

# A host-parasite list of the haematozoa of domestic poultry in sub-Saharan Africa and the isolation of *Plasmodium durae* Herman from turkeys and francolins in South Africa

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## ABSTRACT

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An annotated host-parasite list of the blood parasites of domestic poultry in sub-Saharan Africa is presented. This list contains the haematozoa found in domestic waterfowl (ducks, geese and muscovies) and phasianids (turkey, fowl and peafowl).

In South Africa *Plasmodium durae* was isolated from 4 out of 8 backyard turkeys, from 3 out of 26 Swainson's francolins and from 1 redwing francolin, but not from 20 helmeted guineafowls and 9 greywing francolins. This points at Swainson's and redwing francolins as being the main natural hosts of *P. durae* in South Africa.

The increase in the period of prepatency after intramuscular subinoculation as compared with the intravenous route was found to correspond to that of a 1 000 fold dilution of an intravenous inoculum of parasitized blood. This delay was not due to an intervening cycle of exoerythrocytic schizogony, but to large numbers of the injected erythrocytes apparently not finding their way into the circulation of the new host.

## INTRODUCTION

Bennett, Earlé, Du Toit & Huchzermeyer (1992a) compiled a host parasite catalogue of the blood parasites of wild sub-Saharan birds. This list did not include the haematozoa of domestic poultry.

*Plasmodium durae* was first found, isolated and described in domestic turkeys in Kenya (Herman 1941; Purchase 1942). As the turkey is an exotic species and as *P. durae* has not been found outside of Africa, the latter is presumed to have an indigenous African host and only occasionally to be infecting turkeys. Markus & Oosthuizen (1972) reported finding *Plasmodium* sp. in a Swainson's francolin from the Nylstroom district. They also

quoted a personal communication by Southgate, that the yellow-necked francolin is the natural host of *Plasmodium durae* in Kenya. However, Ashford, Palmer, Ash & Bray (1976) found this parasite in the helmeted guineafowl *Numida meleagris* in Ethiopia.

Sub-inoculation of blood from infected birds into susceptible birds of the same or a related species was recommended by Herman, Knisley & Snyder (1966). These authors also reported longer prepatent times and lower parasitaemias after intramuscular (i.m.) inoculation than after intravenous (i.v.) injection.

This paper reviews the blood parasites found in domestic poultry in Africa south of the Sahara and reports on attempts to isolate *P. durae* from domestic turkeys and several species of wild gallinaceous birds in South Africa, as well as on the effect of the

route of inoculation of infected blood on the length of the prepatency period of the infection.

## MATERIALS AND METHODS

### Trial birds

Day-old poults, keats and chicks were obtained for transmission and passaging from various commercial sources and later bred at the Onderstepoort Veterinary Institute. Trials could only be carried out when suitable birds were available, as supplies were mostly seasonal. All birds were reared and kept under mosquito proof conditions.

### Isolation attempts

Birds sampled included live back-yard turkeys, shot francolins and guineafowls as well as guineafowls presented for examination. Blood samples were collected from the brachial vein or from the heart and kept in heparinized tubes or citrate saline and inoculated i.m. or i.v. into susceptible turkey poults. When available, susceptible juvenile guineafowls and domestic fowls were also used on some occasions in the isolation attempts.

Thin blood smears were taken 2–5 × per week after inoculation. The smears were fixed with May-Grünwald Giemsa, stained with Giemsa and examined at a magnification of 1 000. Of each smear 5–100 fields of view with approximately 100 erythrocytes per field were examined and parasites counted and recorded as number of parasites per 100 fields of view. Examination of smears continued until after the peak of parasitaemia or at least until 30 d after inoculation for most cases when no parasites appeared.

At first isolates were maintained by passaging only. However, isolate T was lost in 1981 when a group of carriers was disbanded in the author's absence. Isolates were subsequently stored in liquid nitrogen after varying numbers of passages.

### Route of inoculation

On several occasions, using different isolates at different passages, birds to be inoculated were divided into 2 groups—the birds in 1 group being inoculated i.m. and in the other 1 by the i.v. route. The i.m. injection was carried out by inserting the needle into the pectoral muscle near the cranial point of the sternum parallel to the clavicle in the direction of the shoulder joint. For the i.v. injection, an assistant grasped the bird close to the carpal joints and held the bird firmly in a lateral position with both wings extended backwards and the legs extended in the opposite direction. The ventral side of the bird was then facing the operator who, with the tips of 3 fingers of his left hand, gently pulled and fixed the skin of the median side of the upper

arm to expose the brachial vein which is visible through the skin. The needle was then inserted, under visual control, into the vein close to the shoulder joint in the direction of the elbow. The same technique was used for collecting venous blood for passaging. Monitoring was carried out as described above.

In order to investigate the possibility of development of exoerythrocytic schizonts after i.v. and i.m. injection, 16 poults of approximately 250 g live mass were divided into 2 groups of 8 birds each. Each bird received 0,25 ml fresh heparinized blood containing approximately  $1,6 \times 10^7$  parasites per dose. The birds in Group A were inoculated via the i.v. route and those in Group B via the i.m. route. One bird from Group A was slaughtered on Days 3, 6, 8, 10 and 13 after infection. Brain smears were taken, fixed with May-Grünwald Giemsa and stained with Giemsa's stain. Brain was also fixed in formalin, processed routinely and stained with haematoxylin and eosin (HE). Both the smears and the sections were then examined for exoerythrocytic schizonts (EES) as first described by Huchzermeyer (1976). One bird from Group B was slaughtered on Days 1, 2, 3, 6, 8, 10 and 13 after infection. Muscle was excised from the site of injection, fixed in formalin, processed and stained as above and the sections also examined for EES.

In the dilution trial 3 birds (mean live mass 1 033 g) were injected i.v. with fresh heparinized blood containing approximately  $2,8 \times 10^6$  parasites per dose. The remaining blood was diluted 1 000 fold in sterile phosphate buffered saline and injected i.v. into each of 4 birds (mean live mass 1 317 g). Monitoring was carried out as described above.

## RESULTS

The annotated host-parasite list of domestic poultry in Africa south of the Sahara is presented in Table 1. The isolation attempts are detailed in Tables 2–4. Four isolates of *P. durae* (50 %) were obtained from 8 turkeys. In addition, 3 isolates of *P. durae* resulted from sub-inoculation of blood of 26 Swainson's francolins (11,5 %) and 1 further isolate from 1 redwing francolin. However, 20 guineafowls yielded 3 isolates of *Plasmodium circumflexum* (15 %) and 6 isolates of *Aegyptianella botuliformis* (30 %) as reported previously by Huchzermeyer & Van der Vyver (1991), Huchzermeyer, Horak & Braack (1991), Earlé, Horak, Huchzermeyer, Bennett, Braack & Penzhorn (1991) and Huchzermeyer, Horak, Putterill & Earlé (1992).

The effect of the route of inoculation on the length of the prepatency period is shown in Table 5.

Examination of the brain smears and histological sections of brains and muscles did not reveal the

TABLE 1 Annotated host-parasite list of domestic poultry in sub-Saharan Africa

**ANATIDAE*****Anas platyrhynchos***

*Aegyptianella pullorum*: Coles 1934; Rousselot 1953; Bennett & Herman 1976

*Plasmodium* sp.: Bennett & Herman 1976

***Anser anser***

*Aegyptianella pullorum*: Coles 1937a

***Carina moschata***

(*Aegyptianella pullorum*: Curasson & Andrjesky 1929: cited by Rousselot 1953, in error)

**PHASIANIDAE****MELEAGRINAE*****Meleagris gallopavo***

*Haemoproteus* sp.: Macfie 1916

*Leucocytozoon smithi*: Huchzermeyer & Sutherland 1978; OVI

*Plasmodium durae*: Herman 1941; Purchase 1942; Garnham 1950, 1966; Bennett & Herman 1976; OVI

*Plasmodium* sp.: Mackenzie & Simpson 1953 (probably *P. durae*); Garnham 1966 (not *P. durae*); Barnes 1973, 1974 (identified as *P. juxtannucleare* by Laird 1978) Huchzermeyer 1975, 1976 (now *P. durae*)

*Trypanosoma* sp.: OVI

**PHASIANINAE*****Gallus gallus***

*Aegyptianella pullorum*: Balfour 1907, 1911; Jowett 1910; Curasson & Andrjesky 1929; Robinson & Coles 1932; Bedford & Coles 1933; Coles 1939; Receveur & Thomé 1948; Rousselot 1953; Gothe 1967; Huchzermeyer 1967; Adene & Dipeolu 1981; Bartkowiak, et al. 1988; OVI

*Haemoproteus santosdiasi*: Son 1960 (*species inquirenda*)

*Leucocytozoon gallinarum*: Rousselot 1953 (now *L. schoutedeni*)

*Leucocytozoon schoutedeni*: Rodhain, Pons, Vandenbranden & Bequaert 1913; Huchzermeyer 1966; Fallis, Jacobson & Raybould 1973; Bennett, Huchzermeyer, Burger & Earlé 1992b; OVI

*Leucocytozoon sabrazesi*: Bennett & Herman 1976 (now *L. macleani*)

*Leucocytozoon* sp. (fusiform): Son 1960 (now *L. macleani*)

*Plasmodium* sp.: Adene & Dipeolu 1981

*Trypanosoma gallinarum*: Duke 1912

*Trypanosoma numidae*: Fallis et al. 1973

*Trypanosoma* sp.: OVI

Unidentified parasite: Coles 1937b

***Pavo christatus***

*Plasmodium durae*: Laird 1978

presence of exoerythrocytic schizonts. Numerous erythrocytes from the inocula were found to be degenerating in the muscle sections with increasing time. Parallel to this was an increasing invasion by lymphocytes and macrophages and phagocytosis of the red blood cells.

The effect of 1 000 fold dilution of the i.v. inoculation on the length of the prepatency period is shown in Table 6.

**DISCUSSION****Host-parasite catalogue**

The morphology of all species of *Haemoproteus* and *Leucocytozoon* named in the host-parasite catalogue was briefly reviewed by Bennett, Earlé, Du Toit & Huchzermeyer (1992a). Few blood parasites have been reported in domestic Anatidae in Africa. The susceptibility of ducks and geese to *Aegyptianella pullorum* is well documented as reviewed by Gothe (1971). The absence of records of blood parasites of *Carina moschata*, in spite of its popularity as a back-yard fowl, is surprising. Rousselot (1953) cites Curasson & Andrjesky (1929) in error, as their record is for *Balearica pavonina*.

The absence of records for *Aegyptianella pullorum* from the turkey was to be expected. Brumpt (1930) had already found turkeys and guineafowls resistant to experimental infection with this parasite. Similarly a different species must have been recorded by Castle & Christensen (1985) in wild turkeys in North America. In addition, there is an absence of *A. pullorum* from domestic fowls on that continent.

The report of *Haemoproteus* sp. in turkeys by Macfie (1916) could be in error. He might have seen gametocytes of a *Plasmodium* which sometimes remain visible in chronic infections after the disappearance of trophozoites and schizonts from peripheral circulation. *Haemoproteus meleagridis* has not been reported outside North America, probably because of very specific vector requirements.

*Leucocytozoon smithi* occurs widely throughout South Africa and was probably introduced by early importations of adult turkeys which first occurred around 1656/57 (Oosthuizen, 1985).

The susceptibility of the turkey to different strains of *Plasmodium juxtannucleare* has been documented repeatedly (Versiani & Gomez 1941, 1943; Beltran 1943; Dhanapala 1962; Manuel, Tongson & Bale-diata 1968). Beltran (1943) erroneously quotes the authors of *P. juxtannucleare* as Versiani and Furtado because of the Spanish custom of placing the father's family name in 2nd place and the mother's family name in 3rd place, while in Portuguese custom the sequence is reversed.

The common fowl is the type host of *Aegyptianella pullorum* Carpano 1929. The record by Bartkowiak, Huchzermeyer, Potgieter, Van Rensburg, Labuschagne & Van Biljon (1988) is of an Onderstepoort isolate used in experiments.

The *Haemoproteus santosdiasi* reported by Son (1960), is from the same bird in which that author found a fusiform *Leucocytozoon*. This appears to be the only report of a *Haemoproteus* in *Gallus gal-*

Host-parasite list of haematozoa and isolation of *Plasmodium durae* Herman

TABLE 2 Isolation of *Plasmodium durae* from domestic turkeys

Date		n	Origin	Route of inoculation	Result	Days to parasitaemia or days checked	Isolate	Passages
m	y							
07	76	1	Pretoria	i.v.	+	10	T	40
11	83	2	Pretoria	i.v.	-	18	-	-
					+	16	M	44
01	86	1	Pretoria	i.v.	+	27	N	22
11	86	1	Warmbaths	i.v.	-	32	-	-
01	87	2	Pretoria	i.v.	-	31	-	-
02	89	1	Cullinan	i.v.	+	24	Q	3

n = number of birds sampled

TABLE 3 Isolation of *Plasmodium durae* from francolins

Date		n	Species	Origin	Route of inoculation	Result	Days to parasitaemia or days checked	Isolate	Passages
m	y								
08	76	1	F.s.	Orange Free State	i.m.	-	130	-	-
06	79	3	F.s.	Orange Free State	i.m.	-	60	-	-
08	80	3	F.s.	KNP	i.v.	+	2	E	2
					i.m.	+	34	-	-
					i.m.	+	34	-	-
01	84	2	F.s.	Pretoria	i.m.	-	30	-	-
02	84	1	F.s.	Pretoria	i.m.	-	35	-	-
11	84	2	F.s.	Rust de Winter	i.m.	-	30	-	-
09	85	7	F.s.	Rust de Winter	i.v.	-	32	-	-
02	86	3	F.s.	Rust de Winter	i.v.	+	16	O	39
					i.v.	-	31	-	-
					i.v.	-	31	-	-
07	88	2	F.s.	Pietersburg	i.m. (mixed)	+	8	J	7
08	88	1	F.II.	Stofberg	i.m.	+	49	N	4
08	88	1	F.s.	Stofberg	i.m.	-	30	-	-
10	88	1	F.s.	Brits	i.v.	-	35	-	-
05	90	9	F.a.	Stormberg	i.v.	-	25	-	-

F.s. = *Francolinus swainsoni*

F.II. = *F. leuallantii leuallantii*

F.a. = *F. africanus*

KNP = Kruger National Park

n = number of birds sampled

TABLE 4 Isolation of blood parasites from guinea-fowls

Date		n	Origin	Route of inoculation	Result	Days to parasitaemia or days checked	Isolate	Passages
m	y							
11	84	1	Rust de Winter	i.m.	-	31	-	-
11	84	1	Pretoria	i.v.	-	14	-	-
02	88	2	Piensaarsrivier	i.v.	+	10	<i>P. circumflexum</i> :	G
05	88	1	Meyerton	i.v.	-	28	-	-
06	88	1	Pretoria	i.m.	-	31	-	-
06	88	2	Piet Retief	i.m.	-	32	-	-
07	88	1	Pietersburg	i.m.	-	32	-	-
10	88	1	Pretoria	i.v.	-	5	-	-
03	89	3	KNP	i.v.	+	15 (bird died)	<i>P. circumflexum</i> :	R
							<i>Aegyptianella</i>	R
							<i>botuliformis</i> :	S
								T
06	89	1	Pretoria	i.v.	-	33	-	-
01	90	2	Pretoria	i.v.	-	31	-	-
08	90	3	KNP	i.v.	+	15	<i>P. circumflexum</i> :	C
							<i>Aegyptianella</i>	C
							<i>botuliformis</i> :	A
								B

KNP = Kruger National Park

n = number of birds sampled

TABLE 5 Effect of infection dose and route of infection on prepatency time in *P. durae* infections

Isolate	Passage	Dose	i.v. n	Prepatency days		i.m. n	Prepatency days	
				$\bar{x}$	Range		$\bar{x}$	Range
M	38A	$1,2 \times 10^7$	2	6	5-7	2	14	14
M	15BC	$1,6 \times 10^7$	6	6	6	1	27	27
M	35/42	$10^8$	3	3,7	2-5	3	12,3	11-14
O	22	$1,4 \times 10^8$	4	3,5	3-5	4	12	12
O	19	$1,7 \times 10^8$	10	3,1	1-4	10	13,8	11-15
M	41	$2,0 \times 10^8$	1	3	3	2	14	14
M	38	$2,3 \times 10^8$	2	3	3	1	17	17
N	20	$2,4 \times 10^8$	2	4	3-5	2	18	12-24
O	37	$4,8 \times 10^8$	1	2	2	1	13	13
		TOTAL	31	3,92 ( $\pm 1,62$ )	1-7	26	14,3 ( $\pm 3,67$ )	11-27

n = number of birds used in the trail

$\bar{x}$  = arithmetic mean

Standard deviation given in brackets below the mean

TABLE 6 Effect of 1000 fold dilution of an i.v. inoculum of *P. durae* on length of prepatency period

Isolate	Dose	Prepatency days		
		n	$\bar{x}$	Range
M	$2,8 \times 18^8$	3	3	3
	$2,8 \times 10^5$	4	14	11-16

n = number of birds used in the trial

$\bar{x}$  = arithmetic mean

*lus*, while a fusiform *Leucocytozoon* was reported as *L. sabrazesi*—by Bennett & Herman (1976) from Tanzania. According to Bennett, Earlé, Peirce, Huchzermeyer & Squires-Parsons (1991), *L. sabrazesi* is a synonym of *L. macleani*. The *Haemoproteus* reported by Son (1960) could also have been the gametocyte of a *Plasmodium*.

The *Plasmodium* sp. of *Gallus gallus* reported by Adene & Dipeolu (1981) in Nigeria could have been *P. juxtannucleare*, as identified by Laird (1978) from turkeys in the same country. The only African records of *Plasmodium gallinaceum* are from domestic fowl in Egypt (Haiba 1948; Ezzat 1961).

### Isolation of blood parasites from domestic turkeys and game birds

Domestic turkeys yielded the highest percentage of isolates (50 %) of *P. durae*. However, the isolation of 3 strains from Swainson's francolin and 1 from redwinged francolin demonstrates that these birds are indigenous hosts of this parasite in South Africa, proving correct the speculation by Markus & Oosthuizen (1972). De Jong (1971) isolated *P. durae* from *Francolinus leucoscepus* obtained from Kenya, an achievement which remained hidden in an unpublished thesis, but was the basis of the personal communication by Southgate to Markus & Oosthuizen (1972).

Conversely, 20 guineafowls from areas which yielded *P. durae* from turkeys and/or francolins did not

produce any isolates of this parasite, while *P. circumflexum* was not isolated from any of the francolins. Thus *P. circumflexum* appears to be restricted to *Numida meleagris*, *P. durae* to *Francolinus swainsoni* and *F. levaillantii levaillantii* and *P. juxtannucleare* to *F. africanus* (Earlé, Huchzermeyer, Bennett & Little 1992). The fact that *P. juxtannucleare* was not isolated from the 9 greywing francolins might have been due to the use of only turkeys and guineafowls at the exclusion of *Gallus gallus* which may in this case have been a more susceptible host.

Initially it was feared that blood from a different species could not be injected i.v. without danger of anaphylactic shock. As game-bird samples were difficult to obtain, it was safer to inject them i.m. and thereby safeguard the survival of the recipient bird. Later, however, it was found that the i.v. route was quite safe amongst phasianids *sensu lato*, except when the donor blood was already partially clotted.

Successful interspecies i.v. sub-inoculation from turkey to guineafowl and fowl (successful in the sense of survival of the recipient bird) was reported by Huchzermeyer (1976). However, during the early stages of the present work a muscovy duck died from anaphylactic shock within minutes of receiving turkey blood intravenously. This incident caused (probably unnecessary) caution to prevail for a considerable period.

In attempts to passage *P. durae* from an outbreak of turkey malaria in Zimbabwe, Huchzermeyer (1976) found exoerythrocytic schizonts early in the infection which killed the experimental birds before higher parasitaemias could be witnessed. It was, therefore, considered possible that a cycle of exoerythrocytic schizogony could occur at the site of the i.m. injection thereby delaying the onset of patent parasitaemia. However, no exoerythrocytic stages were found in the present trials.

Herman *et al.* (1966) observed longer prepatent periods and lower parasitaemias after i.m. sub-inoculation than after using the i.v. route. In the

present trials the increased delay caused by i.m. sub-inoculation corresponded closely with that caused by a 1 000 fold dilution of the inoculum. The many degenerating and phagocytized red blood cells, histologically found at the site of injection, show that only a small proportion of the injected erythrocytes and thereby also of the parasites find their way into the bloodstream of the new host after i.m. sub-inoculation. This then appears to give the physical effects of a 1 000 fold dilution with the concomitant lengthening of the prepatent period. With blood samples from chronic carriers with very low parasitaemias i.m. sub-inoculation may therefore not be sensitive enough, particularly when blood smears are monitored only for 30 d after infection. With hindsight it can be assumed that possibly more isolates could have been obtained from the available material if the i.v. route and longer monitoring periods had been used throughout.

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## ADDENDUM

Due to circumstances beyond the author's control all isolates of *P. durae* stored in liquid N were destroyed in a recent accidental thaw out.