

## Host range, survival in dead hosts, cryopreservation, periodicity and morphology of *Plasmodium durae* Herman in experimental infections

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### ABSTRACT

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In experimental infections, fowl, duck, guineafowl and canary were refractory to *Plasmodium durae*, which in Japanese quail produced low and transient infections, but a high and long-lasting parasitaemia in a Lady Amherst pheasant. Heart, blood and brain of dead hosts injected into turkeys, allowed the recovery and further passaging of the live parasite. This technique could be useful for the recovery of malaria parasites from suspect postmortem material. Intravenous infection produced parasitaemias in chicken and turkey embryos, while attempts at allantoic-sac infections of chicken embryos were unsuccessful. A certain degree of periodicity of schizogony was demonstrated. The South African isolates of *P. durae* had smaller schizonts than those described from East and West Africa, with 2–14 merozoites (mostly four).

Some strains did not produce mature gametocytes in the experimental hosts. Exoerythrocytic schizonts of *P. durae* are depicted in this paper for the first time.

**Keywords:** Cryopreservation, experimental infections, host range, morphology, periodicity, *Plasmodium durae*, survival in dead hosts

### INTRODUCTION

*Plasmodium durae* was first encountered in naturally infected domestic turkeys in Kenya (Herman 1941; Purchase 1942). Huchzermeyer (1993a) reviewed the occurrence of natural infections in African phasianids *sensu lato* and reported on isolations of this parasite from francolins (*Francolinus* spp.) and domestic turkeys in South Africa.

#### Host range

Purchase (1942) found ducks resistant to experimental infection with *P. durae*, but was able to induce

a transient infection in young chicks (*Gallus gallus*). However, Simpson (1944) reported transmitting the infection from a duck of unspecified origin to turkeys. Garnham (1966) stated that canaries and pigeons were refractory. Laird (1978) found common peafowl (*Pavo cristatus*) naturally infected with *P. durae* in northern Nigeria.

Embryonated eggs have been infected successfully with a number of avian plasmodia: Duck embryos were infected via the allantoic membrane with blood stages of *P. cathemerium*, *P. elongatum* and *P. lophurae* (Wolfson 1940) and chick embryos intravenously (i.v.) with blood stages of *P. lophurae* (Kovic & Zeuthen 1964). Chick embryos were infected i.v. with blood stages of *P. hexamerium*, but were refractory

to infection with *P. elongatum* (Manwell & Robinson 1961). Zuckerman (1946) successfully infected chick embryos with the exoerythrocytic forms of *P. gallinaceum* by chorio-allantoic implants, after Haas, Feldman & Ewing (1945) had already reported on the i.v. infection of chick embryos with blood stages of the same parasite, while Gavrillov, Bobkoff & Laurencin (1938) failed in their attempt to do the same. The infection of turkey and chick embryos with the exoerythrocytic forms of *P. fallax* by chorio-allantoic implants, was reported by Graham, Stauber, Palczuk & Barnes (1973).

### Survival in dead hosts

Young (1985) demonstrated the survival of *P. berghei* in dead mice at room temperature for up to 24 h in the only reported attempt to recover malaria parasites after the death of the host.

### Cryopreservation

The preservation of *Aegyptianella pullorum* in liquid nitrogen with and without cryoprotectants was described by Huchzermeyer (1965) and later Bartkowiak, Huchzermeyer, Potgieter, Van Rensburg, Labuschagne & Van Biljon (1988) reported the successful freeze-drying of the same organism.

The preservation of malarial parasites on dry ice at a temperature of  $-76^{\circ}\text{C}$  was achieved by a number of authors:

- *P. knowlesi* and *P. inui* without cryoprotective agents for 70 d (Coggeshall 1939)
- *P. falciparum* for up to 739 d and *P. ovale* for 997 d (Jeffery 1957)
- *P. cynomologi*, *P. knowlesi*, *P. vinckei* and *P. gallinaceum* for at least 1 year (Molinari 1961)
- *P. berghei* and *P. gallinaceum* for 28 d (Jeffery 1962).

With the use of liquid nitrogen, the freezing temperature was lowered to  $-196^{\circ}\text{C}$ . Malarial parasites were preserved at this temperature as follows:

- *P. berghei* and *P. relictum* for 14 d (Mieth 1966)
- *P. gallinaceum* for 767 d (Weatherby & McCall 1967)
- *P. falciparum* and *P. knowlesi* for over 200 d (Booden & Geiman 1973)
- *P. falciparum* for 2 years (Pavanand, Permpnich, Chuanak & Sookto 1974)
- *P. berghei* for almost 11 years (Jadin, Timperman & De Ruysser 1975)
- *P. berghei* and *P. gallinaceum* for up to 274 d (Oganesian 1983).

However, an attempt to freeze-dry *P. gallinaceum* was not successful (Weatherby 1970).

### Periodicity

Herman (1941) and Garnham (1966) reported *P. durae* to have a cycle of 24 h without, however, describing their trial procedures. De Jong (unpublished thesis 1971) confirmed a 24-h cycle with the schizonts rupturing between 14:00 and 16:00.

### Morphology

The blood stages of *P. durae* were described by Herman (1941) and this description was repeated by Garnham (1966). The trophozoites were amoeboid or oval with one pigment granule and sometimes a refractory globule. The schizont contained 6–14 (mostly eight) merozoites and its pigment was concentrated in one lump. The gametocytes were elongate, irregular, amoeboid and often in an oblique position, when they tended to displace the host-cell nucleus. Laird (1978) found *P. durae* in a peafowl, and his description of the blood stages coincided with that of the preceding authors, with the exception of the merozoite numbers of schizonts, which in his description were 4–16 (mostly six). While reviewing Herman's deposited material, Laird (1978) also found several schizonts containing four merozoites.

Huchzermeyer (1975) encountered a highly pathogenic *Plasmodium* sp. in naturally infected turkeys in Zimbabwe, which he then believed to be *P. durae* (Huchzermeyer 1993b). The schizonts of this parasite had 7–21 merozoites and the gametocytes occupied two-thirds or more of the host-cell area.

This paper reports on trials to infect a number of host species with South African isolates of *P. durae*, the cryopreservation of the parasite, the recovery of live parasites from dead hosts, an attempt to investigate the periodicity of its schizogony, and certain morphological aspects.

## MATERIALS AND METHODS

The origins of the isolates of *P. durae* were described in a previous paper (Huchzermeyer 1993a). The trial turkeys were obtained from the institute's own breeding flock. All birds were reared and kept under mosquito-proof conditions. Unless mentioned otherwise, syringe passaging was carried out by i.v. injection of fresh, infected blood without anticoagulant. The parasitaemias were monitored by blood smears taken daily during week days unless stated otherwise. They were fixed with May-Grünwald Giemsa and stained with Giemsa stain in buffered, distilled water.

### Host range

The birds used in the host-range trials were obtained from the following sources:

Guineafowls – the institute's own flock

Japanese quail	– Irene Animal Production Institute (IAPI)
Aylesbury ducklings	– IAPI
Fowls	– commercial sources
Lady Amherst pheasant	– donated by a fancier
Canaries	– donated by a fancier
Embryonated eggs	– the institute's own flocks

The layout of the host-range trials is detailed in Table 1.

Nine-day-old chick embryos were injected into the allantoic sac either with washed, infected erythrocytes or with ground, infected brain tissue. Twelve-day-old chick embryos and 15–17-day-old turkey embryos were injected with infected heparinized blood via the i.v. route as described by Eichhorn (1940).

### Survival in dead hosts

When infected birds died during routine passaging before a further passage had been attempted, infectious material was collected from these dead birds and prepared as follows:

- In trial 1, brain tissue was taken from two birds which had been found dead on the same morning and first examined microscopically on a Giemsa-stained impression smear for the presence of exoerythrocytic schizonts (EES). When these were found present in both brains in large numbers, the two brains were ground with a sterile pestle and mortar in a small quantity of Alsever's solution, after which 0,5 ml of the cell suspension was injected subcutaneously (s.c.) into each of two susceptible turkey poults.
- In trials 2–4, a small quantity of heart blood was collected into phosphate-buffered saline solution in a heparinized tube and shaken vigorously to loosen some of the erythrocytes, after which the cell suspension was used for injection. In trial 2, blood was collected in this way from two dead birds and injected intramuscularly (i.m.), and in trials 3 and 4, from one dead bird and injected i.v. In trial 3, the donor bird had been dead for 12 h when the blood was collected.

### Cryopreservation

In order to compare the effect of the cryopreservant with that of freezing, 4 ml of blood was drawn from the brachial vein of an infected turkey (strain M, passage 14) and 0,5 ml of this blood injected immediately i.v. into one turkey poult. The remaining blood was mixed with 1,2 ml of acid-citrate-dextrose solution (ACD), cooled on crushed ice and mixed into 6,2 ml of dimethylsulphoxide (DMSO) buffer solution (5 ml of phosphate-buffered saline plus 1,2 ml of DMSO). It was calculated that 1 ml of this blood mixture contained 32,1% of the parasites of 1 ml of blood, or 64,2% of the 0,5 ml injected into the first bird. The

blood-ACD-DMSO mixture was filled into plastic tubes in 1 ml aliquots, of which one was injected i.v. into a second turkey. The remaining tubes were hung overnight in the gas phase in a liquid-nitrogen container to slow down the rate of freezing. In the morning, one tube was removed and thawed, and its contents injected into a third poult, while the remaining tubes were immersed into the liquid nitrogen for storage. After an interval of 382 d, two tubes were taken out of the liquid nitrogen, thawed and their contents injected into two further poults. Regular monitoring of the infection was carried out as described above.

In a second instance, 26 ml of blood of strain N, passage 7, was mixed with 4 ml of ACD and 20 ml of 20% DMSO, divided into 2 ml aliquots and frozen rapidly by direct immersion into liquid nitrogen. After 287 d, one tube was thawed and the contents injected i.v. into one poult.

### Periodicity

One turkey poult was infected i.v. with blood of strain N, passage 21, and monitored until on day 11 post infection, the parasitaemia had reached 440 parasites per 100 fields. Blood smears were then taken at 4-h intervals during the nights and 2-h intervals during the days for a period of 75 h, by which time the parasitaemia had dropped to 100 parasites per 100 fields. The parasites were counted in five categories:

- Trophozoites
- Presegmenters
- Schizonts
- Immature gametocytes
- Mature gametocytes

The results were plotted on graphs.

### Morphology

During passaging of the different isolates of *P. durae*, outstanding morphological features were noted in the protocols. This accumulated material was reviewed, the merozoite numbers in schizonts of different sizes were counted, and semischematic drawings of the different stages were prepared. Microphotographs of exoerythrocytic schizonts were taken and they were also measured with the help of a drawing tube (camera lucida) and a micrometer slide.

## RESULTS

### Host range

The host-range trials are summarized in Table 1. Transient, low parasitaemias were obtained in Japanese quail (*Coturnix japonica*) and a high, long-lasting parasitaemia in a Lady Amherst pheasant (*Chrysolophus amherstiae*). The common fowl (*Gallus gallus*),

TABLE 1 Layout of host-range trials

Trial no.	Strain	Passage	Dose	Route	n	Host	Age	Parasitaemia			Monitored to d.p.i.	Re-isolation
								Onset d.p.i.	Peak parasites per 100 fields	Duration d.p.i.		
1	T	4	3,0 x 10 <sup>8</sup>	i.v.	1	Lady Amhurst pheasant	3 months	3	6 680	84	Not done	
2	T	2	1,6 x 10 <sup>8</sup>	i.v.	1	Fowl	3 months	—	—	—	Not done	
3	T	2	7,2 x 10 <sup>8</sup>	i.m.	2	Canary	3 months	—	—	35	Not done	
4	T	2	3,6 x 10 <sup>8</sup>	i.m.	1	Guineafowl	4 months	—	—	83	Not done	
5	M	11	1,7 x 10 <sup>8</sup>	i.v.	1	Fowl	2 months	—	—	116	Not done	
6	O	20	8,6 x 10 <sup>8</sup>	i.v.	1	Muscovy	5 weeks	Death from shock	—	—	Not done	
7	O	23	2,7 x 10 <sup>8</sup>	i.m.	6	Japanese quail	3 months	16	16	31 (4 of 6)	Day 28 pos.	
8	O	23	2,7 x 10 <sup>8</sup>	i.m.	2	Duck	7 d	—	—	—	Not done	
9	O	26	1,1 x 10 <sup>8</sup>	i.m.	2	Guineafowl	2 months	—	—	39	Not done	
10	O	38	1,6 x 10 <sup>8</sup>	i.v.	2	Guineafowl	6 weeks	—	—	41	Not done	
11	N	9	3,6 x 10 <sup>8</sup>	i.v.	2	Guineafowl	2 months	—	—	14	Day 13 neg.	
											Not done	

d.p.i. = days post infection  
n = number of birds

TABLE 2 Attempts to infect embryonated eggs with *Plasmodium durae*

Trial no.	Isolate	Passage	Dose	Species of egg	n	Age of embryo	Route	Killed	Smear	Subpassaged	Hatch	Smear	Results
1	M	7	10 <sup>7</sup>	c	10	9 d	a.s.	7	1 EES in lymphocyte		None		Neg.
2	M	7	Ground brain	c	18	9 d	a.s.	7	nps	Pos.	2	Neg.	Neg.
3	N	15	4,8 x 10 <sup>8</sup>	t	12	14 d	i.v.	8	Pos. (820 and 555)	Pos.			
3	N	15	4,8 x 10 <sup>8</sup>	c	8	12 d	i.v.	11	6:8 pos. (bsm)				
4	N	15	6,6 x 10 <sup>6</sup>	t	20	17 d	i.v.	8	Brain smear neg.				
4	N	15	6,6 x 10 <sup>6</sup>	c	20	12 d	i.v.	8	6:10 pos. (bsm: 4-720)				
5	O	29	9,3 x 10 <sup>6</sup>	t	40	15 d	i.v.	8	Brain: 2:10				

n = number of eggs  
c = chicken  
t = turkey  
a.s. = allantoic sac

TABLE 3 Survival of *Plasmodium durae* in dead hosts

Trial no.	Strain	Passage	Parasitaemia of host before death <sup>a</sup>	n <sup>b</sup>	Material used	Medium	Route	n <sup>b</sup>	d.p.i. to patency
1	M	8	740 920	2	Brain	Alsever's	s.c.	2	20
2	M	10	12 440	2	Heart blood	PBS + heparin	i.m.	2	17
3	M	26	1 080	1	Heart blood	PBS + heparin	i.v.	2	15

<sup>a</sup> parasites per 100 fields of view<sup>b</sup> number of birds

d.p.i. = days post infection

TABLE 4 Cryopreservation of *Plasmodium durae* in liquid nitrogen: isolate M, passage 14

Treatment	Delay	No. of parasites per dose	Route	n	Patency d.p.i.	Peak of parasitaemia d.p.i.
Fresh unpreserved	None	2,8 × 10 <sup>8</sup>	i.v.	1	1	12
ACD + DMSO	0,5 h	1,8 × 10 <sup>8</sup>	i.v.	1	15	28
ACD + DMSO frozen in gas phase	18 h	1,8 × 10 <sup>8</sup>	i.v.	1	15	28
Stored in liquid N	382 d	1,8 × 10 <sup>8</sup>	i.v.	2	17 17	33 35

n = number of birds

d.p.i. = days post infection

the duck (*Anas platyrhynchos*), guinea fowl (*Numida meleagris*) and canaries (*Serinus canaria*) were found to be refractory to experimental infections with the South African isolates of *P. durae*.

The results of the embryonated-egg trials are presented in Table 2. Blood or brain material injected into the allantoic sac failed to infect chick embryos. However, i.v. injection of infected blood produced low parasitaemias in chick embryos and high parasitaemias in turkey embryos. The subinoculation of blood from both into turkey poults by the i.v. route was successful. EES were found in the brains of two out of ten turkey embryos in trial 5.

### Survival in dead hosts

The survival of blood and tissue stages of *P. durae* in dead hosts was demonstrated in all four trials, as shown in Table 3.

### Cryopreservation

The results of the cryopreservation trials are given in Table 4. The admixture of ACD and DMSO to fresh blood, caused a lengthening of the prepatent time by 14 d. This delay was not increased further by slow freezing in the gas phase in the liquid-nitrogen container and only slightly after 382 d of storage in liquid nitrogen. A similar delay was obtained after rapid freezing and storage for 287 d.

### Periodicity

While most stages were found at all times of day and night in the periodicity trial, there was a certain degree of 24-h periodicity, with the trophozoites peak-

TABLE 5 Measurement in µm of EES of *Plasmodium durae*, *P. circumflexum* (Huchzermeyer & Van der Vyver 1991) and an unidentified *Plasmodium* sp. from Zimbabwe (Huchzermeyer 1976)

Parasite	Length	Width
<i>P. durae</i>	16,5–32,0	6,0–11,5
<i>P. circumflexum</i>	17,0–19,0	9,0–29,0
<i>Plasmodium</i> sp. Zimbabwe	10,0–16,0	5,0–11,0

ing around noon (Fig. 1) and presegmenters peaking just after midnight (Fig. 2). The lowest numbers of mature schizonts occurred during the late afternoons and peaked during the early morning hours (Fig. 3). Mature gametocytes were present only during the last night of observations (Fig. 4).

### Morphology

A composite photograph and semischematic drawings of the blood stages of South African isolates of *P. durae* in experimental infections of turkeys are shown in Fig. 5–7. All stages were very pleomorphic. While the trophozoites were found to fit the original descriptions, the numbers of merozoites in mature schizonts appeared to be reduced and were found in a range of 2–14 (mostly four), but with an arithmetic mean of 5,0. Mature gametocytes were found only rarely. Large schizonts were found most often in birds developing very high parasitaemias, while small ones predominated in the early stages of parasitaemia.

EES were found in birds dying at the peak of parasitaemia, usually associated with very high levels of parasitaemia. In 44 passages of isolate M, EES were



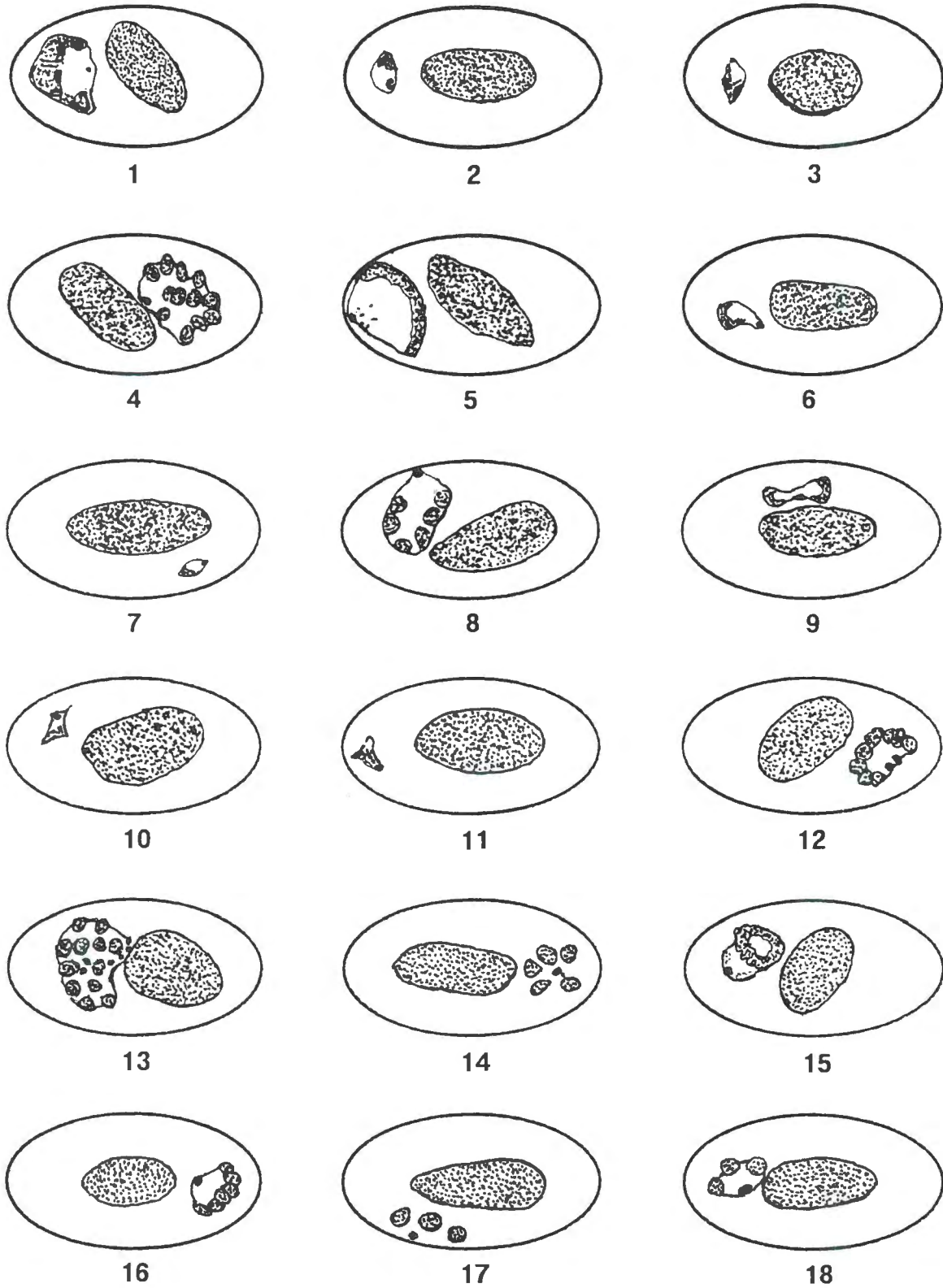


FIG. 6 Asexual blood stages of *P. durae*

Trophozoites 2; 3; 6; 7; 10; 11  
 Presegmenters 1; 5; 9; 15  
 Mature schizonts 4; 8; 12; 13; 14; 16-18

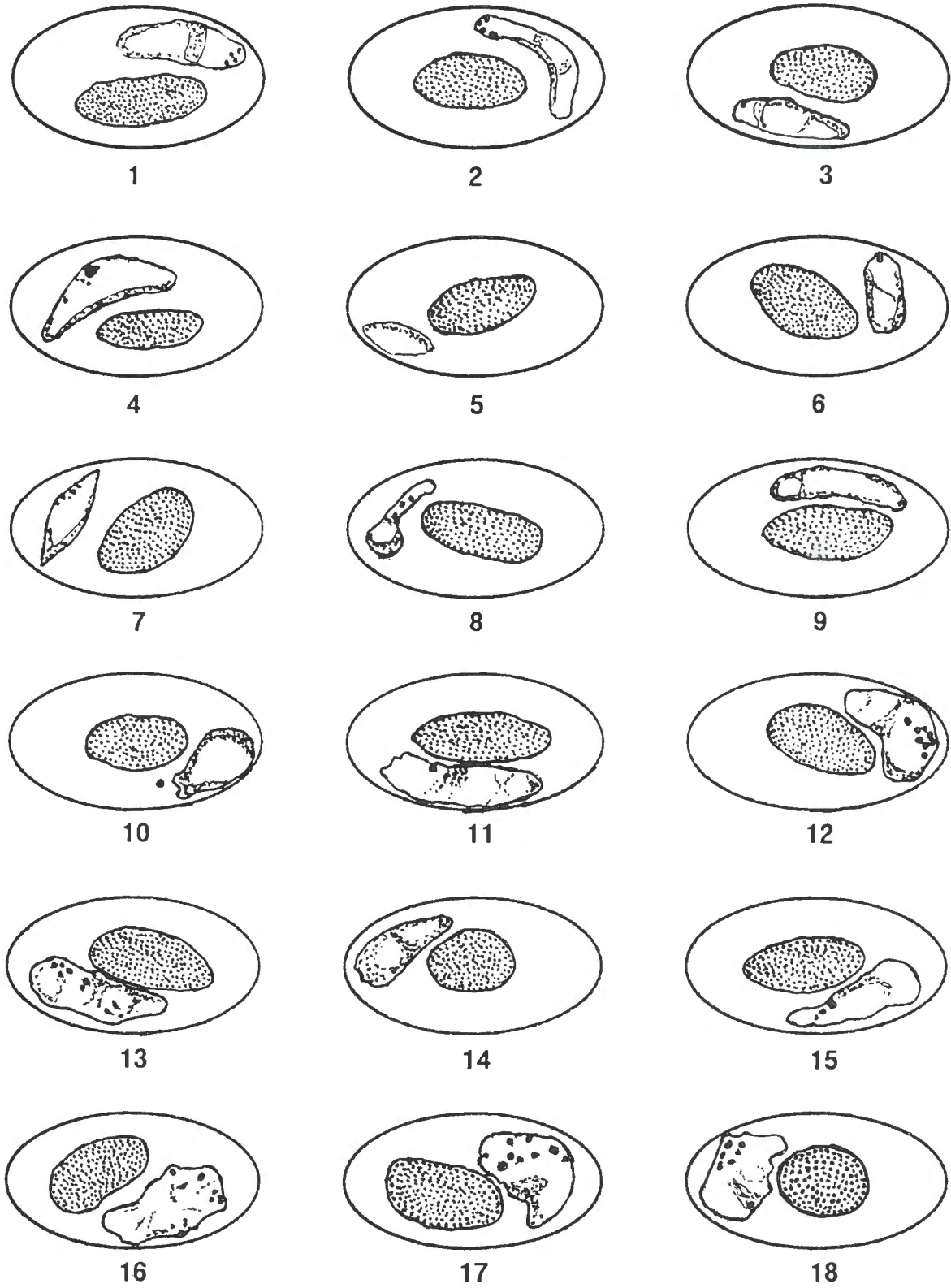


FIG. 7 Sexual blood stages of *P. durae*

Immature gametocytes	5-10
Macrogametocytes	1; 11; 12; 14; 17; 18
Microgametocytes	2-4; 13; 15; 16



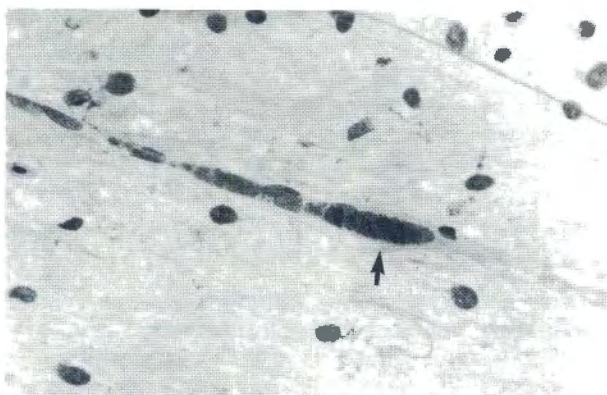


FIG. 8 Exoerythrocytic schizont of *P. durae* (arrow) in a brain capillary; turkey, brain smear, Giemsa, x 1000



FIG. 9 Exoerythrocytic schizonts of *P. durae* (arrows), turkey, x 1000



FIG. 10 Exoerythrocytic schizonts of *P. durae* (arrows), turkey, x 1000

found in 39 birds which had died during high parasitaemia, also in 58 birds during 30 passages of isolate O and in two birds during 14 passages of isolate N. No EES were found during 40 passages of isolate T.

The mean measurements of 23 EES found in endothelial cells of brain capillaries were: Length 23,1  $\mu\text{m}$  ( $\pm 4,43$ ), range 16,5–32,0  $\mu\text{m}$  and width 8,7  $\mu\text{m}$  ( $\pm 1,56$ ), range 6,0–11,5  $\mu\text{m}$ . In Table 5 these measurements are compared with those of the EES of *P. circumflexum* (Huchzermeyer & Van der Vyver 1991) and of the unidentified isolate from a case of turkey malaria from Zimbabwe (Huchzermeyer 1976), all from experimental infections in turkeys. Microphotographs of the EES of *P. durae* in brain capillaries (brain smears) are shown in Fig. 8–10.

In cases with very high parasitaemia, just before the peak, forms were occasionally seen in the circulating blood, in red-blood cells, which were characterized by a pink-staining nucleus surrounded by blue cytoplasm. These forms were interpreted to represent ectopic EES.

## DISCUSSION

### Host range

*P. durae* appears to have a very narrow host range, limited mainly to a small number of phasianid species. The results of the present trials differ to some extent from those of Purchase (1942), who was able to infect chicks, and of Simpson (1944), who reported infection in ducks. These differences could be due to strain differences or to the fact that the above authors may have used younger birds. In contrast to the finding of natural infection with *P. durae* in a wild guinea-fowl in Ethiopia (Ashford, Palmer Ash & Bray 1976), four attempts at experimentally infecting guinea-fowl were unsuccessful, as were 20 attempts to isolate the parasite from guinea-fowl (Huchzermeyer 1993a).

During 1973 and 1974 Huchzermeyer (unpublished data) encountered fatal malaria cases in a collection of exotic pheasants in the Chinoi area of Zimbabwe. Birds of several species, including Lady Amherst pheasants, were in a good nutritional state and, on postmortem examination, showed severe anaemia. All the examined birds had been frozen before submission. Smears of haemolyzed heart blood contained a *Plasmodium* sp. in low parasitaemias. Brain smears were not examined for exoerythrocytic schizonts. The species of pheasants were not specified on the submission forms and after the author's move to South Africa, the postmortem records were destroyed. Neither were transmission experiments undertaken, particularly because of the fact that the carcasses had been frozen. When the material was reviewed during the present studies, the parasites were identified as *P. durae*, especially on the morphology of their erythrocytic schizonts. The susceptibility of Lady Amherst pheasants was confirmed during the present host-range trials.

Embryonated eggs appear to offer less resistance to malarial infections, although Manwell & Robinson

(1961) found chick embryos refractory to i.v. infection with *P. elongatum*. The shorter incubation period of chicken eggs, allowing less time for the development of *P. durae* with its slow multiplication rate due to its small schizonts, may have been responsible for the low parasitaemias achieved in chick embryos in comparison with turkey embryos. This slow rate of multiplication may also have been the reason for the apparent failure to infect chick embryos via the chorio-allantoic sac.

### Survival in dead hosts

In survival trials 1, 2 and 4, it was not possible to estimate the time of death of the hosts from which infective material had been taken. They could have died at any time during the 17 h since the last inspection in the afternoon of the preceding day. No noticeable putrefaction had set in. In the third trial, blood was collected 12 h after death. Young (1985) demonstrated the survival of *P. berghei* in dead hosts until the onset of putrefaction. In the present trials, *P. durae* appeared to behave similarly. The four successful attempts demonstrate that this technique could be of practical importance for the isolation of avian-malaria parasites from suspect postmortem material.

More recent studies have demonstrated that *P. berghei* can be transmitted orally by feeding infected host tissues or by cannibalism (Malagón, Castillo & Tapia 1993; 1994). This mode of transmission could also apply in certain cases of avian malaria and needs further investigation.

### Cryopreservation

Huchzermeyer (1965) demonstrated that the addition of glycerol as cryoprotectant to fowl blood containing *Aegyptianella pullorum*, lengthened the prepatent period as much as did deep-freezing in liquid nitrogen without the addition of glycerol. In the meantime, the use of ACD and DMSO has become routine in the deep-freezing of blood parasites. It is noteworthy that the addition of this cryoprotectant combination to turkey blood containing *P. durae*, produced a similar effect. However, freezing of this parasite without a cryoprotectant was not attempted. As shown by Huchzermeyer (1993a), this lengthening of the prepatent period indicates a loss of parasites corresponding to a 1 000-fold dilution. The slight dilution of the dose caused by the addition of ACD and DMSO could not possibly account for this effect. There was no difference in parasite survival between slow and rapid freezing.

### Periodicity

The periodicity trial confirmed the previously reported 24-h cycle (Herman 1941; Garnham 1966; De Jong 1971), although most stages were found at all hours of the day and night. Schizonts peaked in the early

morning hours and not in the early afternoon as reported by De Jong (1971). The lengthy passaging of the strain used in the present trial in an isolation unit with artificial 24-h lighting could have reduced its periodicity. Although the bird used in the investigation of the guineafowl isolate of *P. circumflexum* was kept in the same isolation unit under identical conditions, the strain used in that trial had been passaged only 12 times (Huchzermeyer & Van der Vyver 1991).

The finding of mature gametocytes towards the end of the observation period of the periodicity trial confirmed frequent own observations made during routine passaging, that their presence increased towards the end of patent parasitaemia.

### Morphology

Schizonts of the strains of *P. durae*, examined in the present study, had fewer merozoites than those in the original description (Herman 1941) and those of the Nigerian peafowl material (Laird 1978). Similar differences in geographically different strains of *P. juxtanucleare* have been reported by Bennett & Warren (1966). Mohan & Manwell (1969) reflected on the possibility of a variation in schizont size ("merozoite brood size") at different stages in an infection, a phenomenon which was distinctly observed in the course of the present studies.

Some of the present isolates, particularly isolate T of turkey origin (Huchzermeyer 1993a), appeared to be unable to produce mature gametocytes, a fact that rendered the identification of the parasite more difficult. This apparent inability to complete the whole intraerythrocytic developmental cycle is interpreted as being due to the host-parasite relation, the parasite being poorly adapted to its exotic host. Thompson & Huff (1944) found the same phenomenon in saurian malarial which were transmitted to abnormal hosts.

The EES of *P. durae* were markedly more slender than those of *P. circumflexum*, as measured by Huchzermeyer & Van der Vyver (1991). They occurred at peak parasitaemia and not after the disappearance of the blood forms, as was the case with the latter parasite. Conversely, in the case of the unidentified turkey malaria in Zimbabwe (Huchzermeyer 1976), the EES developed before the onset of the parasitaemia. Haas, Wilcox, Laird, Moore Ewing & Coleman (1948) found the appearance of the EES of *P. galinaceum* to depend on the life-cycle stage used in the infection. However, all three the isolates referred to above, had been transmitted by their erythrocytic stages.

A revision of the material from the turkey malaria described by Huchzermeyer (1975) showed that neither the size of the gametocytes nor the shape and merozoite numbers of the schizonts fitted the descriptions of *P. durae*, but that they were more like *P. circum-*

*flexum*. The South African guineafowl isolate of that parasite described by Huchzermeyer & Van der Vyver (1991) was highly pathogenic to turkeys in experimental infections, but the Zimbabwean turkey isolate did not produce a parasitaemia in an attempted guineafowl passage (Huchzermeyer 1976). It therefore remains debatable to which species those exoerythrocytic schizonts belonged, that were depicted by Huchzermeyer (1976), as they were clearly not of *P. durae*, although they showed more resemblance to this parasite than to *P. circumflexum*. Microphotographs of the exoerythrocytic schizonts of *P. durae* have not been published previously.

The finding of ectopic EES in the circulating blood just before the peak of parasitaemia, was mentioned also by De Jong (1971). In the course of the present study, only early stages with a single nucleus were seen, and it is possible that these ectopic EES were not able to develop in erythrocytes.

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## AUTHOR'S NOTE

After the closure of the Poultry Section of the OVI, the liquid-nitrogen container with all the isolates was taken over by the institute's vaccine factory (Onderstepoort Biological Products). Soon afterwards, the author was informed of an accidental thaw-out in which all his isolates of avian-blood parasites were destroyed.

## REFERENCES

- ASHFORD, R.W., PALMER, T.T., ASH, J.S. & BRAY, R.S. 1976. Blood parasites of Ethiopian birds. 1. General survey. *Journal of Wildlife Diseases*, 12:409–426.
- BARTKOWIAK, R.A., HUCHZERMEYER, F.W., POTGIETER, F.T., VAN RENSBURG, L., LABUSCHAGNE, F.J. & VAN BILJON, B.J. 1988. Freeze-drying of *Aegyptianella pullorum*. *Onderstepoort Journal of Veterinary Research*, 55:125–126.
- BENNETT, G.F. & WARREN, M. 1966. Biology of the Malaysian strain of *Plasmodium juxtancleare* Versiani & Gomes, 1941. I. Description of the stages in the vertebrate host. *Journal of Parasitology*, 52:565–569.
- BOODEN, T. & GEIMAN, Q.M. 1973. *Plasmodium falciparum* and *P. knowlesi*: Low temperature preservation using dimethylsulfoxide. *Experimental Parasitology*, 33:493–498.
- COGGESHALL, L.T. 1939. Preservation of viable malaria parasites in the frozen state. *Proceedings of the Society for Experimental Biology and Medicine*, 42:499–501.
- DE JONG, A.C. 1971. Some haemosporidian parasites of parakeets and francolins. Unpublished Ph.D. thesis, London, III: *Plasmodium durae*: 157–214.
- EICHHORN, E.A. 1940. A technique for the intravenous inoculation of chick embryos. *Science*, 92:245–246.
- GARNHAM, P.C.C. 1966. *Malaria parasites and other haemosporidia*. Blackwell Scientific Publications: Oxford: 652–657.
- GAVRILOV, W., BOBKOFF, G. & LAURENCIN, S. 1938. Essai de culture en tissus de *Plasmodium gallinaceum* (Brumpt). *Annales de la Société Belge de Médecine Tropicale*, 18:429–434.
- GRAHAM, H.A., STAUBER, L.A., PALCZUK, N.C. & BARNES, W.E. 1973. Immunity to exoerythrocytic forms of malaria. I. Course of infection of *Plasmodium fallax* in turkeys. *Experimental Parasitology*, 34:364–371.
- HAAS, V.H., FELDMAN, H.A. & EWING, F.M. 1945. Serial passage of *Plasmodium gallinaceum*. *Public Health Reports*, 60:577–371.
- HAAS, V.H., WILCOX, A., LAIRD, R.L., MOORE EWING, F. & COLEMAN, N. 1948. Symposium on exoerythrocytic forms of malarial parasites. IV. Response of exoerythrocytic forms to alterations in the life-cycle of *Plasmodium gallinaceum*. *Journal of Parasitology*, 34:306–320.
- HERMAN, C.M. 1941. *Plasmodium durae*, a new species of malaria parasite from the common turkey. *American Journal of Hygiene*, 34:22–26.
- HUCHZERMEYER, F.W. 1965. Das Tiefgefrieren von *Aegyptianella pullorum* in flüssigem Stickstoff mit einigen Bemerkungen über die künstliche Infektion beim Huhn. *Berliner und Münchener Tierärztliche Wochenschrift*, 78:433–435.
- HUCHZERMEYER, F.W. 1975. An outbreak of malaria in turkeys. *Rhodesian Veterinary Journal*, 6:15–17.
- HUCHZERMEYER, F.W. 1976. Further observations on an outbreak of malaria in turkeys. *Rhodesian Veterinary Journal*, 7:52–57.
- HUCHZERMEYER, F.W. & VAN DER VYVER, F.H. 1991. Isolation of *Plasmodium circumflexum* from wild guineafowl (*Numida meleagris*) and the experimental infection in domestic poultry. *Avian Pathology*, 20:213–223.
- HUCHZERMEYER, F.W. 1993a. A host-parasite list of the haematzoa of domestic poultry in sub-Saharan Africa and the isolation of *Plasmodium durae* Herman from turkeys and francolins in South Africa. *Onderstepoort Journal of Veterinary Research*, 60:15–21.
- HUCHZERMEYER, F.W. 1993b. Pathogenicity and chemotherapy of *Plasmodium durae* in experimentally infected domestic turkeys. *Onderstepoort Journal of Veterinary Research*, 60:103–110.
- JADIN, J., TIMPERMAN, G. & DE RUYSSER, F. 1975. Comportement d'une ligne de *P. berghei* après préservation à basse température pendant plus de dix ans. *Annales de la Société Belge de Médecine Tropicale*, 55:603–608.
- JEFFERY, G.M. 1957. Extended low temperature preservation of human malaria parasites. *Journal of Parasitology*, 43:488.
- JEFFERY, G.M. 1962. Survival of trophozoites of *Plasmodium berghei* and *Plasmodium gallinaceum* in glycerolized whole blood at low temperatures. *Journal of Parasitology*, 48:601–606.
- KOVIC, M. & ZEUTHEN, E. 1964. Malarial periodicity and body temperature. An experimental study of *Plasmodium lophurae* in chicken embryos. *Proceedings of the 1st International Congress on Parasitology*, B2:241–244.
- LAIRD, M. 1978. *Plasmodium durae* Herman from the introduced common peafowl in Northern Nigeria. *Journal of Protozoology*, 25:21–24.
- MALAGÓN, F., CASTILLO, L. & TAPIA, J.L. 1993. Experimental transmission of murine malaria by the oral route. *Parasitology Research*, 79:527–528.

- MALAGÓN, F., CASTILLO, L. & TAPIA, J.L. 1994. Experimental transmission of murine malaria by cannibalism on mice infected by *Plasmodium berghei yoelii*. *Acta Protozoologica*, 33:191–193.
- MANWELL, R.D. & ROBINSON, F.O. 1961. The behaviour of *Plasmodium elongatum* and *Plasmodium hexamerium* in chick and duck embryos. *Proceedings of the 1st International Congress on Parasitology*: 465–458.
- MIETH, H. 1966. Tiefgefrierkonservierung verschiedener Blut- und Gewebeprotzoen in flüssigem Stickstoff. *Zeitschrift für Tropenmedizin und Parasitologie*, 17:103–108.
- MOHAN, R.N. & MANWELL, R.D. 1969. *Plasmodium juxtannucleare* in African partridges (*Francolinus* sp.). *Journal of Parasitology*, 55:543.
- MOLINARI, V. 1961. The action of low temperatures on plasmodia. *Journal of Tropical Medicine and Hygiene*, 64:225–232.
- OGANESIAN, A.S. 1983 [Prolonged storage of malarial parasites at liquid nitrogen temperature. Infectivity and capacity for gametocyte formation]. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni*, 4:28–31.
- PAVANAND, K., PERMPANICH, B., CHUANAK, N. & SOOKTO, P. 1974. Preservation of *Plasmodium falciparum* infected erythrocytes for *in vitro* cultures. *Journal of Parasitology*, 60:537–539.
- PURCHASE, H.S. 1942. Turkey malaria. *Parasitology*, 34:278–283.
- SIMPSON, M.L. 1944. Exoerythrocytic stages of *Plasmodium durae*. *Journal of Parasitology*, 30:177–178.
- THOMPSON, P.E. & HUFF, C.G. 1944. A saurian malarial parasite, *Plasmodium mexicanum*, n. sp., with both elongatum- and gallinaceum-types of exoerythrocytic stages. *Journal of Infectious Diseases*, 74:48–67.
- WEATHERBY, A.B. & MCCALL, J.W. 1967. Survival of sporozoites of *Plasmodium gallinaceum* Brumpt for 767 d in liquid nitrogen (–197 C). *Journal of Parasitology*, 53:638–640.
- WEATHERBY, A.B. 1970. Cryopreservation of malaria. Section II. 1. *Journal of Parasitology*. 57:34.
- WOLFSON, FRUMA 1940. Successful cultivation of avian plasmodia in duck embryos. Section C. *American Journal of Hygiene*, 32:60–61.
- YOUNG, M.D. 1985. Survival of *Plasmodium berghei* in dead hosts. *Journal of Parasitology*, 71:135–136.
- ZUCKERMAN, A. 1946. Infections with *Plasmodium gallinaceum* in chick embryos induced by exoerythrocytic and blood stages. *Journal of Infectious Diseases*, 79:1–11.