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# Risk factor analysis of equine strongyle resistance to anthelmintics

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ABSTRACT

Intestinal strongyles are the most problematic endoparasites of equids as a result of their wide distribution and the spread of resistant isolates throughout the world. While abundant literature can be found on the extent of anthelmintic resistance across continents, empirical knowledge about associated risk factors is missing. This study brought together results from anthelmintic efficacy testing and risk factor analysis to provide evidence-based guidelines in the field. It involved 688 horses from 39 French horse farms and riding schools to both estimate Faecal Egg Count Reduction (FECR) after anthelmintic treatment and to interview farm and riding school managers about their practices. Risk factors associated with reduced anthelmintic efficacy in equine strongyles were estimated across drugs using a marginal modelling approach. Results demonstrated ivermectin efficacy (96.3% ± 14.5% FECR), the inefficacy of fenbendazole (42.8% ± 33.4% FECR) and an intermediate profile for pyrantel (90.3% ± 19.6% FECR). Risk factor analysis provided support to advocate for FEC-based treatment regimens combined with individual anthelmintic dosage and the enforcement of tighter biosecurity around horse introduction. The combination of these measures resulted in a decreased risk of drug resistance (relative risk of 0.57, $p = 0.02$). Premises falling under this typology also relied more on their veterinarians suggesting practitionners play an important role in the sustainability of anthelmintic usage. Similarly, drug resistance risk was halved in premises with frequent pasture rotation and with stocking rate below five horses/ha (relative risk of 0.53, $p < 0.01$). This is the first empirical risk factor analysis for anthelmintic resistance in equids. Our findings should guide the implementation of more sustained strongyle management in the field.

## 1. Introduction

The diversity of helminth species infecting horses is large, and differences in life cycles, epidemiology, pathogenicity and drug susceptibility make it increasingly challenging to define good sustainable parasite control programs. Strongyles remain a major concern. They can be classified into two sub-families, namely Strongylinae (large strongyles) and Cyathostominae known as small strongyles or cyathostomins (Lichtenfels et al., 2008). The large strongyle *Strongylus vulgaris* is associated with a high mortality rate resulting from parasite associated verminous arteritis (Nielsen et al., 2016). This species has been successfully controlled by anthelmintics, and recent reports suggested a putative re-emergence associated with reduced frequency of anthelmintic treatments (Nielsen et al., 2012, 2016). On the contrary, cyathostomins have become a growing concern in the field (Matthews, 2014; Peregrine et al., 2014). This group of nematodes encompasses 40 species that can infect both young and adult horses (Lichtenfels et al., 2008; Corning, 2009). These nematodes have a ubiquitous distribution throughout geo-climatic conditions (Sallé and Cabaret, 2015). Their life cycle is direct and involves encystment of infective larvae into the caeco-colic mucosa of their hosts (Corning, 2009). In heavily infected horses, *en-mass* emergence of these encysted larvae can cause severe clinical pathology characterized by loss of weight, colic, diarrhoea, protein-losing enteropathy (Murphy and Love, 1997; Love et al., 1999). If not appropriately controlled, larval cyathostominosis can be fatal (Love et al., 1999). For instance, an English report of 15 proved or

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suspected clinical cases seen in early 1980's, recorded recovery for only six horses (Giles et al., 1985).

The use of anti-infectious drugs puts pathogen populations under selection pressure that can ultimately lead to the emergence of resistant or multi-drug resistant populations (Kennedy and Read, 2017). Over the past decades, small strongyle populations, like other livestock-infecting parasitic nematodes (Kaplan and Vidyashankar, 2012), have demonstrated a gradual increase in their resistance to available anthelmintics in every part of the world (Matthews, 2014; Peregrine et al., 2014). Under French settings (Traversa et al., 2012) like in other European (Traversa et al., 2007, 2009; Relf et al., 2014) or American countries (Slocombe and de Gannes, 2006; Lyons et al., 2008; Molento et al., 2008; Canever et al., 2013), resistant strongyle populations have been reported for every available class of anthelmintics, namely benzimidazoles, tetrahydropyrimidines or macrocyclic lactones.

Although previous studies have demonstrated how widespread anthelmintics resistance is, critical assessment of associated risk factors and species composition of resistant parasitic populations is still lacking (Nielsen, 2012), thereby preventing the implementation of clear field guidelines. There has been a limited number of reports focusing on factors associated with prevalence of strongyle infection in horses in Germany (Fritzen et al., 2010; Hinney et al., 2011a) or the impact of faeces removal on prevalence in the UK (Corbett et al., 2014). Available studies have considered drenching practices (Lendal et al., 1998; O'Meara and Mulcahy, 2002; Lind et al., 2007; Hinney et al., 2011b; Relf et al., 2012) or estimation of anthelmintic efficacy (Relf et al., 2014; Tzelos et al., 2017). But no attempt has been made to reconcile drenching practices and drug efficacy data. As a consequence, a knowledge gap about putative risk factors and their impacts remains (Nielsen, 2012).

The results reported herein have been gathered as part of survey involving 688 horses from 39 French horse farms and riding schools. At each location, an anthelmintic efficacy test and a questionnaire interview about their practices were performed. From this data, risk factors associated with reduced anthelmintic efficacy in equine strongyles have been estimated across drug classes. The objective of this study was to bring together anthelmintic efficacy testing and risk factor analysis to provide evidence-based guidelines to the field.

## 2. Materials and methods

### 2.1. Farm and riding school sampling

Our study aimed to evaluate drug efficacy for three drug classes and if possible, to have a control group. Therefore, we aimed to build four groups of at least five horses with a minimal faecal egg count (**FEC**) of 150 eggs per gram as recommended by the World Association for the Advancement of Veterinary Parasitology, WAAVP guidelines (Coles et al., 1992). To reach this number of infected individuals, bigger stud farms, i.e. with at least 20 producing mares, were pre-selected from the French Horse and Riding Institute (IFCE) database, in Normandy, Loire Valley, Aquitaine and Burgundy.

Two additional criteria were defined to increase the chance of finding horses with sufficient faecal egg excretion load to undertake FEC reduction test. First, premises with less than 40 horses were discarded. Indeed, FEC is usually over-dispersed and selecting farms with smaller herd sizes would have resulted in smaller treatment group sizes. In Aquitaine however, two farms with slightly smaller herd sizes (25 and 31 horses) were enrolled to replace two previously selected farms where faecal egg counts were too low. Second, last anthelmintic treatment should have been performed three months earlier as this corresponds to the minimal post-moxidectin treatment egg reappearance period (Boersema et al., 1998) advertised on product information (Anonymous, 2016). Flyers explaining the purpose of the project were then sent to pre-selected farms before a phone call was made to each manager to make sure that their premises fulfilled requested criteria (at least 40 horses not drenched in the last three months) and to confirm their willingness to participate. Nineteen stud farms were enrolled, *i.e.* five in Normandy, four in Loire Valley, four in Burgundy and six in Aquitaine. Approximately half of these farms (n = 11) were involved in horse racing (Thorougbreds, Anglo-Arabians, French trotters or other breeds), while the remainder produced leisure ponies (n = 4) or leisure horses (n = 3) or reared dairy mares (n = 2). For each of these, matching riding schools located within each stud farm area were subsequently identified and enrolled for anthelmintic efficacy test, with an additional riding school enrolled in Aquitaine, providing a set of 20 riding schools. This set of matched riding schools was used to investigate putative differences between stud farms and riding schools.

### 2.2. Horse sampling and anthelmintic resistance tests

A first round of faecal sampling was made one week before drenching, to select for individuals with a minimum excretion level of 150 eggs per gram as recommended by the WAAVP guidelines (Coles et al., 1992). Faecal material was stored at 4 °C before being processed for faecal egg counting within 24 h. Based on individual FEC measured, three treatment groups, *i.e.* fenbendazole (Panacur Equine Guard®, Intervet, France), pyrantel (Strongid®, Zoetis, France), or ivermectin (Eqvalan pâte, Merial, France), balanced for FEC were built. To do so, individuals were sorted according to their FEC and the 15 (or 20 if a control group was available) horses with highest FEC were sequentially allocated to each treatment group. A Kruskal-Wallis test was run to ensure that average FECs were balanced across groups within each farm. A control group was built in every farm where additional horses with minimal excretion level were available. On day 0, each horse was weighed using a girth tape (Buckeye® Nutrition) and orally administered an anthelmintic dose following manufacturer's requirements. Treatment was administered by the veterinarians enrolled as part of this project.

Faecal material was subsequently taken from each horse 14 days after treatment. Every ivermectin-treated individual still present on the premise 30 days after drenching (n = 157 out of 159) was sampled again to identify cases of shortened egg-reappearance period. This short time interval was chosen to minimize disturbance with activities on the premises (horse sales or movements).

### 2.3. Processing of faecal material

FEC were measured by sampling 5 g of faecal material for each individual horse, subsequently diluted and thoroughly mixed into 75 mL of a NaCl solution (density of 1.18). Prepared solution was loaded on a McMaster slide and strongyle eggs were counted with a sensitivity of 15 eggs per gram.

### 2.4. Questionnaire survey and variable definition

A questionnaire, built upon previous published surveys (Fritzen et al., 2010; Maddox et al., 2012), was used to interview each manager as part of a larger survey on antibiotic and anthelmintic resistance. The anthelmintic-associated questions fell into four categories that addressed global premise overview, available pasture and management, horse health management, and drenching strategy.

For statistical inference, a few variable levels were redefined to avoid redundancy and to provide the analysis with more statistical power. Therefore, one farm that did not apply systematic drenching upon horse arrival was considered as not drenching any horse upon arrival. Rotation between pastures was recoded as occurring either never, or more (frequent) or less (rare) than every 3 months.

In addition, pasture strategies either involved own private pastures dedicated to horses or alternative strategies that included co-grazing with cattle, or access to pastures shared between several breeders.