INVESTIGATIONS ON THE RRESISTANCE OF NORWAY RATS *(RATTUS NORVEGICUS)* TO ANTICOAGULANT RODENTICIDES IN HUNGARY

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Abstract

Anticoagulant resistant strains of Rattus norvegicus carriyng mutations in a key element (VKORC1) of the blood clotting cascade were regularly reported in the past decades in Western-European countries, but related records from Central and Eastern-Europe are apparently scarce.

In Hungary, a land where anticoagulant rodenticides have been widely used since the mid 1960s, SNP analysis-based resistance studies had been conducted in 2006-2007 and in 2014-2015 on rat corpses which were collected at multiple sites of urban and rural environments.

During the first study in 2006-2007 28 collected rat corpses were studied out of which 11 (39.3 %) proved to be hetero and/or homozygote for Y139C mutation, a mutation at the 139th base position in the 5' end of the VKORC1 3rd exon.

Later in 2014-2015 64 rat corpses were subjected to an SNP analysis and Y139C mutation was demonstrated again in 41 cases (64 %) in hetero- (17) or homozygous (24) form.

These results, however, cannot be regarded as representative since, on the one hand, 79 % of the rat corpses originated from Budapest and, on the other, the corpses were mainly collected at sites where regular rodent control measures were carried out with bromadiolone by qualified PCOs. The later finding might question the view represented by certain registration/licensing authorities, according to which the increasing resistance to anticoagulant rodenticides is due to inadequate rodent control measures performed by amateurs.

1. The history of introduction of anticoagulants

From the mid 60s until the early 80s, first generation anticoagulants (FGARs) – like **warfarin** and **coumatetralyl** and later **coumachlor** and **chlorophacinone** active substances – were in use by amateurs and PCOs in Hungary.

The table below shows the time of registration and introduction in Hungary of the anticoagulant rodenticide active ingredients.

Year	Active ingredient
1958	warfarin
1965	coumatetralyl
1969	chlorophacinone
1977	coumachlor
1978	difenacoum

Year	Active ingredient
1980	brodifacoum
1983	bromadiolone
1992	difethialone
1996	flocoumafen
1997	diphacinone

The registration of the second generation anticoagulant rodenticides started with **difenacoum** in **1978,** but none of the formulations containing this active gained broad practical use for a long time. It is a historical point of interest that – on the European continent – **brodifacoum** was first registered in Hungary in **1980,** and was mainly used for mouse control. The **bromadiolone** active ingredient had been registered in **1983,** and the mouse control product based on this active was put on the market in **1984.** Widespread use of this active ingredient in Hungary started in **1991.**

2. Anticoagulant resistance studies

Anticoagulant resistance studies of synanthropic rodents in Hungary have been carried out in three cycles.

2.1. Studies before 2006

Until **2006,** so-called **no choice feeding tests** had been conducted in the rodent laboratory of Bábolna Bio Ltd. in Budapest on live rats caught by periodic trappings at various locations (Budapest and animal breeding facilities in the countryside). During the tests, the trapped rodents were offered a bait containing 0.05 % (500 ppm) **coumatetralyl** active ingredient for *ad libitum* consumption.

In the course of these resistance studies, all Norway rats (*Rattus norvegicus*) died within 12 days at the very most. Accordingly, we did not observe any sign indicative of resistance. It should nevertheless be noted that such studies were performed in limited numbers only.

2.2. Studies in 2006-2007

Comparative SNP analyses of the VKORC1 locus were conducted by Dr. Hans-Joachim Pelz (Institute for Nematology and Vertebrate Research, Münster, Germany) in collaboration with Bábolna Bio Ltd.

Between March and May **2006 12**, while in March **2007** another **16** tail fragments coming from dead Norway rats were examined in Germany. The dead rodents were collected by co-workers of Bábolna Bio Ltd. and dissected tail fragments were subjected to analysis in Münster.

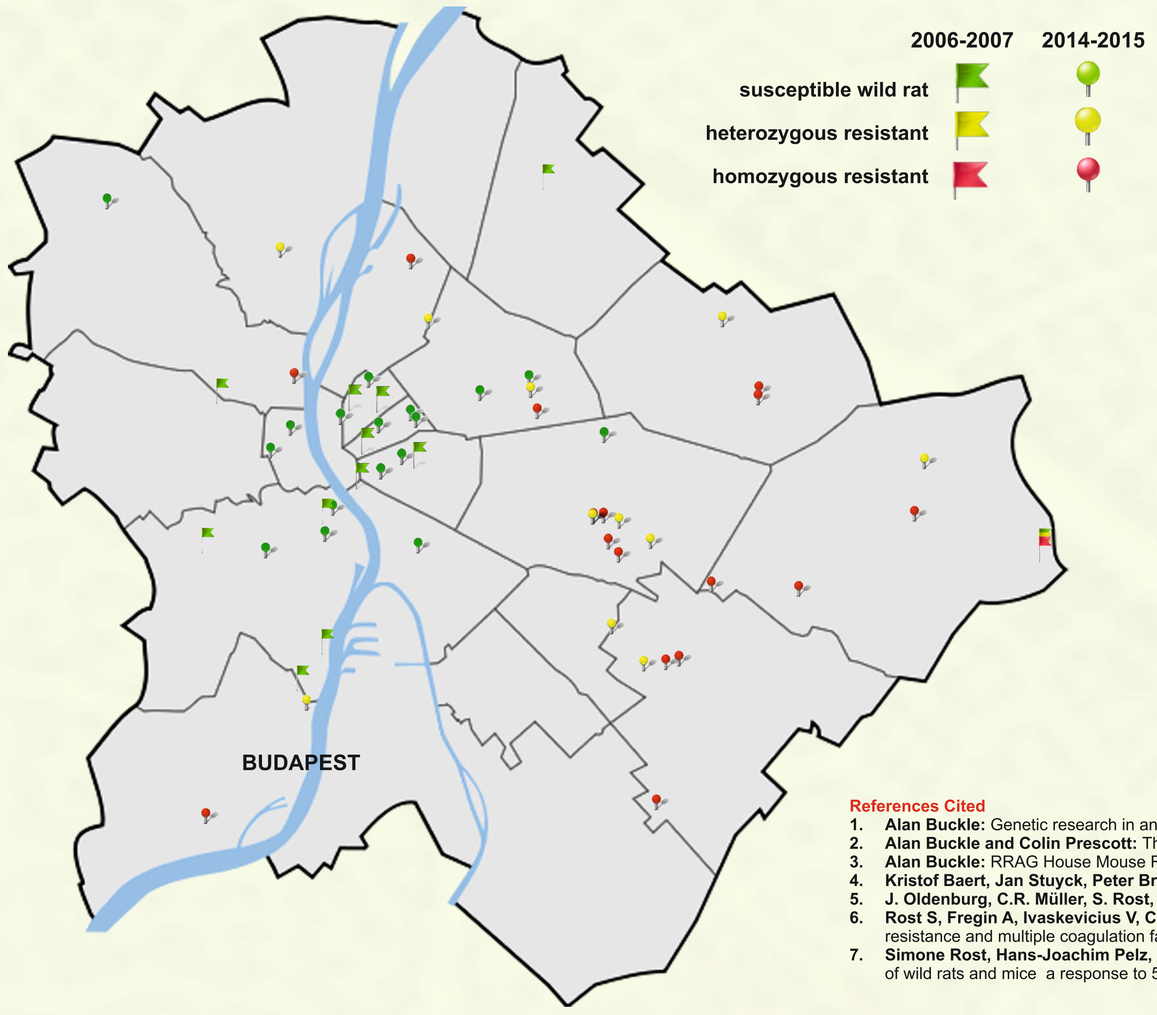
Out of the 12 samples collected in March-May **2006** in Pécel and Maglód, two villages on the outskirts of the capital, Budapest, 3 (25%) showed Y139C mutation, 1 (8.33%) and 2 (16.67%) of the 3 samples were heterozygous and homozygous, respectively. The rat originating from the city of Békés in the south of Hungary was a homozygous mutant. All of the four rats carrying mutant genes were collected from animal breeding facilities. Two samples coming from the western (Bábolna) and southern (Mohács) part of Hungary were wild types.

In March **2007**, **16** samples were collected from Budapest and its immediate surroundings. Seven samples collected from a pig farm in the villages of Pécel/Maglód on the outskirts of Budapest proved to be hetero (4/16; 25%) or homozygous (3/16; 18.75%) Y139C carriers. Out of these mutant specimens, 4 and 3 were heterozygous and homozygous, respectively. None of the other 9 samples coming from various parts of Budapest showed any mutation.

2.3. Studies in 2014-2015

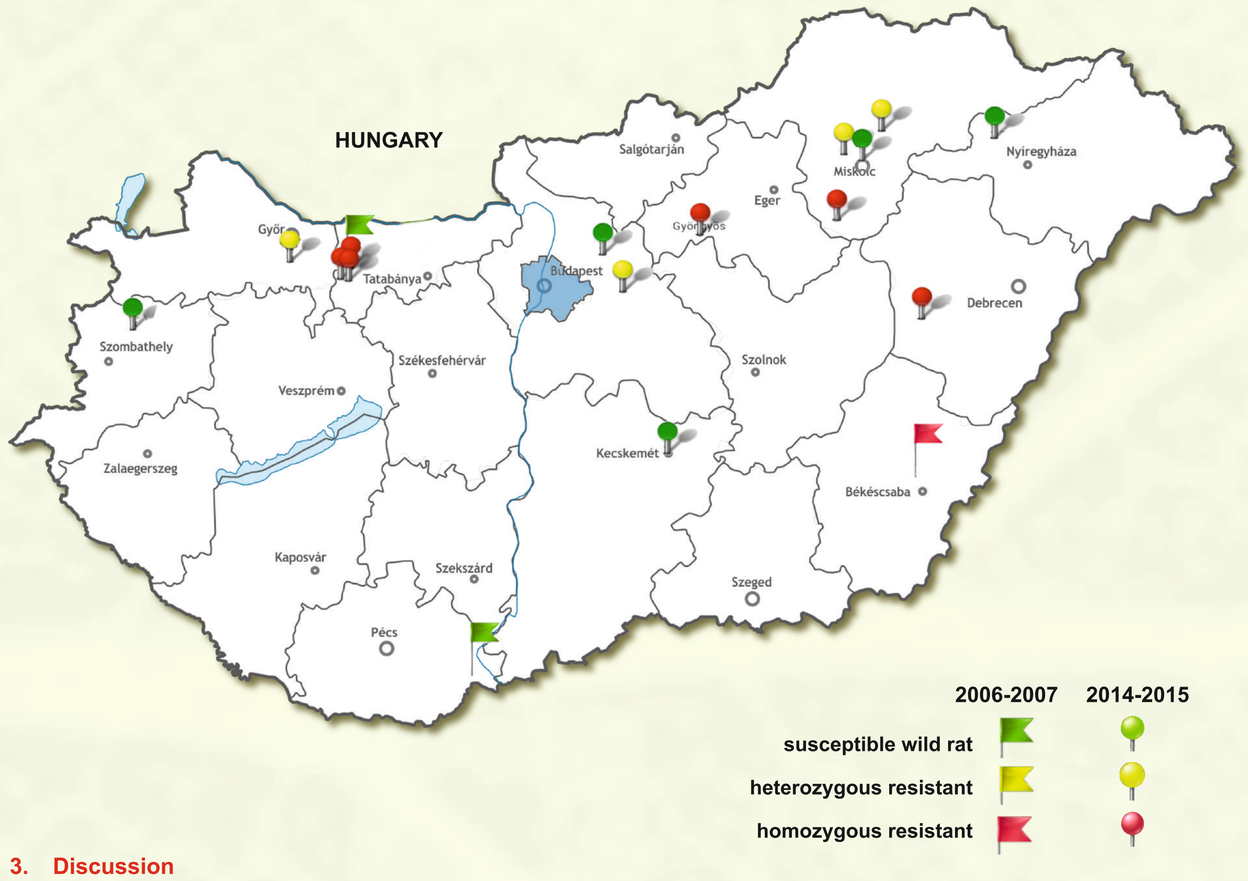
In **2014-2015 64** rat carcasses collected by the pest control operators of Bábolna Bio Ltd. in Budapest (48/64; 75%) and at in countryside agricultural facilities (16/64; 25%) were subjected to comparative SNP analysis of the VKORC1 locus.

DNA extraction of rat tails was performed with QIAmp DNA Mini kit (QIAgen, Hilden, Germany) following the tissue protocol described by the manufacturer. A cca. 300 bp long fragment localized at the '5 end of the VKORC1 third exon was amplified with HotStar Taq HighFidelity polymerase (QIAgen) and with the primer pair mus-rat-3F ('5-CACCTGCTGYCTGTCATT-'3) / mus-rat-3R ('5-ATGTGCYAAGGCAAAGCAA-'3) according to the method and cycle conditions described by Rost et al. 2009. Nucleotide sequence determination of PCR products was performed by Macrogen Inc. (Seoul, South Korea). Comparative analysis of single nucleotide variations was accomplished with CodonCode Aligner 5.0.2. (CodonCode Corp. Centerville, MA, USA).



In 23 (36 %) out of 64 studied specimens wild genotype of the VKORC1 gene was demonstrated, while 41 (64 %) proved to be hetero- (17 specs; 18%) or homozygous (23 specs; 36%) or Y139C mutation carriers.

Out of the 48 samples collected in Budapest 17 (35%) showed susceptible wild type of VKORC1 locus, while 31 (64.58%) specimens proved to carry Y139C mutation as hetero- (13 specs; 27.08%) or homozygous (18 specs; 37.5%). No other known mutations of VKORC1 3rd exon regarded for causing warfarin-resistance in rats were detected.



3. Discussion

The anticoagulant resistance studies carried out in Hungary between 2006 and 2015 in compliance with the genetic method are **non-representative**. In each case, the **92** tissue samples (rat tail) originated from Norway rats. Except for two special cases – one rat killed by a ferret and another caught with a trap –, **the tissue samples originated from rats which were killed with anticoagulant rodenticide**.

Out of the **92** samples **75** (82 %) came from inhabited or industrial (urban) areas. Within the urban area, three typical locations of origin can be distinguished: buildings (mainly basement) – 41.4 %, sewage system (public and within buildings) – 37.3 % and around buildings (mainly burrows) – 21.4 %. **17** (18 %) samples came from animal breeding facilities (mainly pig farms).

Together **73** (79 %) samples were collected from Budapest or its immediate surroundings (Pécel, Maglód), and only **19** (21 %) from other parts of the country.

According to the genetic resistance studies performed in Germany (Münster) and in Budapest, **40** samples (43.5 %) belonged to the susceptible wild type, **52** samples (56.5 %) carried the Y139C mutation; out of the latter group, **22** and **30** happened to be heterozygous and homozygous, respectively. Beside Hungary and Denmark, this resistance mutation is present in Germany, the Netherlands, Belgium and the United Kingdom.

The place of origin and the resistance status of the studied rat carcasses are demonstrated in the two maps.

In Hungary the presence of the Y139C mutation was first demonstrated in a rodenticide resistant rat population in a pig farm on the outskirts of Budapest (Pécel, Maglód) in 2006-2007. This rat population was finally eliminated with brodifacoum based bait. In the light of the later study however, the resistance seems to have rapidly emerged and considerably expanded in Budapest, as the figures of the following table demonstrate.

Year	No. of samples	Y139C carriers		Heterozygous carriers		Homozygous carriers	
	pcs	pcs	%	pcs	%	pcs	%
2006 and 2007	28	11	39.3	5	17.9	6	21.4
2014 and 2015	64	41	64.0	17	26.5	24	37.5
Total	92	52	56.5	22	23.9	30	32.6

The data in the above table show that the rate of anticoagulant resistant rats increased from 39.3 % in 2006-2007 to 64 % in 2014-2015, **but the small number of the samples and their non-representative character in both periods under investigation should be emphasized**.

Out of the examined 92 rat corpses 52 samples were found to be carrier of Y139C mutant chlorophacinone/bromadiolone resistant gene. The trapped rat was carrier of homozygous resistant gene while the rat caught by the ferret was heterozygous carrier of Y139C mutation. The remaining 50 rats carrying resistant gene undoubtedly perished as a result of treatment with bromadiolone based rodenticide!

In 1971-72 an overall deratization action covering the total 525 km2 area of the city was carried out in Budapest. As a result of the rat control action, the initial 33 % rat infestation rate of the premises dropped to less than 0.5 %. To preserve the rat-free conditions, maintenance works started as of 1st January 1973, using coumatetralyl based baits until 2000, and bromadiolone active ingredient thereafter.

Both the deratization action and the maintenance activity had been and are still financed by the city of Budapest and have been carried out by a **professional service company**, Bábolna Bio Ltd. Considering that the costs related to the maintenance of rat-free conditions are borne by the municipality, neither the inhabitants nor the institutions are charged for this activity. As a consequence, the general public has not practically carried out rat control in Budapest for decades.

The data in relation to the emergence of the resistance obtained in Budapest support that rat control performed by the general public does not contribute to the emergence and the spread of anticoagulant resistance.

4. Conclusion

The non-representative genetic study of the rat population in Hungary proved that – like in most Western European countries – rat resistance to warfarin and less potent second generation anticoagulant rodenticides should be reckoned with, since 56.5 % of the tested samples were Y139C carriers. For that very reason, it is especially worthy of note that 50 out of the 52 rats carrying the mutant gene died as a result of control carried out by - according to the current control-regime - bromadiolone active substance. Accordingly it was well documented that at certain locations a combination of higher quantity and longer exposure time for rodenticides was needed to provide appropriate rat control performance.

Having comprehensive knowledge of the location, the type and the degree of anticoagulant resistance is a precondition to the successful protection against rodents. This is why the efficiency of the treatments carried out with first generation anticoagulants (FGARs) should be followed up continuously. In case of unsatisfactory efficiency it seems reasonable to conduct the genetic resistance study as soon as possible and to change over to the use of a more potent second generation anticoagulant rodenticide. The application of the IPM approach in practice is helpful to achieve the satisfactory result.

According to the well documented resistance studies performed in Hungary, the Norway rats become carriers of resistant genes even if rat control over a given area is carried out by highly trained PCOs for decades.

5. Acknowledgement

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