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A CASE OF WARFARIN RESISTANCE IN *RATTUS RATTUS DIARDII* (JENTINK)

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RINGKASAN

Kesan kekebalan warfarin terhadap tikus *R. rattus diardii* yang diperangkap dari ladang koko Kuala Bernam, Teluk Anson, Perak, telah diperiksa melalui ujian-ujian makmal. Dari 14 ekor tikus tersebut lima didapati kebal terhadap warfarin bila dibandingkan dengan tikus-tikus dari jenis yang sama yang sebelumnya tidak pernah terkena (termakan) warfarin. Dalam ujian yang dijalankan selama 6 hari, warfarin pada kepekatan 0.025%, didapati bahawa takaran membunuh tikus jantan dan tikus betina masing-masing ialah 49.8 mg/kg dan 30.8 mg/kg. Purata tempoh mati bagi tikus jantan ialah 7.4 hari sementara tikus betina pula ialah 14 hari. Dalam ujian pemakanan 'tanpa pilihan' yang dibuat selama 30 hari menggunakan kepekatan racun yang sama, empat dari lima tikus yang diuji mati. Dua tikus betina mati pada hari ke 14, setelah masing-masing memakan racun pada kadar 107.2 mg/kg dan 119.3 mg/kg. Seekor tikus jantan mati pada hari yang ke 24 setelah memakan racun pada kadar 205 mg/kg dan seekor lagi tikus jantan mati pada hari ke 34 setelah memakan racun pada kadar 241.7 mg/kg. Sementara itu tikus betina yang terselamat terus hidup setelah memakan racun pada kadar 347.4 mg/kg. Corak pemakanan yang tidak tetap (terputus-putus) oleh tikus-tikus yang tahan terhadap warfarin adalah penting dari sudut amali untuk mengawal tikus-tikus ini dengan menggunakan warfarin.

INTRODUCTION

Anticoagulant resistance was first discovered in Scotland in 1958 in which a population of *Rattus norveqicus* Berk. failed to succumb to prolonged treatments with both warfarin and diphacinone (BOYLE, 1960). Warfarin-resistant *R. norveqicus* populations were subsequently detected in England and Wales in 1960 (DRUMMOND, 1966; DRUMMOND and BENTLEY, 1967), Denmark in 1962 (LUND, 1964), Netherlands in 1966 (OPHOF and LANGEVELD, 1969), Germany in 1971 (TELLE, 1971) and the United States in 1971 (JACKSON, *et. al.*, 1971). Anticoagulant resistance has also been confirmed in the house mice, *Mus musculus* L. in England (ROWE and REDFERN, 1965) and ship rats, *Rattus rattus* L. in Liverpool (GREAVES, *et. al.*, 1973).

Warfarin, an anticoagulant rodenticide, has been widely used for rat control in the oil palm and cocoa plantations since the late 1960s in Peninsular Malaysia. In August 1980, a sample of *Rattus rattus diardii* (Jentink) was trapped from a cocoa area in Kuala Bernam Estate, Teluk Anson, Perak, where control with warfarin has been unsatisfactory. This paper presents laboratory evidence of warfarin resistance in a population of *R. rattus diardii.*

MATERIALS AND METHODS

Warfarin, 3-(1-phenyl-2-acetylethyl)-4 hydroxycoumarin, of 95% purity (Agricultural Chemicals Malaysia Sdn. Bhd.) was used to prepare the master-mix of 0.5% warfarin in finely ground rice. All doses are expressed as milligrams of warfarin per kilogram of body weight. The test conditions were as given in LAM (1979) and largely followed the World Health Organisation (1970) test procedures. The rats (*R. rattus diardii*) were caged singly and were conditioned in the laboratory for two months.

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During the conditioning period mouse pellets (Gold Coin Sdn. Bhd.), unmilled rice and water were given *ad libitum*.

The rats (nine males and five females) were then given a sole diet of rice/corn oil baits containing 0.025% warfarin for 6 days. Bait consumption and mortality were recorded and dead animals were autopsied for evidence of anticoagulant poisoning. Rats that survived the above 6-day test were allowed a one-month recovery period. During the recovery period mouse pellets, unmilled rice and water were given *ad libitum*. The rats (those that survived the 6-day test) were then fed broken mouse pellets/rice/corn oil baits containing 0.025% warfarin continuously for 30 days. The bait based was made up of equal parts of broken mouse pellets and rice, and corn oil was added at the rate of 2% in the final bait containing 0.025% warfarin. Bait consumption and mortality were recorded and dead animals were autopsied for evidence of anticoagulant poisoning.

RESULTS

Five (two males and three females) out of 14 rats survived the 6-day 'no-choice' test with 0.025% warfarin (*Table 1*). The mean lethal dose for males and females that succumbed was 49.8 mg/kg (range 42.9-60.9) and 30.8 mg/kg (range 23.7-37.9), respectively. Mean days to death (average time to die) for males was 7.4 days (range in days to death 5-11) and females 14 days (range in days to death 13-15).

In the 30-day 'no-choice' test, four rats succumbed, two females on the 14th day after consuming a dose of 107.2 mg/kg and 119.3 mg/kg of warfarin, respectively; and two males, one on the 24th day after consuming 205 mg/kg and the other on the 34th day after consuming 241.7 mg/kg of warfarin (*Table 2*). The remaining female rat survived the 30-day test after having consumed 347.4 mg/kg of warfarin.

Feeding pattern of the five rats indicated rats that survived over a longer period tended to feed intermittently with consumption falling after 5-8 days and then subsequently increased (*Figure 1*). With the exception of the female that survived, consumption fell to almost zero in those rats that died. In the case of the female that survived, bait consumption only fell to about 50% of the highest previous bait consumed (*Figure 1*).

DISCUSSION

Based on the data of BENTLEY and LARTHE (1959), the World Health Organisation (1970) proposed as a guide the use of warfarin at 0.025% in baits for susceptibility testing against *Rattus rattus* L., *Rattus rattus diardii,* being a subspecies of *R. rattus,* was expected to show similar susceptibility. However, there is still little information on the susceptibility of *R. rattus diardii* to warfarin. Trials are currently being conducted with laboratory bred warfarin-naive *R. rattus diardii* to determine the baseline toxicity of warfarin against this species. Results (LAM, unpublished) from a 6-day 'no-choice' test with 0.025% warfarin against laboratory bred warfarin-naive rats gave a mortality of 80% in males and 90% in females, and in a 10-day 'no-choice' test 100% mortality was achieved in both sexes. For animals that succumbed in the 6 and 10-day tests, mean days to death for males was 6.8 days (range in days to death 5-9) and females 8.2 days (range in days to death 6-12). Mean lethal dose of warfarin for males and females was 61.3 mg/kg (range 42.9-103.3) and 67.3 mg/kg (range 47.1-91.3), respectively.

BENTLEY and LARTHE (1959) reported that for *R. rattus,* warfarin at 0.025% killed only 1/12 in a 5-day feeding, 10/12 in a 7-day feeding, 9/12 in a 10-day feeding and 11/11 in a 12-day feeding test. This indicated that the warfarin-naive *R. rattus diardii* is more susceptible to warfarin when compared with *R. rattus.* However, the present study with field caught *R. rattus diardii* indicated a very high tolerance to warfarin (*Table 2*). Even the three females that succumbed in the 6-day test took more than 13 days to die which is outside the range of 6-12 days in warfarin-naive rats, but the seven males did not show

TABLE 1. BAIT CONSUMPTION AND MORTALITY OF *RATTUS RATTUS DIARDII* GIVEN A SOLE DIET OF 0.025% WARFARIN IN RICE/CORN OIL BAIT FOR SIX DAYS

Sex	Mean body weight (g)	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	152.8	7/9	7.60	5.56	49.80	42.89-60.85	69.11	60.23-78.00	7.4	5-11
F	188.8	2/5	9.26	5.82	30.80	23.72-37.87	63.09	56.12-74.97	14.0	13-15

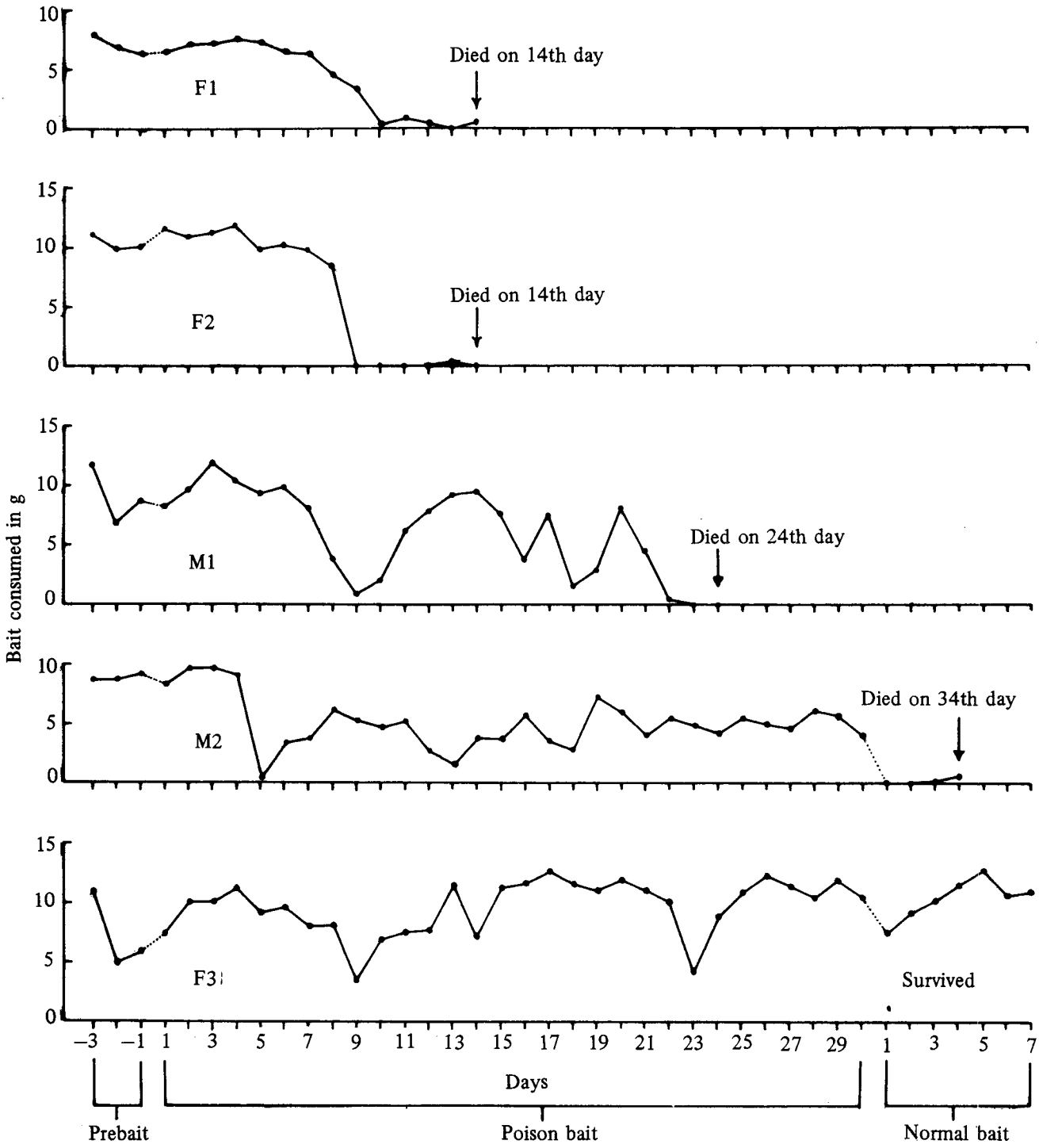

Figure 1. Bait consumption of **Rattus rattus diardii** *given a diet of 0.025% warfarin in broken laboratory pellets/rice/corn oil baits over a 30-day period (M-male, F-female).*

TABLE 2. BAIT CONSUMPTION AND MORTALITY OF *RATTUS RATTUS DIARDII* GIVEN A SOLE DIET OF 0.025% WARFARIN IN BROKEN PELLETS/RICE/CORN OIL BAIT FOR 30 DAYS

Sex	Body weight (g)	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 prison			
M	158.0	8.88	5.09	241.71	—	34
M	176.3	9.10	6.02	205.00	—	24
F	207.7	7.25	9.62	—	347.39	—
F	150.8	7.14	4.62	107.16	—	14
F	176.4	10.39	6.01	119.25	—	14

the same increase in the time taken to die (*Table 1*). In view of the high tolerance in the five rats that survived the 6-day test, these rats would have survived the ordinary control treatment with warfarin that is practised in the estates, based on their feeding behaviour as observed in the laboratory (*Figure 1*). The interspersed feeding pattern of 5—9 days is of pratical importance in the poisoning of wild populations of *R. rattus diardii*. Similar feeding behaviour was observed in *R. norvegicus* (DRUMMOND and WILSON, 1968; BROOKS and BOWERMAN, 1973) and in *R. argentiventer* (LAM, 1979).

The female rat that survived the 30-day test after ingesting 347.4 mg/kg of warfarin indicated that it is resistant to warfarin baits (*Table 2* and *Figure 1*). Judging from the high incidence (35.7%) of warfarin tolerant/resistant rats from this small sample, warfarin resistance may have become widespread in the estate. This could be easily confirmed by further sampling of the rat population in the estate. However, through the widespread use of warfarin in the oil palm and cocoa areas it is expected that warfarin-resistant *Rattus tiomanicus* and *Rattus argentiventer* may be encountered in near future.

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SUMMARY

Warfarin resistance in *R. rattus diardii* was confirmed in the laboratory. Five from a sample of 14 *R. rattus diardii* trapped from a cocoa area in Kuala Bernam Estate, Teluk Anson, Perak, showed a high tolerance to warfarin when compared with warfarin-naive rats of the same species. The mean lethal dose for males and females that succumbed in the 6-day screening test with 0.025% warfarin was 49.8 mg/kg and 30.8 mg/kg respectively. Mean days to death for males was 7.4 days and females 14 days. In the 30-day 'no-choice' test with 0.025% warfarin, four out of five rats succumbed, two females on the 14th day after consuming a dose of 107.2 mg/kg and 119.3 mg/kg of warfarin respectively; and two males, one on the 24th day after consuming 205 mg/kg and the other on the 34th day after consuming 241.7 mg/kg of warfarin. One female survived the 30-day test after having consumed a total dose of 347.4 mg/kg of warfarin. The intermittent feeding pattern of the warfarin tolerant/resistant rats is of practical importance in control treatments using warfarin.

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