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## Research Note

### Treatment failure of ivermectin for *Oxyuris equi* in naturally infected ponies in Czech Republic

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#### Summary

Nine ponies (20 months of age) with naturally acquired cyathostome and pinworm infections were treated orally with ivermectin at a dosage of 400 µg/kg. Ivermectin was highly effective on the cyathostome infection. However, adult *Oxyuris equi* were present in six horses. In recent years, anthelmintic treatment with macrocyclic lactones have not appeared to deliver the expected efficacy against equine pinworms (*Oxyuris equi*) in the USA. This is the first European study to demonstrate anthelmintic resistance in *Oxyuris equi* to macrocyclic lactones in naturally infected ponies (Czech Republic).

#### Introduction

Parasite resistance to anthelmintic medication is of great concern to the anthelmintic industry and to horse owners. Cyathostome resistance to benzimidazoles and pyrantel salts has been documented worldwide (Brazik *et al.*, 2006, Slocombe & Gannes, 2006, Traversa *et al.*, 2007, 2009). Ivermectin resistance in horse cyathostomes has also been described (Canever *et al.* 2013; Lyons *et al.*, 2013, Traversa *et al.*, 2012). The first recorded incidence of resistance in cyathostomes in the Czech Republic was reported in studies by Chroust (2000) and Langrova *et al.* (2002), and in Slovakia by Várady *et al.* (2000), Konigova *et al.* (2001), Corba *et al.* (2002).

Non-strongylid parasite resistance in horses is rarely reported. Since 2002, selected populations of *Parascaris equorum* in several countries have been reported to survive treatment with macrocyclic lactone anthelmintics (Boersema *et al.*, 2002; Hearn & Peregrine, 2003; Von Samson-Himmelstjerna, 2012). The equine pinworm (*Oxyuris*

*equi*) is a worldwide distributed horse parasite, which usually occurs in farms that implement below standard zoo-hygiene practices. Adult pinworms live in the large intestines (colon a caecum) and rectums of horses. Females migrate to the anus and lay their eggs in cement-like masses in the skin of the perineal region (each mass consists of a thick yellow or gray fluid containing up to 60,000 eggs). The affected horse rubs its tail on any stationary object and severe rubbing may cause irritation and secondary infection of the anus, tail, and surrounding skin. Severe infections with third and fourth stage larvae can produce inflammation of the cecal and slonic mucosa, resulting in mild colic symptoms (Briggs *et al.*, 2004; Stoltenow & Purdy, 2003; Urquhart *et al.*, 1996).

Benzimidazoles, macrocyclic lactones and pyrantel salts have all demonstrated activity against *Oxyuris equi* (Reinemeyer, 2012). However, in the USA, Reinemeyer *et al.* (2006), Reinemeyer (2012) and Lyons *et al.* (2009) observed that pinworm infections persist despite repeated treatments with anthelmintics dosed at higher levels than directed on the label.

The main goal of the current investigation was to assess the efficacy of double doses of ivermectin administered orally to ponies naturally infected with cyathostome and pinworm infections.

#### Materials and methods

Nine ponies (males, aged 20 months at the time of infection) with naturally acquired cyathostome and pinworm infections were used in this study. All animals were weighed and dewormed with a double dose (400 µg/kg IVM) of

Table 1. Distribution of the adult pinworms

Section	Pony									<b>Total <i>O. equi</i> in section</b>
	P1	P2	P3	P4	P5	P6	P7	P8	P9	
<b>Colon</b>	0	0	0	0	1	0	4	6	6	<b>17</b>
<b>Caecum</b>	5	0	0	0	0	3	0	0	0	<b>8</b>
<b>Total <i>O. equi</i> in host</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>3</b>	<b>4</b>	<b>6</b>	<b>6</b>	<b>25</b>

Noromectin. Horses were housed individually in stalls in order to avoid natural parasite infection. Each stall flooring was bedded with shavings. Horses were given hay and grain, and drinking water was provided ad libitum in buckets and was replenished twice daily. Feces were removed daily, and stall bedding was replaced every three days.

Horses were slaughtered 4 weeks post treatment. Immediately after slaughter, the entire contents of the large intestine of each animal (colon, caecum) were examined and washed. The mucosal scrapings of both the abomasum and intestines were digested at 39 °C in HCl-pepsin for 4 hrs (Langrova & Jankovska, 2004). The intestinal contents and digested material collected from each animal were kept in Barbagall solution (7.5 g NaCl; 30 ml formaldehyd; 1000 ml dd H<sub>2</sub>O) and later observed under a microscope.

All of the experimental procedures were conducted in accordance with Czech legislation (section 29 of Act No. 246/1992 Coll., on protection of animals against cruelty, as amended by Act No. 77/2004 Coll.). We hereby declare that animal handling during research complies with relevant European and international guidelines on animal welfare, namely Directive 2010/63/EU on the protection of animals used for scientific purposes, and the guidelines and recommendations of the Federation of Laboratory Animal Science Associations.

## Results

Nine naturally infected ponies received doses of ivermectin that provided 200 % of the target dosage. No adverse health events were noted subsequent to treatment. In the present study, ivermectin demonstrated 100 % efficacy against cyathostomes. However, six of the nine ponies treated yielded a total of 25 adult pinworms from both sections of the large intestine (colon and caecum). Two ponies passed six adults pinworms each, and each of the remaining four ponies yielded five, four, three and one pinworm, respectively. In four animals, parasites were found in the colon only, and pinworms were observed exclusively in the caecum of the remaining two ponies. Distribution of the adult pinworms recovered from different parts of the large intestines of the ponies is shown in Tab. 1.

## Discussion

Benzimidazoles, pyrantel salts and macrocyclic lactones have all demonstrated the high effectiveness against pin-

worms. All of these preparations were tested in naturally infected horses and displayed various results (Reinemeyer, 2012). However, Reinemeyer *et al.* (2006), Reinemeyer (2012) and Lyons *et al.* (2009) have reported cases from the USA, in which anthelmintic treatment did not appear to deliver the expected efficacy against equine pinworms (*Oxyuris equi*). It is still unknown whether the survival of some pinworm specimens is due to resistance or to incomplete efficacy. However, it is apparent that M/L treatments may not achieve total removal. The efficacy of the benzimidazole and pyrimidine classes against *Oxyuris* is not known to have diminished, but it should be recognized that their activity level has always been less than 100 % (Reinemeyer, 2012). There is also a possibility that biological changes in *Oxyuris equi* take place. One potential change in the biology of *O. equi* is the shortening of the prepatent period; another possibility may be the expanded age distribution among hosts infected with adult pinworms (Reinemeyer, 2012).

In recent years, some studies in the USA have reported failures of anthelmintic treatments in removing adult pinworm (*Oxyuris equi*) infections. Our study is the first in Europe to observe that pinworm infections (*Oxyuris equi*) persist despite treatments with a ivermectin dosed at higher levels than directed on the label.

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