

POOR EFFICACY OF THE MOST COMMONLY USED ANTHELMINTICS IN SPORT HORSE NEMATODES IN MOROCCO IN RELATION TO RESISTANCE

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Summary:

Sport and leisure horses in Morocco are treated with several anthelmintics, organophosphates (dichlorvos), benzimidazoles (mostly thiabendazole) or tetrahydropyrimidines (mostly pyrantel pamoate) against nematodes. We studied three horse stables in Rabat, one in Meknes and one in Bouznika. Two of the Rabat and Bouznika stables had introduced a large number of horses from countries (Argentina or Europe) where resistance to benzimidazoles is frequent, whereas the Meknes stud farm remained without foreign introduction. The number of treatments was not very frequent (twice a year in adult horses) but the same anthelmintics were used repeatedly. No resistance to dichlorvos was detected whereas benzimidazole and pyrantel pamoate resistances were detected for the first time in African horses, outside South Africa.

KEY WORDS : anthelmintic, horse, nematode, Morocco, resistance.

Résumé : EFFICACITÉ RÉDUITE DES ANTHELMINTHIQUES LES PLUS UTILISÉS CHEZ LE CHEVAL AU MAROC EN RELATION AVEC LA RÉSISTANCE

Les chevaux de sport et de loisirs au Maroc sont traités pour les infections par les nématodes avec plusieurs anthelminthiques, des organophosphorés (dichlorvos), des benzimidazoles (principalement le thiabendazole) ou des tétrahydropyrimidines (principalement le pamoate de pyrantel). Nous avons étudié trois écuries à Rabat, une à Meknes et une autre à Bouznika. Deux des écuries de Rabat et celle de Bouznika ont introduit des chevaux en grand nombre d'Argentine ou d'Europe, qui sont des régions avec des résistances très fréquentes, alors que le site de Meknes restait sans introduction. Le nombre de traitements n'était pas très élevé (deux par an chez les adultes), mais les mêmes molécules ont été utilisées de manière répétitive au sein de chaque écurie. Aucune résistance au dichlorvos n'a été enregistrée, mais par contre des résistances aux benzimidazoles ou au pamoate de pyrantel ont été mises en évidence pour la première fois chez des chevaux africains (hors Afrique du Sud).

MOTS CLÉS : anthelminthique, cheval, nématode, Maroc, résistance.

INTRODUCTION

Horses are important in Morocco, as a working or a leisure animal (360.000 heads in 2001). The internal parasitic diseases are frequent in working donkeys and horses of Morocco (Pandey & Dakkak, 1979; Cabaret & Pandey 1980; Pandey & Cabaret, 1993). Horses harbour small and large strongyles, and other nematodes such as *Habronema* sp. or *Trichostrongylus axei*, and insect larvae, *Gasterophilus* spp. (Ouhelli *et al.*, 1979). In Morocco, small strongyles are considered as playing an important role in severe diarrhoea and colitis (Berrag & El Kohen, 1999). Large strongyles were a major cause of diseases in horses and frequent treatments programs were implemented in

order to control infection; it resulted in an increase of small strongyles which were considered as much less pathogenic than large strongyles (Kaplan, 2002). The first reports of thiabendazole resistance in the cyathostomes (= small strongyles) were recorded by Drudge & Lyons, 1965; later, it has been extensively reported in many countries (Lyons *et al.*, 1999). Resistance to pyrantel was reported only in recent years although it has been used since the 1970's, and mostly in places where daily feeding with pyrantel is practised (Kaplan, 2002). Resistance to organophosphate is not documented in horse nematodes. The majority of resistance studies are based on the faecal egg reduction test (FECRT) and Bauer *et al.* (1986) stated that a reduction of less than 80 % after treatment was indicative of resistance. Resistance is then a common feature in horse small strongyles in South Africa (Matthee *et al.*, 2000), USA (Drudge & Elam, 1961; Wood *et al.*, 1998), Brazil (Pereira *et al.*, 1991), Denmark (Bjorn *et al.*, 1991), Australia (Waller, 1993), Belgium (Geerts, 1995) and Ukraine (Borgsteede *et al.*, 1996) among others.

The sport and leisure horses in Morocco benefit from anthelmintic treatments, some of them are imported from countries where prevalence of resistance is high,

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Site	Breeds and origins	Activity	Previous use of the anthelmintics in the last ten years	Tested anthelmintics for efficacy in present study
Raf-Unit 1	English, Argentina, Arab, and Barb thoroughbreds and their crosses (introduction from Europe and Argentina in 1996 and before)	Polo, Escort, Fantasia, Racing competition	Dichlorvos 53 % Thiabendazole 29 % Oxibendazole 18 %	Dichlorvos
Raf-Unit 2	Argentina, Holland, Barb thoroughbreds and their crosses (introduction from Europe and Argentina in 1996 and before)	Polo, Escort, Fantasia, Racing competition	Thiabendazole 68 % Piperazine 11 % Dichlorvos 11 % Oxibendazole 8 % Mebendazole 2 %	Thiabendazole Dichlorvos
El Harka	Arab-Barb, Arab, English thoroughbreds	Riding school	Pyrantel pamoate 60 % Oxibendazole 40 %	Pyrantel pamoate
Bouznika Stud-farm	English and Arab thoroughbreds (massive importation from Europe in 1994)	Exchange of studs between stables	Pyrantel pamoate 50 % Dichlorvos 40 % Oxibendazole 10 %	Pyrantel pamoate
Meknes Stud-farm	Barb and Arab thoroughbred and their crosses (no importation from other countries)	Exchange of studs between stables	Pyrantel pamoate 100 %	Pyrantel pamoate

Table I. – Characteristics of horse stables studied in Morocco.

and they may distribute resistance genes along their movements from one racing place, polo competition, or through movements of studs. According to pro and cons for acquiring resistance (Silvestre *et al.*, 2002) these horses are good candidates for harbouring resistant cyathostomes. The same family of anthelmintics are apparently repeatedly used, which would be a major pro for selection of resistance. We recorded the main anthelmintics used in different leisure or stud horse farms from Morocco and we checked their efficacy using faecal egg counts or in vitro tests and tentatively deduced the resistance status of nematode communities.

MATERIALS AND METHODS

Several sites in Morocco were investigated in Rabat (Raf-1, Raf-2, El Harka), one in Meknes and one in Bouznika (Table I) from 1999 to 2002. Only adult horses were studied.

The efficacy was first evaluated on faecal egg counts (FEC) using McMaster technique (one egg seen = 50 eggs per gram or EPG, Manual of veterinary parasitology laboratory techniques, 1986) and the most used anthelmintic(s) during the last 10 years was (were) tested (Table I). The decrease of the FEC (FECRT faecal egg count reduction test in faeces) was evaluated on day of treatment (arithmetic average T1) and 10-12 days after (arithmetic average T2). The reduction was calculated as $((T1-T2)/T1) \times 100$. According to Sangster, 1996 or Bauer *et al.*, 1986, resistance is suspected when FECRT < 80 % whereas Coles *et al.*, 1992 propose FECRT < 90 %. The faecal individual reduction

(Cabaret & Berrag, 2004) and bootstrap confidence interval were also calculated using the Resivers program (Cabaret *et al.*, 2001). The evaluation of resistance was also done on egg development for benzimidazole (using thiabendazole at various concentrations: Coles *et al.*, 1992) and motility of third stage larvae for pyrantel pamoate based on a similar test provided by Coles *et al.*, 1992, replacing development by motility and thiabendazole by pyrantel pamoate. The evaluation of confidence interval at 95 % of the LD50 (lethal dose on eggs) or ID50 (immobilizing dose of 50 % of larvae) was performed with the probit program (Raymond, 1985).

RESULTS

The number of treatments was twice a year for adults and three times a year for yearlings in all the sites. Nematodes were mostly cyathostomes (more than 80 % of larvae) as evaluated in Raf-2.

Sites	No of horses	Eggs per gram D0	Eggs per gram D10	FECR (%)
Raf-Unit 1	30	945* (0-6,150)**	5 (0-150)	99.5
Raf-Unit 2	8	970 (1,350-6,050)	0	100

* arithmetic average and ** range.

Table II. – Dichlorvos faecal egg count reduction test (FECR 10 days after treatment) in horses from two sites (33 mg/kg body weight) (1999).

. Efficacy of dichlorvos (Table II)

The efficacy of dichlorvos (Equigard®) remained high, and no resistance was evidenced in the two horse stables we investigated.

. Efficacy of pyrantel pamoate (Table III)

The efficacy of pyrantel pamoate (Strongid®) was low (69 or 72 reduction of FEC) and the in vitro test on

motility of larvae (the values were three times higher than in a susceptible community) corroborated that the Meknes stud farm harboured a resistant worm community.

. Efficacy of thiabendazole (Tables IV and V)

The efficacy thiabendazole (Thibenzole®) was low (32-53 % on average value) in the five stables of Raf-2 as

Sites	No of horses	Eggs per gram D0	Eggs per gram D10	FECR (% , average, and individual based estimations and 95 % confidence interval**) (paralysis dosis µg/mL***)
Bouznika	6	175* (50-500)**	8 (0-50)	95* (97: 94-99)** (Not done)
Meknes	10	970 (300-3,200)	300 (0-950)	69 (72: 62-82) (0.95: 0.48-2.02)
Rabat (El Harka)	10	725 (300-2,650)	60 (0-150)	92 (88: 78-98) (0.34: 0.25-0.42)

* arithmetic average and ** range.

Table III. – Faecal egg count reduction test (FECR 10 days after treatment) with pyrantel pamoate in horses from three sites (6.6 mg/kg body weight).

Stable	Eggs per gram D0	Eggs per gram D12	FECR (% , average or individually based-with 95 % confidence interval)
N° 1 (n = 20) Argentina thoroughbreds	1,952* (50-5,500)**	1,140 (0-3,850)	42 37 (19-55)
N° 2 (n = 8) Mostly English thoroughbreds and Anglo-arab crosses	594 (0-1,750)	281 (0-900)	53 64 (45-83)
N° 3 (n = 25) European thoroughbreds	2393 (500-8,850)	1,412 (0-6,950)	41 % 22 (9-41)
N° 4 (n = 20) Arab-Barb crosses	2,640 (500-8,150)	1,808 (450-4,900)	32 % 26 (13-37)
N° 5 (n = 26) Argentina thoroughbreds	2,376 (550-4,900)	1,162 (150-3,850)	51 % 42 (22-59)

* arithmetic average and ** range.

Table IV. – Faecal egg count reduction test after treatment (FECR based on D12, 12 days) in five stables of Raf-2 after thiabendazole treatment using a unique dose of 18.75 g per horse which corresponded to 26-55 mg/kg body weight depending on horse (1999).

Sites	No of horses	Eggs per gram D0	Eggs per gram D10	FECR (% , average or individually based-with 95 % confidence interval) (%)
Raf-Unit 2 50 mg/kg, 1999	10	2,340* (600-10,250)**	1,965 (450-9,500)	16 17 (10-41)
Raf-Unit 2 100 mg/kg, 1999	10	2,815 (900-8,150)	1,430 (0-4,150)	49 43 (20-65)
Raf-Unit 2 100 mg/kg, 2002	10	3,780 (300-8,700)	1,785 (150-440)	53 52 (34-68)

* arithmetic average and ** range.

Table V. – Faecal egg count reduction test (FECR) after treatment with thiabendazole in horses from two sites (50 mg-100 mg/kg body weight) (1999-2002).

the practice is to give the same amount of drug to horses of diverse weights, which correspond to 26-55 mg dosage/kg body weight. The low efficacy was either due to low dosage or resistance. The average reductions at 50 and 100 mg/kg body weight were indicative of resistance. The lethal dose that inhibited the egg development *in vitro* was high in Raf-2 in 1999: 0.42 µg/mL compared to the 0.06 µg/mL of the susceptible El Harka strongyle community.

DISCUSSION

The horse stables we choose were often dedicated to a unique or nearly unique scheme of anthelmintic treatments for the last ten years. Lack of efficacy was then expected due to repeated use of the same anthelmintic and to the repeated introductions of new horses from other countries (Europe or Argentina) where resistance to benzimidazoles is a very common feature. The helminth control practices are not that much different from Europe (O'Meara & Mulcahy, 2002 in Ireland); the worming interval is somewhat larger (twice a year which represents 28 % of parasite control in Ireland) and the drug rotation is lower (rapid-59 % or annual-13 % of horse stables) in Morocco conditions. The intensity of treatments is also lower than that recorded in South Africa (Matthee *et al.*, 2002), five times per year in adult horses; an average of 3.4 different drugs were used annually which is not the case in Morocco (one in most cases or two different drugs per year). Morocco is then a candidate for resistance in stud or leisure horses.

The extent of resistance in horse nematodes throughout the world is recognized, particularly for benzimidazoles (Conder & Campbell, 1995; Kaplan, 2002) which were used from 1960's. Resistance to benzimidazoles is common in horses (Dorny *et al.*, 2000; Fisher *et al.*, 1992; Varady *et al.*, 2000; Woods *et al.*, 1998, and see review by Kaplan, 2002) as it has been used for decades (Uhlinger, 1992). Resistance to benzimidazole in horses cyathostoms is then recorded for the first time in Morocco and Africa (South Africa excluded), which was expected when considering pro and cons for building up of resistance. It might be a case of introduced resistance if we consider the introductions of horses harbouring resistant worms from Argentina (Tolosa *et al.*, 1999) or Europe (see Kaplan for review, 2002). The selective pressure remained low but permanent (the Raf-1 or Raf-2 cases) and it probably helped the diffusion of introduced resistant genes in nematodes. The introduction of resistant worms as a major factor is the most probable hypothesis.

Although pyrantel pamoate has been used since the 1970's, it is only in recent years that reports of resis-

tance are available in United States, Norway and Denmark (in Kaplan, 2002) or Italy (Genchi *et al.*, 1992). The first record of resistance to pyrantel pamoate in Morocco is acknowledged, in a situation where intensive use of the drug and limited introduction of infected horses is the rule. This resistance is probably of local origin due to selective pressure for many years. This again is the first record of resistance to pyrantel pamoate in Africa, outside South Africa.

The repeated use of dichlorvos did not result into selection of resistant nematodes; and no others records worldwide are indicative of resistance.

Resistance of sport or stud horse nematodes (mostly small strongyles) to anthelmintics in Morocco is already present. The small strongyle resistant species should be identified in future. The present investigation was dedicated to horses with a good environment and investigations in ordinary small holder horses should be undertaken, in order to see if resistance remained within sport/leisure horses or invaded common horses and donkeys.

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