepared daily from exposed poults beginning 6 days postexposure (PE) and continuing for 8 wk. Blood films were stained and examined as previously described.

Studies of exoerythrocytic stages

Seven 2-wk-old poults were subinoculated with infective blood and individual birds were necropsied at weekly intervals beginning 4 wk postinoculation. Impression smears were made of brain, liver, lung, spleen, kidney, and bone marrow, fixed with methanol, and stained with Giemsa's stain at pH 7.2 (1:10 dilution for 1 hr). Tissue smears were screened at 400× and studied with oil immersion optics (1,000×).

RESULTS

Experimental vertebrate hosts

In addition to domestic turkeys, all stages of the parasite developed in chukars, bobwhites, guinea fowl, peacocks, and canaries. A transient infection was produced in mallards and geese. No parasite development occurred in ring-necked pheasants, chickens, pigeons, Japanese quail, or starlings. Data on experimental vertebrate hosts are presented in Table 1.

No obvious clinical signs or mortality occurred in any avian host exposed and (or) infected in these studies.

Experimental vectors

Exposure indices of infective turkey poults and mosquito species exposed to individual birds are given in Table 2. Exposure indices were very similar in all poults except 2580. This was the only infective bird available when our limited population of *C. restuans* was ready to

TABLE 1. Susceptibility of different bird species to a *Plasmodium* from wild turkeys in Iowa to subinoculation of 0.2–0.5 ml of heparinized blood from turkeys with an active parasitemia (third, fifth, or sixth passage from original isolate).

Experiment no	Blood passage	No exposed	Species	Age	Route of subinoculation	No. infected (%)
I	3rd	5	pigeons	adult	IM	0 (0)
		12	chickens	1 day	IM	0 (0)
		15	turkeys	9 days	IM	15 (100)
		8	turkeys	42 days	IM	8 (100)
II	5th	6	geese	7 days	IV	6 (100)[a]
		8	ducks	7 days	IV	8 (100)[a]
		4	turkeys	45 days	IV	4 (100)
III	6th	5	canaries	90 days	IP	5 (100)
		10	pheasants	17 days	IV	0 (0)
		10	chukars	14 days	IV	8 (80)
		4	turkeys	11 days	IV	3 (75)
IV	6th	3	pigeons	42 days	IV	0 (0)
		12	chickens	14 days	IV	0 (0)
		12	guinea fowl	14 days	IV	12 (100)
		20	bobwhite	14 days	IV	19 (100)[b]
		2	turkeys	17 days	IV	2 (100)
V	6th	14	Japanese quail	21 days	IV	0 (0)
		8	peacocks	21 days	IV	8 (100)
		6	turkeys	14 days	IV	6 (100)
VI	6th	16	starlings	adult	IV	0 (0)
		4	turkeys	14 days	IV	4 (100)

[a] Transient infections, no gametocytes produced.
[b] One quail died before it was tested.

blood feed. Of 60 *C. restuans* exposed to turkey 2850, only five individuals fed, and none would take a second feeding; therefore, the ability of *C. restuans* to transmit the *Plasmodium* was not determined. All five mosquitoes were dissected and all contained oocysts and (or) sporozoites.

Culex tarsalis and *C. p. pipiens* both supported the complete development of the *Plasmodium*, although a greater percentage of *C. tarsalis* was infected at nearly all developmental times (Table 3). Of 61 *C. tarsalis* examined, only one mosquito was negative for all developmental stages.

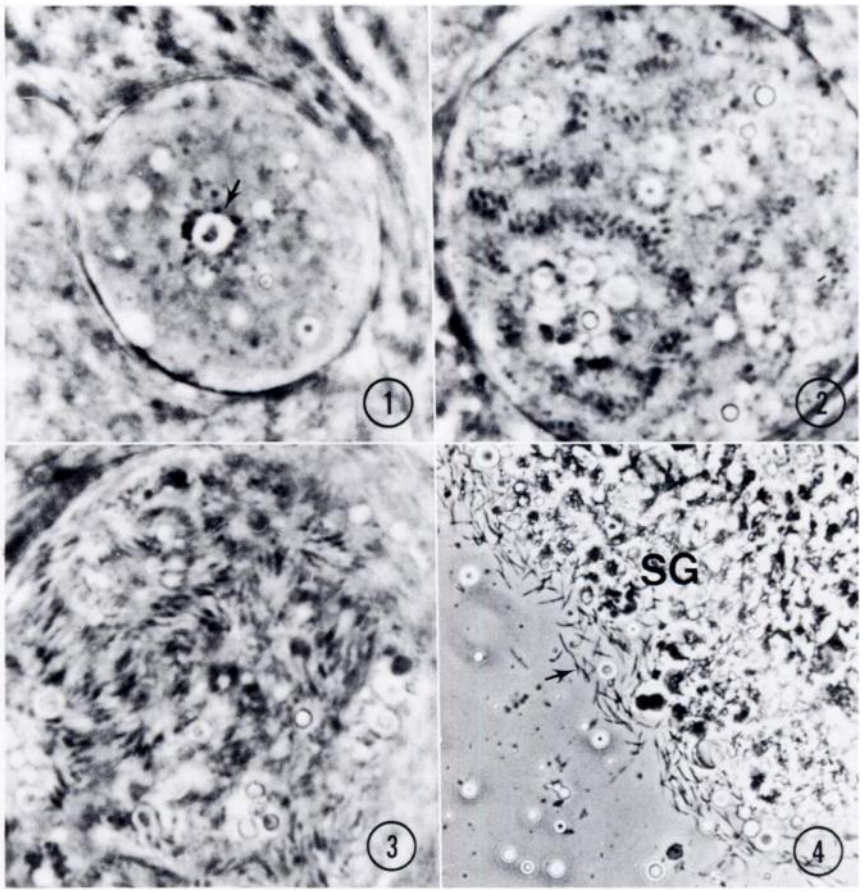
Growth and development of oocysts was more rapid in *C. tarsalis* than *C. p. pipiens* (Table 4). Mature oocysts, containing fully formed sporozoites, were noted on day 5 PE in *C. tarsalis*, but not until 6 days PE in *C. p. pipiens* (Figs. 1–3). Consequently, vacuolated oocysts on the midgut and sporozoites in the salivary glands were first seen on day 6 PE and day 7 PE in *C. tarsalis* and *C. p. pipiens*, respectively (Fig. 4). Nearly 100% of the *C. tarsalis* examined contained sporozoites in their salivary glands after 7 developmental days. There was a general decrease in percentage of *C. p. pipiens* infected with sporozoites after day 9 PE. It was not determined if a mosquito defense reaction against mature oocysts or sporozoites could have accounted for this decrease.

Both *C. tarsalis* and *C. p. pipiens* transmitted the *Plasmodium* to susceptible turkey poults by natural feeding (Table 5). Six of seven transmission experiments with *C. tarsalis* and three of five with *C. p. pipiens* were successful. A single infective mosquito of both species trans-

TABLE 2. Parasitemia, percentage of gametocytes, and exposure index of turkeys used to expose mosquitoes to a *Plasmodium* from wild turkeys in Iowa.

Turkey no	Mosquito	Parasitemia[a]	Gametocytes (%)	Exposure index[b]
2580	*C. restuans*	30	3.6	108
2129	*C. p. pipiens*	295	5.4	1,593
2132	*A. aegypti* and *A. triseriatus*	310	4.6	1,426
2134	*C. p. pipiens*	220	5.2	1,144
2134	*C. tarsalis*	385	3.0	1,155

[a] Parasites per 10,000 erythrocytes.
[b] Exposure index = parasitemia × % gametocytes.



FIGURES 1–4. Sporogonic stages of *Plasmodium kempi* sp. n. in *Culex tarsalis* and *C. pipiens pipiens*. 1. Oocyst from *C. p. pipiens* at 4 days PE, arrow indicates sporoblast. ×1,650. 2. Oocyst from *C. p. pipiens* at 5 days PE. ×1,435. 3. Mature oocyst from *C. tarsalis* at 5 days PE. ×1,890. 4. Salivary glands (SG) and sporozoites (arrow) from *C. tarsalis*. ×300.

TABLE 3. Infection of *Plasmodium* from wild turkeys in Iowa in *Culex pipiens pipiens* and *Culex tarsalis*.

Days of development	*Culex pipiens pipiens*		*Culex tarsalis*	
	Positive/examined (%)	Stage of parasite development*	Positive/examined (%)	Stage of parasite development
3	8/10 (80)	O	6/6 (100)	O
4	10/10 (100)	O	5/5 (100)	O
5	9/10 (90)	O	6/6 (100)	O, SO
6	9/10 (90)	O, SO	6/6 (100)	O, SO, SG
7	10/10 (100)	O, SO, SG	5/5 (100)	O, SO, SG
8	8/10 (80)	O, SO, SG	5/5 (100)	O, SO, SG
9	6/10 (60)	O, SO, SG	6/6 (100)	O, SO, SG
10	—/—		10/11 (91)	SG
11	1/1 (100)	O, SO, SG	10/10 (100)	SG
12	18/37 (49)	SG	—/—	
13	3/12 (25)	SG	—/—	
14	2/4 (50)	SG	—/—	
Total	84/124 (68)	O, SO, SG	60/61 (98)	O, SO, SG

* O = oocysts; SO = sporozoites in oocysts; SG = sporozoites in salivary glands.

TABLE 4. Measurements (μm) of developing oocysts of *Plasmodium* from wild turkeys in Iowa in *Culex pipiens pipiens* and *Culex tarsalis*.

Days of development	*Culex pipiens pipiens*		*Culex tarsalis*	
	Number measured	Mean ± SD	Number measured	Mean ± SD
3	25	11.8 ± 2.00	57	13.5 ± 2.51
4	52	18.5 ± 3.33	61	21.7 ± 2.85
5	52	26.4 ± 4.19	55	29.5 ± 3.56
6	63	35.2 ± 7.69	80	32.5 ± 4.71
7	39	40.3 ± 6.98	43	38.0 ± 5.17
8	55	39.5 ± 5.26	41	40.5 ± 5.49

TABLE 5. Transmission of a *Plasmodium* from wild turkeys in Iowa to susceptible turkey poults by *Culex tarsalis* and *Culex pipiens pipiens*.

Mosquito species	Number of infective mosquitoes feeding	Days following exposure and degree of parasitemia[a] in poults		
		14–17 days	21–24 days	31–34 days
C. tarsalis	1	0	0	0[b]
	1	12	29	55
	3	17	12	1
	4	30	250	12
	5	1	14	28
	5	2	5	4
	6	0	0	21
C. p. pipiens	1	0	0	0[b]
	1	0	2	1
	2	0	0	100
	3	0	0	0[b]
	17	0.5	15	8

[a] Parasites per 10,000 erythrocytes.
[b] Negative poults were examined through 56 days postexposure.

mitted the parasite and caused a patent infection.

Qualitatively, it seemed that the number of sporozoites was much greater in *C. tarsalis* salivary glands as compared to *C. p. pipiens*. This was possibly due to the difference in the number of oocysts established in each mosquito species. All *C. tarsalis* examined from 3–8 days PE contained over 50 oocysts, with many harboring several hundred. While the majority of *C. p. pipiens* contained over 30 oocysts per mosquito, 23% (14/60) of the individuals examined through day 8 PE contained fewer than five oocysts. The effect this greater number of sporozoites had was reflected in the transmission experiments. Parasitemias were greater and developed faster in poults exposed to infective *C. tarsalis* in contrast to those exposed to *C. p. pipiens* (Table 5).

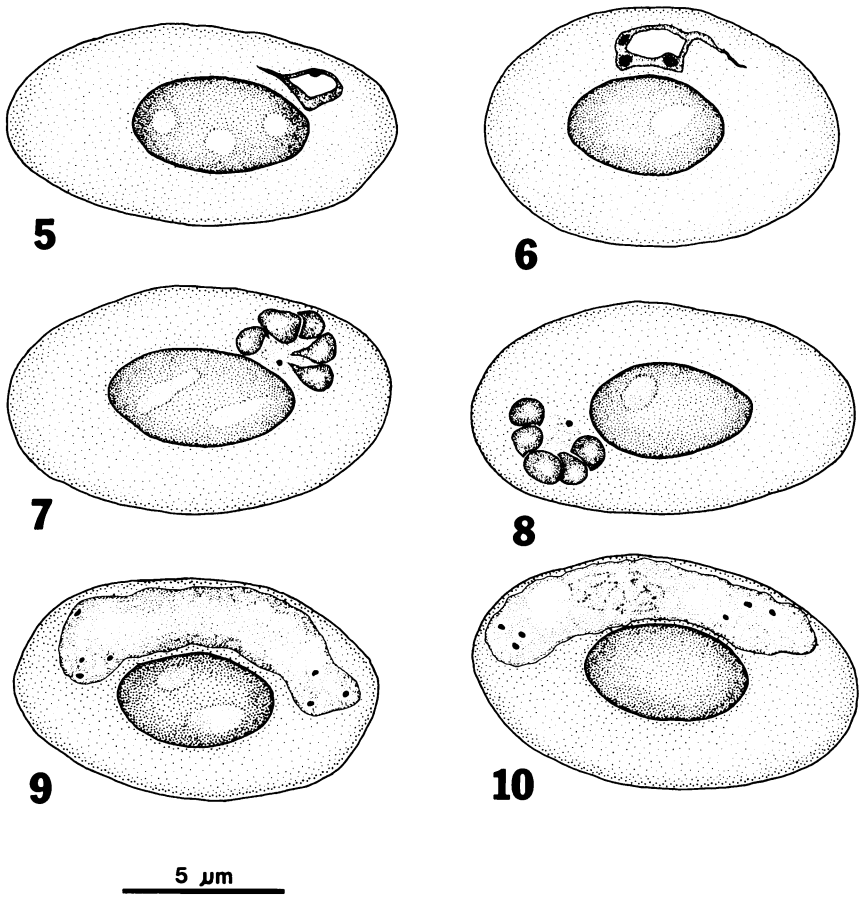
No infected *A. aegypti* or *A. triseriatus* were found by dissection. At 15 days PE, several hundred of each species were allowed to feed on susceptible poults in an attempt to determine if very low infection rates had occurred. No transmission occurred and we concluded that both *Aedes* species were refractive to the *Plasmodium*.

Description of erythrocytic stages

Trophozoites: Trophozoites characteristically contained a ring of gray cytoplasm surrounding a large refractive globule (Figs. 5, 11). A thin cytoplasmic extension was visible on nearly all trophozoites examined. Trophozoites averaged 1.9 × 1.3 (0.7–2.6 × 0.7–1.8), $n = 25$. Trophozoites were found almost exclusively in mature erythrocytes and had a general distribution within the host cell. Usually a single trophozoite was found in an erythrocyte, although as many as four uninucleated parasites were seen in one cell. No nuclear displacement was evident.

Schizonts: All immature schizonts contained a large refractive globule surrounded by a margin of cytoplasm that usually included an extension in the form of a "tail" (Figs. 6, 12–14). Binucleate schizonts measured 1.5–2.0 × 1.3–1.7. As schizonts matured they condensed into a compact "fan-shape" (Figs. 7, 8, 15, 16). Merozoites lacked any visible cytoplasm and numbered from four to eight per schizont, with the majority (95%) of mature schizonts containing five nuclei. A single pigment granule was associated with the base of the fan in all mature schizonts examined (Figs. 7, 8). Mature schizonts averaged 2.5 × 2.6 (1.8–4.0 × 1.6–3.7), $n = 20$. Schizonts were found only in mature erythrocytes.

Gametocytes: Immature gametocytes had an oblong appearance and contained six to 10 pigment granules scattered throughout the cytoplasm. These granules were often clumped together in groups of three or four. Small tags of cytoplasm often projected from developing gametocytes. Mature gametocytes occupied the majority of the cytoplasmic space lateral to the nucleus, but never displaced the host cell nucleus (Figs. 9, 10, 17, 18). Rarely did the gametocytes occupy a polar position in the erythrocyte. Mature gametocytes often curved slightly at the ends (Figs. 9, 17), but seldom curved around the nucleus. Margins of gametocytes were irregular on all sides. Macroga-



FIGURES 5–10. *Plasmodium kempi* sp. n. 5. Trophozoite. 6. Immature schizont. 7, 8. Mature schizonts (compact fan-shaped). 9. Microgametocyte. 10. Macrogametocyte. All parasites in mature erythrocytes.

metocytes stained a deeper blue than microgametocytes, and usually contained pale vacuoles (Figs. 10, 18). A deeper red-staining chromatin area usually was evident in macrogametocytes (Fig. 10). Microgametocytes lacked vacuolated areas, stained light pink, and nuclear material was diffuse throughout the cell, never being concentrated in one precise area (Figs. 9, 17). Macrogametocytes averaged 8.9 × 1.8 (7.3–11.0 × 1.5–2.2), $n = 10$; microgametocytes averaged 8.2 × 1.8 (7.3–9.5 × 1.3–2.2), $n = 10$. Gametocytes were confined to mature erythrocytes.

Exoerythrocytic stages

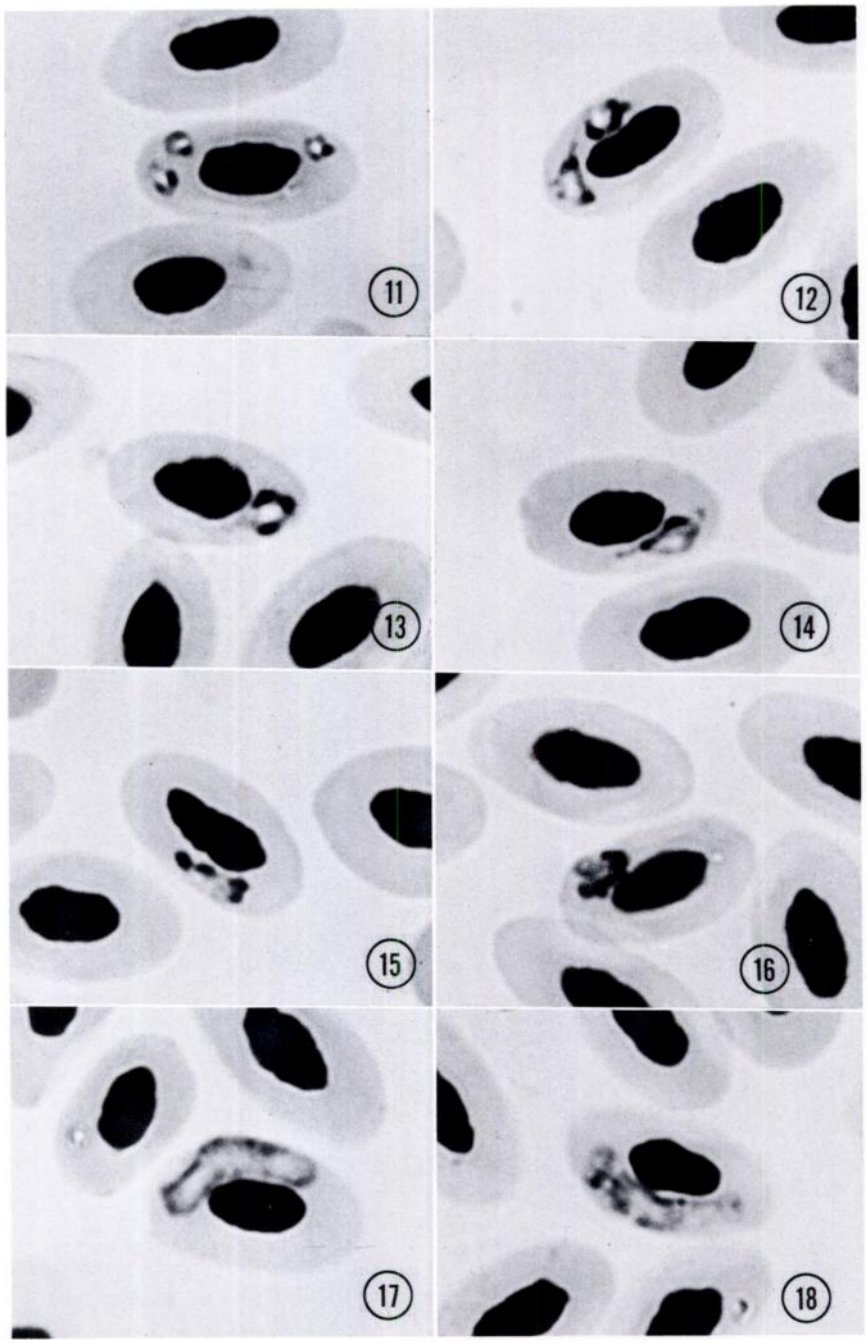
No phanerozoites or other exoerythrocytic stages were found in any smears of brain, liver, spleen, lung, kidney, or bone marrow.

TAXONOMIC SUMMARY

Plasmodium (*Novyella*) *kempi* sp. n.

Diagnosis: Trophozoites with large refractile globule and fine cytoplasmic extensions; mature schizonts in the form of a condensed fan, with four to eight merozoites (usually 5); gametocytes elongate with irregular borders, rarely much longer than the host cell; all parasite stages typically in mature erythrocytes, with no effect on host cell size or position of host cell nucleus; infective for turkeys, bobwhites, chukars, guinea fowl, peacocks, and canaries, but not ring-necked pheasants, chickens, pigeons, Japanese quail, or starlings.

Type host: Meleagris gallopavo silvestris Vieillot, 1817. Stephen State Forest, Lucas County, Iowa, U.S.A. *Syntype slides* in Inter-



FIGURES 11–18. *Plasmodium kempi* sp. n. ×1,850. 11. Trophozoites. 12–15. Immature schizonts. 16. Mature schizont (fan-shaped). 17. Microgametocyte. 18. Macrogametocyte. All parasites in mature erythrocytes.

national Reference Centre for Avian Haematozoa, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, accession numbers 91085–91088; and in U.S. National Parasite Collection, Beltsville, Maryland, USA, accession number 77457; available also from the authors.

Additional experimental vertebrate hosts: Alectoris graeca (chukar), *Colinus virginianus* (Bobwhite), *Numida meleagris* (guinea fowl), *Pavo cristatus* (peacock), *Serinus canaria* (canary), *Anser anser* (domestic goose)—transient, *Anas platyrhynchos* (mallard)—transient.

Geographic range: Lucas County, Iowa, USA.

Etymology: Named in memory of Dr. Russell Kemp, protozoologist, Iowa State University.

DISCUSSION

A brief review of *Plasmodium* infections in wild and domestic turkeys has been presented by Telford and Forrester (1975). The parasite described herein from wild turkeys in Iowa, however, is not similar to any of the *Plasmodium* parasites previously described from turkeys. The morphological characteristics of *P. kempi* clearly place it in the subgenus *Novyella*, with elongate gametocytes, schizonts in mature erythrocytes, and with erythrocytic schizonts lacking noticeable cytoplasm and being smaller than the host cell nucleus (Garnham, 1966). Within this subgenus, the avian parasites *P. vaughani* and *P. hexamerium* resemble most closely *P. kempi*.

The morphological characteristics of the erythrocytic stages of *P. vaughani*, *P. hexamerium*, and *P. kempi* are quite similar. The presence of (1) a large refractile globule in the trophozoite and immature schizont, (2) four to eight merozoites in the mature schizont, and (3) the shape of the gametocyte indicate a closer morphological similarity of *P. kempi* to *P. vaughani*. Although there are subtle morphological differences between these two parasites (e.g., the irregular number of merozoites in *P. vaughani* compared with the almost standard number of five in *P. kempi*, and the small number of round-shaped pigment granules in the *P. kempi* in contrast to the variable, often numerous granules in *P. vaughani*), the separation of these two species solely on the morphology of the erythrocytic stages would be difficult.

Biological data, however, indicate several distinct differences between *P. kempi*, *P. vaughani*, and *P. hexamerium*. *Plasmodium vaughani* and *P. hexamerium* generally are considered to be parasites of non-gallinaceous birds (Greiner et al., 1975), although experimental infections of quail with *P. vaughani* and turkeys with *P. hexamerium* have been reported (Garnham, 1966; Manwell, 1952). *Plasmodium kempi* readily infected several gallinaceous birds (turkeys, chukars, guinea fowl, bobwhites, and peacocks). Starlings were susceptible to both *P. vaughani* and *P. hexamerium*, but not to *P. kempi*. In addition, *P. vaughani* generally does not infect ducks but *P. hexamerium* does (Manwell, 1952), and with *P. kempi* only a transient infection could be established in ducks and geese.

Differences in mosquito susceptibility between the three species are pronounced. *Plasmodium kempi* develops completely in *C. tarsalis*, *C. p. pipiens*, and *C. restuans*; *C. tarsalis* and *C. p. pipiens* can transmit the infection by natural feeding. Previous studies indicated that *P. vaughani* and *P. hexamerium* did not develop in *C. tarsalis* or *C. pipiens* (Huff, 1965; Manwell, 1947), although Janovy (1966) provided evidence that *P. hexamerium* developed in *C. tarsalis*.

Although exoerythrocytic schizogony is unknown for *P. vaughani*, Maxwell (1951) reported phanerozoites of *P. hexamerium* in capillary endothelium of the brain from an infected orange-crowned warbler (*Vermivora celata*) trapped in New York. Despite extensive study, we found no secondary exoerythrocytic parasites in the brain of *P. kempi*-infected turkeys.

It is possible that *P. kempi* could be a strain or subspecies of either *P. vaughani* or *P. hexamerium*, or perhaps all three parasites are simply variants of a single species. Considering *P. vaughani* and *P. hexamerium* as the same species generally is not accepted for a number of reasons (Garnham, 1966). Morphologically we can best align *P. kempi* with *P. vaughani*, but *P. vaughani* shows extreme variations in morphology (Garnham, 1966), a characteristic we did not see in *P. kempi* even when in different hosts. *Plasmodium kempi* exhibited little variation in number of merozoites, pigment granules, or in shape and appearance of schizonts or gametocytes in any of the avian hosts infected. The presence of refractile globules and almost constant number of five nuclei per schiz-

ont in *P. kempi* readily separates morphologically this parasite from *P. hexamerium* that has no refractile globules and rarely contains less than six merozoites in a mature schizont.

Biological differences between these species are significant, however, and seem to outweigh the morphological similarities. Differences in both vertebrate and invertebrate host susceptibility are distinct, and thereby justify designating *Plasmodium* from wild turkeys in Iowa as a distinct species in the subgenus *Novyella*. To simply refer to this parasite as a strain or subspecies of *P. vaughani* or *P. hexamerium* would only further confuse an already difficult taxonomic group. Until more detailed studies of exoerythrocytic schizogony, vertebrate host immune responses, and vertebrate and invertebrate host susceptibility are done, it seems reasonable to take this approach.

The *Culex* species examined in this study were excellent laboratory vectors for *P. kempi*, and the ability of *C. tarsalis* and *C. p. pipiens* to transmit the infection by feeding gives strong support for the ability of these species to function as vectors under natural conditions.

The 98% infection prevalence seen in *C. tarsalis* is very high compared with the majority of studies conducted with this mosquito and avian malaria parasites (Huff, 1965). The only comparable data were obtained by Rosen and Reeves (1954) when they reported 86 of 88 *C. tarsalis* infected with *P. relictum*. Infection prevalences for *C. p. pipiens* seen in our study are more comparable to the majority of reports in the literature. Although our *C. tarsalis* colony might have been selected for susceptibility due to many years of colonization in the laboratory, this would not be the case with the newly established *C. p. pipiens* colony.

Little is known concerning the extrinsic development and transmission of the closely related *P. vaughani*. Huff (1965) was unable to obtain development of this parasite in *A. aegypti*, *A. albopictus*, *C. tarsalis*, or *C. p. pipiens*. Subsequently, Williams and Bennett (1978) reported low prevalences of infection for *Culiseta moristans* (16%) and *Coquilletidia* (=*Mansonia*) *perturbans* (2%) which fed on blackbirds harboring *P. vaughani*, but the ability of these mosquitoes to transmit the infection by feeding was not determined. The importance of transmission experiments has been illustrated by Nayar et al. (1980) in their work with *Wyeomyia vanduzeei* and *P. hermani*. Although *W. vanduzeei* supports the complete development of *P. hermani*, it is not capable of transmitting the infection by natural feeding.

Natural and experimental mosquito vectors of the only other North American turkey malaria, *P. hermani*, are well known. *Culex nigripalpus* functions as a natural vector (Young et al., 1977; Forrester et al., 1980), and *C. salinarius* and *C. restuans* function as experimental vectors (Nayar et al., 1981a, b).

Culex tarsalis and *C. p. pipiens* are common mosquitoes in Iowa (Pinger and Rowley, 1972), and their ornithophilic feeding habits make them suspect as natural vectors of *P. kempi*. Field studies are necessary, however, to determine the role these mosquitoes play in the natural maintenance of this parasite. Likewise, additional studies with *C. restuans* seem warranted from the limited data obtained for this mosquito in the laboratory.

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Journal of Wildlife Diseases, 19(3), 1983, p. 213

CORRECTION . . .

The statement that the finding of *Trichinella* in marten is a new host and North American record in:

POOLE, B. C., K. CHADEE, AND T. A. DICK. 1983. Helminth parasites of pine marten, *Martes americana* (Turton), from Manitoba, Canada. J. Wildl. Dis. 19: 10–13.

is in error. Two previous records are known:

SCHMITT, N., J. M. SAVILLE, J. A. GREENWAY, P. L. STOVELL, L. FRIIS, AND L. HOLE. 1978. Sylvatic trichinosis in British Columbia. Public Health Rep. 93: 189–193.

WORLEY, D. E., J. C. FOX, J. B. WINTERS, AND K. R. GREER. 1974. Prevalence and distribution of *Trichinella spiralis* in carnivorous mammals in the United States northern Rocky Mountain region. *In* Proc. Third Int. Conf. Trichinellosis, C. W. Kim (ed.). Intext Educational Publ., New York, New York, pp. 597–602.