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RESPONSE OF DOMINANT RODENTS TO COUMATETRALYL AND BROMADIOLONE IN GREATER CAIRO, EGYPT

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Abstract

Since the 1950s, anticoagulant rodenticides are used to control rodents in public health and agriculture sectors. The extensive use of these compounds has acted as selective force to rodents, leading to resistance. Resistance mechanisms have been identified in rats and mice, including the modification of the enzyme that activates vitamin K, vitamin K epoxide reductase (VKOR). Susceptibility levels of the Norway rat, *Rattus norvegicus* and the roof rat, *Rattus rattus* to coumatetralyl (first generation anticoagulant) and broamdiolone (second generation anticoagulant) by bioassay detection method under laboratory conditions were studied. Animals were trapped from Greater Cairo, Egypt in which the anticoagulant rodenticides were used to control rodents for long periods. Complete mortality was recorded for both species and sexes within the standard feeding periods (under no-choice feeding test for 6-days to coumatetralyl and 4-days to bromadiolone). Rat species under studied still susceptible to coumatetralyl and bromadiolone. The present work revealed a significant correlation between species in comparison with consumed dose and death time. *R. rattus* showed more active ingredient intake (mg/kg) than *R. norvegicus*. Death time (days), *R. rattus* recorded higher mean values than *R. norvegicus*, also females showed higher mean values compared to males.

Key Words: Egypt, *Rattus norvegicus, Rattus rattus*, Coumatetralyl, Bromadiolone.

Introduction

Rodents are very common in many Egyptian Governorates (Shoukry *et al*, 1986; Morsy *et al*, 1988). Besides their economic hazard causing damage to agriculture and contamination of stored food materials, they also play an important role as reservoir host for many zoonotic diseases as plague and murine typhus (Abdon and Samaan, 1962; El Bahnasawy *et al*, 2012; Butler, 2013), leishmaniasis (Morsy *et al*, 1982; El-Kady *et al*, 1998), toxoplasmosis (Saleh *et al,* 2016) and trichinosis (Morsy *et al*, 2000). They act as reservoir host for hymenolepiasis, giardiasis, amoebiasis and schistosomiasis (Morsy *et al,* 1981; El-Nahal *et al*, 1982). The limitations of the acute rodenticides had made it virtually impossible to achieve 100% clearance of rodent populations and repeated applications of acute rodenticides to rapidly recovering. The chronic anticoagulant rodenticide revolutionized rodent control and for the first time complete control of target rodent populations was possible and practical. They inhibit vitamin K regeneration by the vitamin K *Epoxide Reductase* (VKOR) and cause a fatal hemorrhagic syndrome. Because of repeated use, some populations of commensal rodents have expressed resistance to these compounds (Vein *et al*, 2011). First population of warfarin-resistant to *Rattus norvegicus* was discovered in Scotland 1958 (Boyle, 1960) and in Denmark and UK (Lund, 1964; Greaves *et al*, 1973). Resistance to *Rattus rattus* was in Australia and Malaysia (Saunders, 1978; Lam *et al*, 1982) and *Mus musculus* in Sweden, Finland and Canada (Morgan and Petras, 1979; Müller *et al*, 2014). In Egypt, the continuous use of anticoagulant rodenticides to control rodents in public and agriculture sectors was the main reason selected individuals with resistance genes.

This work aimed to determine the suscept-

ibility level of coumatetralyl and bromadiolone anticoagulant rodenticides used in public health to control the dominant rodent species in Great Cairo, Egypt.

Materials and Methods

Wire box traps were deodorized by cleaning with hot water and soap before used. Traps were baited, distributed in selected houses at sunset at Greater Cairo, Egypt. Traps collected next morning and transported to the laboratory (Rifaat *et al*, 1969).

Rats were kept for two weeks before being tested and during this period they were caged individually and given water and a suitable diet. Two days before testing, animal's weight and sex were determined. Immature ones (less than 150gm. For the *R. norvegicus* & 100gm. for *R. rattus)*, pregnant females and unhealthy rats were excluded from the experiment (WHO, 1970). The studies were done from February to September 2016. The susceptibility levels of *R. norvegicus* and *R. rattus* (20 rodents for each level) was carried out by bioassay detection method under laboratory conditions (WHO, 1982). Coumatetralyl 0.0375% "4-hydroxy-3-(1, 2, 3,4-tetrahydro-1-naphthyl) coumarin" was used for 6-days by no-choice feeding test. Bromadiolone 0.005% anti-coagulantrod-endicide"3-[3-(4-bromobiphenyl 4-yl)-3-hy-droxy1-phenylpropyl]-4-hydroxycoumarin" was used for 4-days by no-choice feeding test. The amount of anticoagulant bait eaten was recorded daily and food pots were replenished each day with fresh poison bait. After the completion of feeding, animals were fed on normal laboratory diet.

The death's day was recorded. The resistant rats were those survived for 24 days after six-feeding days for coumatetralyl and four-days feeding for Bromadiolone at the normal concentration.

Statistical analysis: Data were subjected to analysis for variance and the method of least significant differences (L.S.D.), the method of Duncan (1955) was used.

Results

The results are shown in tables (1, 2, 3 & 4).

Table1: Criteria of 6-feeding days on 0.0375% coumatetralyl for *R. norvegicus* & *R. rattus* in Greater Cairo.

Species	Sex (n=20)	Mean / Range				Mortality %	Resistance %
		Rat weight (g)	Bait consumption (g)	Dose consumed (mg/kg)	Time to Death (days)		
Rattus norvegicus	male	294.1 160 -442	42.1 21.0-71.0	55.6 25.8 – 80.1	5.0 3.0 -6.0	100	0
	female	254.9 152 – 380	30.2 7.0 -49.0	50.9 8.2 - 84.1	5.1 4.0 - 6.0	100	0
Rattus rattus	male	138.6 107 -188	29.9 17.0 - 43.0	80.2 52.7 - 98.1	8.9 6.0 - 12.0	100	0
	female	134 100 -176	25.9 9.0 -39.0	72.6 24.6 - 94.3	9.4 6.0 - 12.0	100	0

Table 2: Criteria of 4-feeding days on 0.005% bromadiolone for *R. norvegicus* & *R. rattus* in Greater Cairo.

Species	Sex (n=20)	Mean / Range				Mortality %	Resistance %
		Rat weight (g)	Bait consumption (g)	Dose consumed (mg/kg)	Time to Death (days)		
Rattus norvegicus	♂	275 187 -386	51.1 37.0 - 72.0	9.7 5.7 - 13.8	6.7 4.0 -9.0	100	0
	♀	286 155- 395	58.3 51.0 -89.0	10.9 4.6 - 17.0	7.0 6.0 - 10.0	100	0
Rattus rattus	♂	128 100 -184	37.2 24.0 - 48.0	15.0 9 - 20.3	7.8 6.0 - 12.0	100	0
	♀	126 100 -160	26.5 14.0 -36.0	10.8 6.9 - 16.4	9.0 6.0 13.0	100	0

Table 3: correlation between species in comparison with consumed dose and time to death

Rodenticide	Variable	Species	Mean	SD	Levene's Test
Coumatetralyl	Consumed dose	*R. norvegicus*	53.1980	21.46855	F= 4.53* P< 0.05
		R. rattus	76.4000	16.45778	
	Time to death	*R. norvegicus*	5.050	1.1756	F= 28.6** P< 0.001
		R. rattus	9.150	2.3594	
Bromadiolone	Consumed dose	*R. norvegicus*	10.3000	3.20384	F= 6.698* P< 0.05
		R. rattus	12.8950	4.29663	
	Time to death	*R. norvegicus*	6.850	1.4772	F= 7.76** P< 0.01
		R. rattus	8.400	2.3621	

*significant ** highly significant

Table 4 correlation between males and females in comparison with consumed dose and time to death

Rodenticide	Variable	Species	Sex	Mean	SD	Levene's Test
Coumatetralyl	Consumed dose	*R. norvegicus*	♂(n=20)	55.5710	17.56690	F=0.223 P>0.05
			♀ (n=20)	50.8250	25.01208	
		R. rattus	♂ (n=20)	80.2100	13.74569	F= 0.01 P>0.05
			♀ (n=20)	72.5900	18.34307	
	Time to death	*R. norvegicus*	♂ (n=20)	5.000	1.3765	F=4.03 P>0.05
			♀ (n=20)	5.158	.9582	
		R. rattus	♂ (n=20)	8.900	1.9708	F=4.476* P>0.05
			♀ (n=20)	9.400	2.7222	
Bromadiolone	Consumed dose	*R. norvegicus*	♂ (n=20)	9.6900	2.55773	F=1.42 P>0.05
			♀ (n=20)	10.9100	3.70730	
		R. rattus	♂ (n=20)	15.0400	3.76555	F= 0.08 P>0.05
			♀ (n=20)	10.7500	3.74538	
	Time to death	*R. norvegicus*	♂ (n=20)	6.700	1.5252	F= 0.17 P>0.05
			♀ (n=20)	7.000	1.4510	
		R. rattus	♂ (n=20)	7.800	1.9894	F= 2.47 P>0.05
			♀ (n=20)	9.000	2.5955	

Discussion

The present study showed that coumatetralyl 0.0375% in diet (under no choice feeding test for 6 days) and bromadiolone 0.005% in diet (under no choice feeding test for 4 days) caused complete mortality (100%) of both species and two sexes of *R. norvegicus* and *R. rattus* trapped from Greater Cairo, by bioassay rechnique under laboratory condition. Results indicate that the two rat species still susceptible to coumatetralyl and bromadiolone anticoagulant rodenticides. Data revealed a significant correlation between species in comparison with consumed dose. *R. rattus* showed more active ingredient intake (mg/kg) than *R. norvegicus*. The mean intake values of coumatetralyl being 80.2 & 72.6 mg/kg to *R. rattus* and being 55.6 & 50.9 mg/kg to *R. norvegicus* for males and females, respectively. While the mean intake values of bromadiolone being 15.0 & 10.8mg/kg to *R. rattus* and being 9.7 & 10.9mg/kg to *R. norvegicus* for males and females, respectively. Time to death was taken as a parameter for anticoagulant efficacy on treated rats. In this respect, *R. rattus* recorded higher mean values than *R. norvegicus* which being 8.9 & 9.4 days for *R. rattus* and 5.0 & 5.1 days for *R. norvegicus* to coumatetralyl for males and females, respectively. While the same values recorded 7.8 & 9.0 days for *R. rattus* and 6.7 & 7.0 days for *R. norvegicus* to bromadiolone for males and females, respectively. The same correlation showed that females recorded higher mean values comparison to males. Such findings agreed to a great extent with other Egyptian studies. Zidan *et al*. (1997) showed that *R. rattus* and *R. norvegicus* from Qualyobia Governorate were susceptible to warfarin and flocoumafen. Hussien (2001) found that *R. rattus* was very susceptible to chlorophacinone. Hussien (2005) reported that the higher effect of warfarin on the prothrombin time was noticed in *R. norvegicus* and *R. rattus* in Qualyobia Governorate, indicating their susceptibility to warfarin. Mikhail and Allam (2007) showed that *R. norvegicus* and *R. rat-*

tus in Qualyobia Governorate were susceptible to bromadiolone, difencoum and coumatetralyl anticoagulant rodenticides**.** Mikhail and Abdel-Hamid (2010) reported that bromadiolone caused complete mortality as indicated by bioassay for *R. norvegicus* and *R. rattus* trapped from Giza and Qualyobia Governorates when anticoagulant rodenticides were used for long period. Saxena *et al*. (2008) in India showed that 0.005% bromadiolone was very effective in controlling the increasing population of *R. rattus* and *Tatera indica* in field crops. Endepols *et al*. (2007) reported that a new clotting response test was used to determine the susceptibility of coumatetralyl and bromadiolone for laboratory strain of Norway rat from Germany and UK (Hampshire), and wild rats trapped from farms in Walex (UK) and Westphalia (Germany). Resistance was calculated in relation to the CD strain of Norway rats. Homozygous and heterozygous animals of a strain of resistant from Westphalia were cross-resistant to coumatetralyl and bromadiolone with higher resistance factor for bromadiolone than that in both UK strains. Pelz (2007) reported that resistance of anticoagulant rodenticides in *R. norvegicus* was estimated to cover an area of roughly 26000 Km2 in northwest Germany. The chemical compounds involved are warfarin, coumatetralyl, bromadiolone and, to a lesser extent, difenacoum. Control failures with house mice (*Mus musculus/domesticus*) suggest that resistance to high potency compounds may occur. Cao *et al*. (2008) reported that there was no significant difference between the susceptibility of male and female of *Apodemus agrarius* after treatment with 0.005% bromadiolone bait at 7 different feeding days. *A. agrarius* was still alive after fed on bait containing 0.005% bromadiolone for 21 days in Harbin (China), and then it could be considered as being resistant. Guidobono *et al*. (2010) reported that application of anticoagulant rodenticides for rodent control has showed a decrease in effectiveness through time in Buenos Aires, Argentina because of the development of resistant populations and the development of aversion behavior to *M. musculus*. A conducted on feeding test wild animals captured in poultry farms and laboratory strain that were fed with bromadiolone bait. Three animals of the field experimental group survived the 21 days period, while for laboratory animals mortality was 100%. Control field animals which were fed without anticoagulant showed 100% survival, evidence of the presence of anticoagulant resistant *M. musculus L* in the study area. Vein *et al*. (2011) reported that in Francecomte (France), rodents have expressed resistance to anti-vitamin Ks (AVKs) rodenticides. The water vole exhibits cyclic population outbreaks. A second generation AVK, bromadiolone, has been used for the last 20 years to control vole population. The results indicate that voles from the most heavily treated area exhibit enzymatic changes in VKOR activity hence arguing for resistance AVKs. Baert *et al*. (2012) investigated the distribution of anticoagulant resistance in Flanders, northern Belgium. Warfarin resistant rats were found in the western and eastern rats of Flanders. The same was found for bromadiolone except in the southeastern area, where resistance was absent. They also detected difenacoum resistance in only six rats and did not observe any resistance rats in the central part of Flanders. Andru *et al*. (2013) reported that rodent control in oil palm plantations in Indonesia is based principally on use of anti-vitamin K (AVK), the main anticoagulant used was coumatetralyl, a first generation AVK. A conducted a comparative study in two well established oil palm plantations in Indonesia, one without chemical control in Riau and another with intensive coumatetralyl use on BangKa. In Riau rats were much more susceptible to coumatetralyl than in BangKa. Buckle (2013) reported that anticoagulant resistance was discovered in UK Norway rats (*R. norvegicus* Berk.) in 1958 and continued over since. The possible detrimental

impact of resistance on effective rodent control was quickly recognized, and, for almost three decades, extensive research was conducted on the geographical distribution and severity of anticoagulant resistance in UK rats. At first, surveys showed resistance only to first-generation anticoagulants, such as warfarin, chlorophacinone and coumatetralyl, but, later, resistance to the more potent second-generation anticoagulants as difenacoum and bromadiolone was also discovered. Wang *et al*. (2014) determined the resistance of *R. norvegicus* to warfarin and bromadiolone in HongKou district of Shanghai and provided evidence for rodent control. Methods of a non-selective feeding trial were conducted with 0.005% warfarin and 0.005% bromadiolone baits. Application of 0.005% warfarin and 0.005% bromadiolone baits for 6-days, occurrence rate of warfarin-resistant was 0% and that bromadiolone-resistant was 3.7% (1/27). *R. norvegicus* in Hong-Kou district is still susceptible to the first generation anticoagulant, but resistant to second generation anticoagulant. Esther (2015) reported that rodents showed genetically determined resistance, so that the control fails to respond. There were numerous resistant to Norway rats and house mice in Germany. Effective management of resistant was only possible with flocoumafen, brodifacoum and difethialone. The use of the first generation active ingredients (warfarin, chlorophacinone & coumatetralyl) and bromadiolone was uneffective. Garg *et al*. (2015) reported that the second generation anticoagulant, bromadiolone controled rodents all over India for the last several years.

Conclusion

Rattus norvegicus and *Rattus rattus* in Greater Cairo were still susceptible to the anticoagulant rodenticides, coumatetralyl 0.0375% and bromadiolone 0.005% by bioassay technique under laboratory conditions. Regular evaluate to determine susceptibility and/or resistance level status to anticoagulant rodenticides to rodent species in all Egyptian Governorates should be done.

References

Abdon, AH, Samaan, M, 1962: Egyptian Rodents and their Public Health Importance. J. Arab. Vet. Med. Assoc. 22, 4:403-31.

Andru, J, Cosson, JF, Calinan, JP, Benoit, E, 2013: Coumatetralyl resistance of Rattus tanezumi infesting oil palm plantations in Indonesia. Ecotoxicology.22, 2:377-86.

Baert,K, Stuyck, J, Breyne, P, Maes, D, Casaer, J, 2012: Distribution of anticoagulant resistance in brown rat in Belgium. Belg. J. Zool. 142, 1:39-48.

Boyle, CM, 1960: Case of apparent resistance of *Rattus norvegicus Berkenhot* to anticoagulant poisons. Nature 188:517-21.

Buckle, A, 2013: Anticoagulant resistance in United Kingdom and a new guideline for the management of resistant infestations of Norway rats (*Rattus norvegicus Berk*.). Pest Manag. Sci. 69, 3:334-41.

Butler, T, 2013: Plague gives surprises in the first decade of the 21st Century in the United States and worldwide. Am. J. Trop. Med. Hyg. 894:788-93.

Cao, Yu, Wang, Yu, Wang Dawe, Cong Line, Dong Tiayi, 2008: The detection of the susceptibility of *Apodemus agarius* to bromadiolone in Harbin. J. Vector Biol. Control. 19, 5:405-07.

Duncan, TMW, 1955: Multiplerange and multiple F-tests. Biomertics. 11:1-42.

El-Bahnasawy, MM, Ahmad, GMS, Abdel-Fattah, MA, Gaber, WAI, Morsy, TA, 2012: Is plague a problem in the Egyptians returning back from Libya? J. Egypt. Soc. Parasitol. 42, 2:329-48.

El-Kady, GA, Makled, KM, Morsy, TA, Morsy ZS, 1998: Rodents, their seasonal activity, ecto-and blood parasites in Saint Catherine area, South Sinai Governorate, Egypt. J. Egypt. Soc. Parasitol. 28, 3:815-25.

El Nahal, HS, Morsy, TA, Bassili, WR, El Missiry, AG, Saleh, MSM, 1982: Antibodies against 3 parasites of medical importance in *Rattus sp*. collected in Giza Governorate, Egypt. J. Egypt. Soc. Parasitol. 12, 2:287-93.

Endepols, S, Presott, CV, Klemann, N, Buckle, AP, 2007: Susceptibility to the anticoagulants bromadiolone and coumatetralyl in wild Norway rats (*Rattus norvegicus*) from the UK and Germany. Int. J. Pest Manag. 53, 4:285-90. **Esther, A, 2015:** Problems of rodenticide resistance, Berichte dem Julius Kuhun. Inst. 176:4-9.

Garg, N, Neena Singia, 2015: Blood clotting

response test for detecting resistance to second generation anticoagulant bromadiolone in house rat (*Rattus rattus*). Indian J. Anim. Res. 49, 5: 607-11.

Greaves, JH, Rennison, BD, Redfern, R, 1973: Warfarin resistance in the ship rat in Liverpool. Int. Pest Control 15, 2:17-21.

Guidobono, JS, Leon, V, Gomez Villafane, IE, Busch, M, 2010: Bromadiolone susceptibility in wild and laboratory *Mus musculus L*. (house mice) in Buenos Aires, Argentina. Pest Manag. Sci. 66, 2:162-67.

Hussien, SSM, 2001: Response of some rodent species to chlorofhacinone rodenticide under laboratory and field conditions. 1st Cong. Integrated Pest Manag., Fac. Agric. Cairo Univ. 125-30.

Hussien, SSM, 2005: Induced tolerance to warfarin rodenticide in different *Rattus rattus* and *Rattus norvegicus* strains trapped from certain Egyptian Governorates, Egypt. J. Agric. Res. 83, 1:241-49.

Lam, YM, Lee, AK, Tan, YP, Mohan, E, 1982: A case of warfarin resistance in *Rattus rattus diardii*. Mardi Res. Bull. 10, 3:378-83.

Lund, M, 1964: Resistance to warfarin in the common rat. Nature 203:778-82.

Müller, E, Keller, A, Fregin, A, Müller, CR, Rost, S, 2014: Confirmation of warfarin resistance of naturally occurring VKORC1 variants by coexpression with coagulation factor IX and in silico protein modeling. BMC. Genet. 15:17-23.

Mikhail, MW, Allam, KAM, 2007: Efficiency of three anticoagulant rodenticides on commensal rodents. J. Egypt. Soc. Parasitol. 37, 2:741-46.

Mihkail, MW, Abdel-Hamid, YM, 2010: Susceptibility of bromadiolone anticoagulant rodenticide two rodent species and its haematological effect. Egypt. J. Egypt. Soc. Parasitol. 40, 1:35-44.

Morgan, DG, Petra, ML, 1979: Variability in response to warfarin in natural populations of the house mouse. Genetics 91, 4:84-8.

Morsy, TA, Micheal, SA, Abou El Seoud, SF, 1981: Leishmaniasis in rodents caught in Suez Canal Zone, Egypt. J. Egypt. Soc. Parasitol. 11: 241-52.

Morsy, TA, Michael, SA, Bassili, WR, Salah, MSM, 1982: Studies of rodents and their zoonotic parasites, particularly *Leishmania*, in Ismailia Governorate, Egypt .J. Egypt. Soc. Parasitol. 12, 2:565-85.

Morsy, TA, Abu El-Ela, R, El-Gozamy, MR, 1988: The commensal rodents and their flea fauna in Alexandria City, Egypt. J. Egypt. Soc. Parasitol. 18, 1:11-26.

Morsy, TA, Ibrahim, BB, Haridy, FM, Rifaat, MA, 2000: *Trichinella* encysted larvae in slaughtered pigs in Cairo (1995-1999). J. Egypt. Soc. Parasitol. 30, 3:753-60.

Pelz, HJ, 2007: Spread of resistance to anticoagulant rodenticides in Germany. Int. J. Pest Manag. 53, 4:299-302.

Rifaat, MN, Mohammed, AM, Shawarby, A A, Arafa, MS, 1969: Incidence and distribution of domestic and commensal rodents in U.A.R. J. Egypt. Pub. Hlth. Assoc. 44, 2:127-46.

Saleh, AMA, Al-Agroudi, MA, Morsy, TA, 2016: Occupational, nosocomial or hospital acquired toxoplasmosis. J. Egypt. Soc. Parasitol. 46, 2:407-18.

Saxena, Y, Kavita Sahni, 2008: Perspectives in Animal Ecology and Reproduction. Edited by V K, Gupta, and Anil, K, Verma, Daya, ISBN: 97881703556325.

Saunders, G R, 1978: Resistance to warfarin in the roof rat in Sidney. NSW, Search. 9: 39-40.

Shoukry, A, Morsy, TA, Abu-Hashish, IA, El-Kady, GA, 1986: Seasonal activities of two commensal rats and flea index in North Sinai, Egypt. J. Egypt. Soc. Parasitol. 16:385-93.

Vein, J, Grandeemange, A, Cosson, JF, Benoit, E, Berny, PJ, 2011: Are water vole resistant to anticoagulant rodenticides following field treatments? Ecotoxicol. 20, 6:1432-41.

Wang, F, Jiang, L, Song, CF, Lu, Juelei, Yao, W, 2014: Resistance of *Rattus norvegicus* to warfarin and bromadiolone. [Chinese] Chin. J. Hyg. Insect. Equip. 20, 4:326-8.

WHO, 1970: Instructions of determining the susceptibility or resistance of rodents to anticoagulant rodenticides: Insecticide resistance and vector control. Tech. Rep. Ser. 443:140-7.

WHO, 1982: Instructions of determining the susceptibility or resistance of rodent to anticoagulant rodenticides**.** WHO/VBC/ 82, 843:1-9.

Zidan, ZH, Sobeiha, GM, Bekheit SS, Mikhail, MW, 1997: Monitoring of resistance of roof and Norway rats to warfarin in some rural areas of Egypt. Arab-Uni. J. Agricul. Sci. 5, 1: 145-55.