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FURTHER EVIDENCE OF RESISTANCE TO WARFARIN IN *RATTUS RATTUS DIARDII*

Y.M. LAM*

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RINGKASAN

Kajian-kajian makmal telah dijalankan untuk menentukan kesan 0.025% warfarin terhadap *R. rattus diardii* (Jentink) yang dibela di dalam makmal dan sebelumnya tidak terkena (termakan) warfarin dan juga tikus-tikus dari populasi liar yang dijangka tahan terhadap warfarin. Garis tapak untuk kepekaan terhadap 0.025% warfarin bagi *R. rattus diardii* yang sebelumnya tidak terkena warfarin telahpun ditentukan. Tempoh median pemakanan maut (LFP_{50}) untuk tikus jantan ialah 1.78 (had-had amanah 95%, 0.85–2.63) hari dan untuk tikus betina ialah 1.84 (1.11–2.56) hari. LFP_{95} untuk tikus-tikus jantan dan betina masing-masing ialah 9.01 (5.52–30.00) dan 6.41 (4.32–14.89) hari. LFP_{50} and LFP_{95} tergembleng (pooled) untuk kedua-dua jantina ialah 1.81 (1.25–2.35) dan 7.59 (5.45–13.45) hari. LFP_{99} yang telah dikira menunjukkan tempoh pemakanan selama 14 hari ke atas 0.025% warfarin, adalah ujian yang sesuai untuk menentukan ketahanan terhadap warfarin. Bagi tikus-tikus liar jantan dan betina, LFP_{50} masing-masing ialah 4.73 (3.16–6.11) dan 7.56 (5.96–9.28) hari. LFP_{95} untuk tikus-tikus liar jantan dan betina pula ialah 16.10 (11.23–36.34) dan 19.03 (13.91–40.11) hari. LFP_{50} dan LFP_{95} tergembleng untuk kedua-dua jantina tikus-tikus liar ialah 6.02 (4.92–7.10) dan 18.91 (14.35–30.82) hari. Faktor ketahanan (R) telah dikira dan nilai-nilai R menunjukkan yang tikus-tikus liar adalah 3.3 kali ganda kurang peka kepada 0.025% warfarin jika dibandingkan dengan tikus-tikus yang sebelumnya tidak kena warfarin. Kesimpulannya, ketahanan terhadap warfarin telah dipastikan dalam populasi liar *R. rattus diardii*.

INTRODUCTION

The occurrence of warfarin-resistant rat and mouse populations in Europe and the United States have been well documented (BOYLE, 1960; DRUMMOND, 1966; DRUMMOND and BENTLEY, 1967; LUND, 1964; OPHOF and LANGEVELD, 1969; TELLE, 1971; JACKSON, SPEAR and WRIGHT, 1971; ROWE and REDFERN, 1965; GREAVES, RENNISON and REDFERN, 1973). In Malaysia, evidence of warfarin resistance in a population of *Rattus rattus diardii* (Jentink) from a cocoa plantation has been reported (LAM, LEE, TAN and ELANJARAN MOHAN, 1982). Subsequently, there were reports of unsatisfactory control with warfarin in Chersonese Estate, Kuala Kurau, Perak (HAN, K.J., *pers. comm.*, 1979).

The Malaysian feral house rat, *R. rattus diardii,* which was once thought to be confined only to human dwellings (CHASEN, 1933, 1940; HARRISON, 1957, 1961), has become increasingly important in the coastal oil palm and cocoa plantations. The successful colonization of the oil palm and cocoa plantations represents a remarkable extension of the ecological niche of this species. In Chersonese Estate, *R. rattus diardii* constituted 97% of the rats trapped from the oil palm and cocoa areas and the remainder were *Rattus tiomanicus* (Miller). In the Klang coastal areas *R. rattus diardii* form 20%–45% of the rat population in oil palm area (SOH, K.G. and HO, C.T., *pers. comm.*, 1981). There is little information available on the susceptibility of *R. rattus diardii* to warfarin, the most common anticoagulant used for rodent control in oil palm and cocoa plantations. This paper describes the determination of the base-line susceptibility of *R. rattus diardii* to 0.025% warfarin and the evaluation of 0.025% warfarin against suspected warfarin-resistant *R. rattus diardii* trapped from Chersonese Estate.

MATERIALS AND METHODS

The laboratory-bred *R. rattus diardii* colony originated from rats that were

*Rice Division, MARDI, Bumbong Lima, Kepala Batas, Pulau Pinang.

trapped from rice stores in MARDI Bumbong Lima. These grain stores have never been treated with warfarin and the rat population was considered warfarin-naive. The wild-caught *R. rattus diardii* were obtained from Chersonese Estate, Kuala Kurau, Perak. Chersonese Estate, covering an area of 2 245 hectares, was planted with a mixed crop of cocoa/coconut and oil palm. The rats were trapped in areas where control with warfarin had been unsatisfactory. A total of 303 rats were caught, 295 were *R. rattus diardii* (comprising 97% of the trapped rats) and eight were *R. tiomanicus*.

Technical grade warfarin with 95% a.i. (Agricultural Chemicals Malaysia Sdn. Bhd.) was used to prepare the master-mix of 0.5% warfarin in finely ground rice. All dosses are expressed as milligrammes of warfarin per kilogramme of body weight. The experimental conditions for the no-choice feeding tests were as given in LAM (1979) and largely followed the WORLD HEALTH ORGANIZATION (1982) test procedures. The rats (laboratory-bred and wild-caught *R. rattus diardii*) were caged singly and were held for two weeks before tests. During the holding period a laboratory diet (mouse pellets, Gold Coin Sdn. Bhd.), unmilled rice and water were given *ad libitum*.

During tests the rats were given a sole diet of bait containing 0.025% warfarin for a fixed number of days. The bait-base was made up of equal parts of broken mouse pellets and rice, and corn oil was added at the rate of two per cent in the final bait containing 0.025% warfarin. Bait consumption and mortality were recorded and dead animals were autopsied for evidence of anti-coagulant poisoning. Rats that survived at the end of the various test periods were further observed for 30 days.

The dosage/mortality data were analysed by probit analysis (FINNEY, 1971), using DAUM's (1970) computer programme.

RESULTS

Results of the feeding tests with 0.025% warfarin against laboratory-bred and wild-caught *R. rattus diardii* are as shown in *Tables 1* and *2*. The dose (feeding period)/mortality data of *Tables 1* and *2* were subjected to probit analysis and the results are as summarized in *Table 3*.

As there was no significant difference between the sexes in the response to warfarin the data of laboratory-bred and wild-caught rats were pooled for probit analysis. The probit dosage/response lines for laboratory-bred and wild-caught rats did not contradict the hypothesis of parallelism (Chi-square value 2.631 with 3 degrees of freedom). Results of parallel lines analyses and the estimated relative potencies of 0.025% warfarin are as shown in *Table 4*. The relative potency (pooled data) indicated that 0.025% warfarin is only 0.32 times as effective against wild-caught rats. This indicated a reduction of 68% in the potency of 0.025% warfarin against wild-caught rats. A resistance factor (R), defined as the larger LFP_{50} divided by the smaller LFP_{50}, was calculated. The R value (sexes combined) indicated that in general, the wild-caught rats were 3.3 times less susceptible to 0.025% warfarin when compared with laboratory-bred warfarin-naive rats *(Table 4)*.

Mean days to death (average time taken for post-treatment mortality to occur) of susceptible laboratory-bred male and female rats was found to be 6.8 (range 3–9) days and 7.3 (3–13) days respectively *(Table 1)*. For the wild-caught rats, the mean days to death for males and females was 7.7 (3–17) days and 7.9 (4–12) days respectively *(Table 2)*. There was no significant difference in the mean days to death between the sexes in the laboratory-bred and wild-caught rats but there was significant difference in the mean days to death between wild-caught and laboratory-bred males at $P=0.05$. However, there was no significant difference in the mean days to death between the wild-caught and laboratory-bred females.

The lowest lethal dose of warfarin to laboratory-bred males and females was 9.86 mg/kg and 8.46 mg/kg respectively. The highest dose survived by laboratory-bred males and females was 80.99 mg/kg and

Table 1. Results of no-choice feeding tests with 0.025% warfarin against laboratory-bred warfarin-naive *R. rattus diardii*

Sex	Mean body weight (g)	Feeding period (days)	Mortality (dead/tested)	Mean bait intake (g)		Lethal dose of poison (mg/kg)		Survived dose of poison (mg/kg)		Days to death	
				Last day of prebait	First day of poison	Mean	Range	Mean	Range	Mean	Range
M	129.1	1	3/10	7.50	7.44	14.42	9.86– 20.59	14.92	12.14–19.35	6.3	6– 7
F	179.2	1	2/10	9.83	8.27	9.57	8.46– 10.68	12.37	8.14–16.81	4.5	4– 5
M	160.9	2	6/10	8.31	7.88	29.65	21.27– 39.67	19.41	15.76–29.03	5.5	3– 8
F	158.1	2	6/10	6.84	6.10	22.01	13.39– 29.29	19.20	15.64–21.08	4.2	3– 7
M	144.0	4	7/10	10.77	8.76	62.92	45.07– 79.44	50.22	42.30–60.04	7.6	6– 9
F	163.5	4	8/10	8.18	6.62	46.72	26.89– 65.57	31.22	26.26–36.18	8.4	7–13
M	183.5	6	8/10	8.21	8.11	58.65	42.86– 70.63	77.51	74.03–80.99	7.0	5– 9
F	190.4	6	9/10	9.64	8.05	66.27	49.77– 79.54	69.89	–	8.1	7–10
M	195.3	8	10/10	9.71	7.92	58.52	19.65– 98.81	–	–	7.0	6– 9
F	182.8	8	10/10	8.97	7.47	63.45	35.93– 91.37	–	–	7.3	5–10
M	175.1	10	10/10	9.74	8.81	63.46	48.74–103.30	–	–	6.7	5– 9
F	191.5	10	10/10	10.30	8.40	68.13	47.08– 91.31	–	–	8.2	6–12

M – male
F – female

Table 2. Results of no-choice feeding tests with 0.025% warfarin against wild-caught *R. rattus diardii* from Chersonese Estate, Perak

Sex	Mean body weight (g)	Feeding period (days)	Mortality (dead/tested)	Mean bait intake (g)		Lethal dose of poison (mg/kg)		Survived dose of poison (mg/kg)		Days to death	
				Last day of prebait	First day of poison	Mean	Range	Mean	Range	Mean	Range
M	163.9	2	1/10	8.09	6.79	27.20	–	22.19	12.94– 31.93	7.0	–
F	158.1	2	0/10	8.90	8.12	–	–	27.72	18.65– 41.05	–	–
M	191.9	4	4/10	11.63	9.29	45.89	32.61– 63.52	53.95	41.82– 60.40	5.5	3– 8
F	160.2	4	1/10	8.50	8.34	62.55	–	54.70	27.21– 74.54	10.0	–
M	175.8	6	7/10	9.44	6.66	52.54	14.92– 73.79	61.55	52.22– 76.91	7.1	5–10
F	170.6	6	3/10	9.03	6.56	59.04	40.37– 68.88	74.95	63.68– 88.27	7.3	6– 9
M	169.1	8	7/10	7.82	7.18	57.46	40.81– 74.44	70.70	42.58– 93.00	7.0	5–12
F	163.2	8	6/10	7.91	7.34	75.45	55.98– 96.06	76.05	65.79– 87.42	8.0	6–11
M	158.7	10	10/10	8.84	8.92	77.63	29.85–115.41	–	–	9.0	5–17
F	144.8	10	9/10	7.71	7.58	63.52	45.02– 90.15	123.44	–	7.2	4–10
M	174.1	12	8/10	8.78	7.98	65.88	23.92– 92.80	116.44	89.12–143.75	7.1	4–11
F	148.9	12	8/10	7.33	6.93	81.78	43.03–115.81	122.53	112.22–132.85	8.6	5–12
M	164.7	14	9/10	10.79	9.81	82.59	47.69–154.30	130.00	–	9.0	5–12
F	160.0	14	7/10	9.90	8.81	70.45	31.46–118.76	169.58	138.63–225.97	7.6	6–10

M – male
F – female

69.89 mg/kg *(Table 1)*. In the case of the wild-caught rats, the lowest lethal dose of warfarin to males and females was 14.92 mg/kg and 31.46 mg/kg respectively. The highest dose survived by wild-caught males and females was 143.75 mg/kg and 225.97 mg/kg *(Table 2)*. Both the lowest lethal dose and the highest survived dose were about 75% higher for wild males and 220% higher for wild females than for laboratory-bred males and females respectively.

DISCUSSION

The development of warfarin resistance in a population of *R. rattus diardii* from Chersonese Estate was clearly demonstrated. The rats from this population were found to be three times more tolerant to warfarin when compared with rats from a warfarin-naive population. Warfarin has been used for rat control in Chersonese Estate since the late 1960s (BONNER,

Table 3. Probit analysis of data from no-choice feeding tests with 0.025% warfarin against laboratory-bred (warfarin-naive) and wild-caught *R. rattus diardii*

Sex	Regression equation	Chi-sq.	d.f.	LFP_{50} (days)	95% fiducial limits of LFP_{50} (days)	LFP_{95} (days)	95% fiducial limits of LFP_{95} (days)	LFP_{99} (days)
M	Y = 2.33X + 4.42 (0.59)*	2.63	4	1.78	0.85–2.63	9.01	5.52–30.00	17.66
F	Y = 3.04X + 4.19 (0.70)	1.00	4	1.84	1.11–2.56	6.41	4.32–14.89	10.74
WM	Y = 3.09X + 2.92 (0.70)	3.42	5	4.73	3.16–6.11	16.10	11.23–36.34	26.76
WF	Y = 4.10X + 1.40 (0.94)	4.71	5	7.56	5.96–9.28	19.03	13.91–40.11	27.89
(M + F)	Y = 2.65X + 4.32 (0.45)	3.41	4	1.81	1.25–2.35	7.59	5.45–13.45	13.74
(WM + WF)	Y = 3.31X + 2.42 (0.53)	5.61	5	6.02	4.92–7.10	18.91	14.35–30.82	30.39

M – laboratory-bred (warfarin-naive) male *R. rattus diardii*.
F – laboratory-bred (warfarin-naive) female *R. rattus diardii*.
WM – wild-caught male *R. rattus diardii* from Chersonese Estate, Kuala Kurau, Perak.
WF – wild-caught female *R. rattus diardii* from Chersonese Estate, Kuala Kurau, Perak.
(M + F) – pooled data of laboratory-bred males and females.
(WM + WF) – pooled data of wild-caught males and females.
*Figures in parenthesis denote the standard error of the slope of regression line.

Table 4. Parallel lines analyses and relative potencies of 0.025% warfarin against laboratory-bred and wild-caught *R. rattus diardii*

Group			Relative potency* and 95% fiducial limits			Resistance factor** (R)
			Estimated	Lower	Upper	
M	vs.	F	0.93	0.58	1.49	1.03
WM	vs.	WF	1.58	1.10	2.34	1.60
M	vs.	WM	2.37	1.55	3.64	2.66
F	vs.	WF	4.03	2.66	6.33	4.11
M	vs.	WF	3.74	2.48	5.83	4.25
F	vs.	WM	2.55	1.66	3.95	2.57
(M+F)	vs.	(WM+WF)	3.11	2.31	4.28	3.32

(M+F) — pooled data of male and female laboratory-bred warfarin-naive rats (M – males; F – females).
(WM+WF)–pooled data of wild-caught male and female rats (WM – wild-caught males; WF – wild-caught females).
*Chi-square value of the four lines (M, F, WM and WF) in the test for parallelism is 2.631, 3 d.f., not significant at p = 0.05. The Chi-square value for pooled data in the test for parallelism is 0.903, 1 d.f., not significant at p = 0.05..
**R = the larger LFP_{50} divided by the smaller LFP_{50}.

P.W.I., *pers comm.*, 1981). Such a development could be expected after more than 10 years of continuous use of warfarin for rat control in the estate. Similar situations were found in Europe and the United States where warfarin and other anticoagulants were widely used for rodent control since the late 1940s. In the United Kingdom where warfarin had been used extensively since 1948, the first case of anticoagulant resistance was found among a *Rattus norvegicus* population in Scotland in 1958 (BOYLE, 1960). The detection of the development of warfarin resistance in *R. rattus diardii* suggests that more serious efforts be made to study the two other species, namely *R.*

tiomanicus and *R. argentiventer,* which also have a long history of exposure to warfarin and other anticoagulants.

The base-line data of the warfarin-naive rats indicated that a 14-day feeding test on a sole diet of 0.025% warfarin is a suitable warfarin resistance detection test for *R. rattus diardii.* Four wild-caught rats (one male and three females) survived the 14-day tests and were considered resistant to warfarin *(Table 2).* The male ingested a total of 130 mg/kg and the three females ingested 138.63, 144.13 and 225.97 mg/kg of warfarin. The results for warfarin-naive rats indicated that *R. rattus diardii* is more susceptible to warfarin when compared with *Rattus rattus* L. (KRISHNAMURTHY, UNIYAL and PINGALE, 1968; WORLD HEALTH ORGANIZATION, 1982).

Based on the LFP_{99}, an estimated one per cent of a population of warfarin-naive *R. rattus diardii* is expected to survive a 14-day feeding with 0.025% warfarin. Using the same criterion, rats from Chersonese Estate that could survive a similar level of treatment have increased to 10% in the males and 30% in the females. This increase in tolerance was clearly shown by results of the laboratory feeding tests. Sixteen wild-caught rats (6 males and 10 females) out of 80 survived feeding tests of 8–14 days but no laboratory-bred rats survived a feeding period of eight days. This indicates that 20% of the rats showed exceptional tolerance to warfarin and would probably survive the warfarin treatments as practised by the estate (the current practice is one baiting round at three to four days intervals until bait acceptance falls below 20% or a maximum of four rounds, whichever comes first). This was clearly shown by the unsatisfactory control with 0.04% warfarin in Chersonese Estate in which there was no decline in the bait consumption after the fourth round and the bait acceptance was about 80%–90% after six or seven rounds (HAN, K.J., *pers comm.,* 1979). The concentration of warfarin in the baits used have been increased to 0.1% since, but bait acceptance did not decline after the fourth round (BONNER, P.W.I., *pers comm.,* 1982). The intermittent feeding behaviour exhibited by this species would further reduce the efficacy of warfarin in the field (LAM *et al.,* 1982).

For the control of warfarin-resistant rats, alternative but more potent novel chronic rodenticides (HADLER and SHADBOLT, 1975) or acute poisons would have to be used. The 'second generation' chronic rodenticides like brodifacoum, difenacoum and bromadiolone and the novel acute rodenticide, bromethalin will be of value in the control of rats resistant to warfarin and other first generation anti-coagulants.

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SUMMARY

Studies were conducted in the laboratory to determine the efficacy of 0.025% warfarin against laboratory-bred warfarin-naive and wild-caught *R. rattus diardii* from a suspected warfarin-resistant population. A base-line for the susceptibility of warfarin-naive *R. rattus diardii* to 0.025% warfarin was determined. The median lethal feeding period (LFP_{50}) for males and females was 1.78 (95% fiducial limits 0.85–2.63) and 1.84 (1.11–2.56) days respectively. The LFP_{95} for males and females was 9.01 (5.52–30.00) and 6.41 (4.32–14.89) days respectively. The pooled LFP_{50} and LFP_{95} for both sexes was 1.81

(1.25–2.35) and 7.59 (5.45–13.45) days respectively. The estimated LFP_{99} indicated that a 14-day feeding on 0.025% warfarin is a suitable test for the detection of warfarin resistance. For the wild-caught males and females, the LFP_{50} was 4.73 (3.16–6.11) and 7.56 (5.96–9.28) days respectively. The LFP_{95} for wild-caught males and females was 16.10 (11.23–36.34) and 19.03 (13.91–40.11) days. The pooled LFP_{50} and LFP_{95} for both sexes of wild-caught rats was 6.02 (4.92–7.10) and 18.91 (14.35–30.82) days respectively. A resistance factor (R) was calculated and the R values indicated that the wild-caught rats were 3.3 times less susceptible to 0.025% warfarin when compared with warfarin-naive rats. In conclusion, warfarin resistance has been confirmed in the wild population of *R. rattus diardii* studied.

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