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FIRST REPORT OF RESISTANCE TO IVERMECTIN IN *PARASCARIS UNIVALENS* IN ICELAND

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KEY WORDS

Parascaris
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ABSTRACT

Horses in Iceland have been isolated for more than 1,000 yr but still harbor a similar range of gastrointestinal parasites as do horses across the world. The long isolation of the horses and their parasites presumably means that no resistance genes have been introduced into the *Parascaris* spp. population. It is therefore of particular interest to investigate the efficacy of ivermectin on *Parascaris* spp. infecting Icelandic foals. Potential treatment failure of ivermectin in Iceland will add substantial new information on how resistance can arise independently. This study aimed to determine the efficacy of subcutaneous injection of ivermectin for the treatment of *Parascaris* spp. infection in foals and to identify the *Parascaris* species present in the west and north of Iceland. A fecal egg count reduction (FECR) test (FECRT) was performed on 50 foals from 8 farms, including an untreated control group of 6 foals, from September to November 2019. The foals were between 3 and 5 mo of age at the start of the study and had not previously been treated with anthelmintic drugs. Each foal was treated subcutaneously with off-label use of Ivomec® injection 10 mg/ml or Noromectin® 1% at a dose of 0.2 mg/kg. The FECR for each farm was calculated in 2 ways, by the eggCounts package in R and by the Presidente formula (FECRT). Both calculation methods resulted in efficacy levels between 0% and 80.78%, indicating ivermectin resistance on all farms. We also confirmed, by karyotyping, that the species of equine ascarid present in the west and north of Iceland is *Parascaris univalens*. This study provides evidence for treatment failure of ivermectin against *P. univalens* infection in foals. Since Icelandic horses have been isolated on the island for more than 1,000 yr, this implies that resistance alleles have developed independently in the Icelandic *Parascaris* population. The actual clinical impact of ivermectin resistance is unknown but another drug of choice should be considered to treat *Parascaris* infection in foals in Iceland.

The Icelandic horse is the only breed of horse in Iceland. The horse population was introduced by settlers in the ninth and 10th centuries and has been isolated for approximately 1,000 yr (Adalsteinsson, 1981). Despite the isolation, Icelandic horses have been found to harbor a similar diversity of equine gastrointestinal helminths commonly infecting horses in other countries of similar climates, such as small and large strongyles, ascarids, and cestodes (Eydal, 1983).

Equine nematodes in the family Ascarididae, *Parascaris* spp. are pathogenic parasites of foals and yearlings worldwide and are also commonly found in foals in Iceland (Eydal, 1983). The genus *Parascaris* contains 2 species, *Parascaris equorum,* and *Parascaris univalens*, which are morphologically identical but can be distinguished by karyotyping, as *P. equorum* has 2 pairs of chromosomes and *P. univalens* has 1 pair (Goday and Pimpinelli, 1986). Recent studies from North America and Europe have shown that *P. univalens* is the dominating species infecting horses (Jabbar et al., 2014; Nielsen et al., 2014; Martin et al., 2018).

Clinical signs of *Parascaris* spp. infection such as nasal discharge and coughing may be observed during the larval migration. The adult parasites present in the small intestine may cause weight loss and impaired growth, whereas large burdens can lead to obstruction and perforation of the intestine (Cribb et al., 2006). *Parascaris* eggs are shed in the foals' feces and can stay infective for several years, generating a high infection pressure on stud farms with many foals (Lindgren et al., 2008). Because of the potential severity of the infection, most foals are dewormed several times during their first year (ESCCAP, 2019).

Overuse of anthelmintic drugs has led to the development of anthelmintic resistance in several parasites of veterinary importance (Reinemeyer, 2009). In 2002 the first case of *Parascaris* spp.

resistance to the anthelmintic drug ivermectin was reported from the Netherlands (Boersema et al., 2002), followed by reports from other European countries (Stoneham and Coles, 2006; Schougaard and Nielsen, 2007; von Samson-Himmelstjerna et al., 2007; Lindgren et al., 2008), North America (Lyons et al., 2008), and Australia (Armstrong et al., 2014). Reports also show treatment failure of pyrantel in North America, Europe, and Australia (Lyons et al., 2008; Armstrong et al., 2014; Martin et al., 2018) and, more recently, of fenbendazole in Australia and Saudi Arabia (Armstrong et al., 2014; Alanazi et al., 2017).

In Iceland, mares and young horses are usually kept outside throughout the year and have access to large areas of uncultivated land for grazing, which would presumably reduce the infection pressure of parasites. Many breeding farms leave the foals relatively unhandled, allowing them to roam the highlands with the herd during their first summer. Therefore the off-label use of subcutaneous-injection ivermectin (such as Ivomec® vet 10 mg/ml and Noromectin® 1%) is preferred as an antiparasitic treatment rather than oral paste formulations registered for horses (Paulrud et al., 1997). The efficacy of both parenterally and orally administrated ivermectin showed a 100% elimination of adult *Parascaris* spp. worms at the recommended dose of 0.2 mg/kg when the drug was introduced on the market (Campbell et al., 1989).

The long isolation of the horses and their parasites presumably means that no resistance genes have been introduced into the *Parascaris* spp. population. It is therefore of particular interest to investigate the efficacy of ivermectin on *Parascaris* spp. infecting Icelandic foals. Potential treatment failure of ivermectin in Iceland will add substantial new information on how resistance can arise independently. This study aimed to determine the efficacy of subcutaneous injection of ivermectin for the treatment of *Parascaris* spp. infection in foals and to identify the *Parascaris* species present in the west and north of Iceland.

MATERIALS AND METHODS

Ethical permission

The study was conducted following the Icelandic Animal Care Guidelines for experimental animals (the Icelandic Animal Welfare Act 55/2013) and the Icelandic regulation on the protection of animals used for scientific purposes 460/2017, and formally approved by the Icelandic Ethical Committee on Animal Research, license 2019-04-10.

Farms and horses

The study was performed on farms in the west and north of Iceland from September to November 2019. A total of 85 foals from 10 farms was screened for the presence of *Parascaris* spp. eggs. Only farms with a minimum of 4 foals excreting $\geq$100 *Parascaris* spp. eggs per gram (EPG) were included in the fecal egg count reduction test (FECRT). A group of 6 foals from one of the farms served as a control for normal variation in *Parascaris* spp. EPG and was not treated with any anthelmintic drug. All foals included in the study were between 3 and 5 mo of age at the start of the study, had not previously been treated with anthelmintic drugs, and were with their dams throughout the observation period.

Table I. Questionnaire to farmers.

Information	Descriptor
Number of horses on the farm	Number
Number of foals	Number
Access to separate winter and summer pasture	Yes/no
Sharing summer pasture with other farms	Yes/no
Grazing with other animal species	Yes, species/no
Segregation management of horse groups on pasture	Yes, age, gender, other groups/no
Deworming routines applied on the farm	(1) Only after examination of fecal samples (2) Routine deworming once/yr (3) Routine deworming 2–4 times/yr
Number of treatments and time of deworming	Number/month

Anthelmintic treatment

Each foal and the corresponding dam (except the control group) were treated with the recommended dosage (0.2 mg/kg) of Ivomec vet 10 mg/ml (Boehringer Ingelheim, Ingelheim, Germany) (farms 1, 2, 3, 6, 7, and 8) or Noromectin 1% (Norbrook Laboratories, Newry, U.K.) (farms 4 and 5). The veterinarian in charge estimated the weight of the foals to be 150–200 kg and a dose was calculated for 200-kg body weight, resulting in a subcutaneous injection of 4 ml for each foal. The dams were treated with 8 ml of the same drug.

Fecal egg count reduction test

Individual fecal samples were collected from the ground or the rectum of the foals, placed in plastic bags, and kept cold during transportation to the laboratory (Institute for Experimental Pathology at Keldur, University of Iceland, Reykjavík, Iceland). Fecal egg counts (FECs) were performed using a McMaster technique slightly modified from Roepstorff and Nansen (1998), where 4 g of feces were suspended in 56 ml of tap water, mixed thoroughly, and then sieved through a mesh with an aperture of 0.8 mm. The strained fluid was centrifuged for 5 min at 500 *g*, the supernatant discarded, and the pellet mixed in FASOL® reagent (Kruuse, Langeskov, Denmark). Two McMaster chambers were filled (0.5 ml/chamber) and counted, including the area around the grid, resulting in a minimum detection limit of 7.5 EPG. Paired FEC samples were analyzed on day 0 (pretreatment) and days 11–15 posttreatment. The follow-up samples were collected from the control group on day 20.

The farm owners answered questions regarding farm size, pasture management, and deworming routines practiced (Table I).

Data analysis

The FECRs for each farm were calculated in 2 ways: (1) data were analyzed in R version 3.6.3 with the eggCounts package version 2.3 using a Bayesian model for paired design (fecr_stan) (R Core Team, 2018; Wang and Paul, 2018); (2) FECR was calculated according to the Presidente formula (Presidente, 1985):

Table II. Descriptive data on farms included in the fecal egg count reduction test.

Farm	Region*	Group size	Individual number	Age (months)	Group mean ± SD EPG pre†	Group mean ± SD EPG post†	Max. individual EPG pre†	Max. individual EPG post†
1	N	7	1-7	4-5	578 ± 348	641 ± 524	1,290.0	1,725.0
2	N	9	8-16	4-5	1,370 ± 705	908 ± 500	2,535.0	1,395.0
3	N	4	17-20	4-5	2,987 ± 758	3,651 ± 4,145	3,735.0	8,182.5
4	N	6	21-26	3-5	478 ± 258	738 ± 499	810.0	1,582.5
5	N	4	27-30	4	488 ± 660	94 ± 111	1,477.5	217.5
6	W	4	31-34	3-5	939 ± 504	889 ± 1,241	1,687.5	2,640.0
7	N	5	35-39	4-5	708 ± 443	309 ± 341	1,140.0	847.5
8	W	5	40-44	4-5	905 ± 407	1,107 ± 645	1,575.0	1,942.5
‡ C	N	6	45-50	4-5	823 ± 685	548 ± 556	1,762.5	1,582.5

* N = north Iceland, W = west Iceland.
† Pre- and posttreatment egg counts.
‡ C, untreated control foals on farm 1.

$$\mathrm{FECRT} = 100 \times (1 - [T_{\mathrm{post}}/T_{\mathrm{pre}}] \times [C_{\mathrm{pre}}/C_{\mathrm{post}}]),$$

where T_{pre} is the pretreatment egg count arithmetic group mean (AGM) of the treated group, T_{post} the posttreatment egg count AGM of the treated group, and C_{pre} and C_{post} are the pre- and posttreatment AGMs of the untreated control group. It should be noted that the control group only contained foals from farm 1.

Results were interpreted according to the guidelines regarding FECRT of strongyle nematodes issued by the American Association of Equine Practitioners (Nielsen et al., 2019), as there are currently no guidelines available for *Parascaris* spp. According to these guidelines, the expected efficacy for ivermectin should be 99.9% and an efficacy below 95% is regarded as confirmation of resistance.

Karyotyping

To determine the *Parascaris* species of roundworm present in west and north Iceland, karyotyping was performed on *Parascaris* spp. eggs. Fecal samples for karyotyping were collected from 1 farm in the north and 1 in the west of Iceland. Karyotyping was performed on approximately 1,000 eggs from each of the 2 farms as described by Martin et al. (2018).

RESULTS

Fecal egg count reduction test

Eight of 10 farms fulfilled the inclusion criteria with a minimum of 4 foals/farm excreting ≥100 *Parascaris* EPG (Table II). In total, 44 treated foals and 6 control foals were included in the FECRT. Among the 44 foals that received treatment, 24 (55%) had an increase in egg excretion at the second FEC and only 4 (9%) had an egg count of 0 after treatment (Fig. 1).

Calculated FECR using eggCounts package in R for all farms including the control group are shown in Table III. None of the investigated farms showed the expected efficacy of the ivermectin treatment (99.9%). Farms 1, 3, 4, 6, and 8 showed very poor efficacy, with a reduction of *P. univalens* eggs of <6.0%, whereas a slightly better efficacy with a reduction between 33.64% and 80.78% was noted on farms 2, 5, and 7 (Table III). The results of decreased efficacy were supported by lower confidence levels of less than 90% in all cases. The normal variation of egg excretion displayed by the control group of 6 foals showed a 33.24% reduction of *Parascaris* EPG at the second sampling (Table III). Calculated FECR using the Presidente formula including the control group in the calculation are displayed in Table III. Overall the FECRT revealed an even lower efficacy of ivermectin treatment that is explained by a lower mean FEC of the control group compared with mean FEC at farms 1, 3, 4, 5, and 8 at the second sampling and thus resulting in negative reduction values.

Deworming routines practiced on the farms

Deworming routines and parasite management practices used on the farms are displayed in Table IV. All farmers dewormed their foals and horses on a routine basis once or twice yearly without prior parasite diagnostic tests. All farms treated their foals in the autumn, and at some farms, a second treatment was added the following spring. All farms had large areas for grazing and had access to separate summer and winter pastures. Many of the farms shared summer pastures for mares, foals, and young horses with other farms in the same region. Three farms applied mixed grazing with sheep when possible. All farms separated the horses in different pastures on the basis of age, gender, and use of the horses.

Karyotype

Karyotypes were successfully obtained from *Parascaris* eggs. A single pair of chromosomes was detected in the samples from both farms, confirming the species to be *P. univalens*.

DISCUSSION

The main purpose of this study was to investigate the efficacy of subcutaneous injection of ivermectin of Icelandic foals naturally infected with *P. univalens*. The FECR calculated by the eggCounts package in R (Wang and Paul, 2018) showed treatment failure of ivermectin on all of the investigated farms, with efficacy of less than 6% on 5 farms and between 33.64% and 80.78% on the 3 remaining farms. It should be mentioned that the number of foals in our study was small. In total 85 foals were screened for *Parascaris* spp. eggs but of these only 50 individuals were excreting ≥100 EPG, which was the inclusion criteria for the FECRT. Of these 50 individuals, 6 foals from 1 farm were included in a control group and left untreated. It has recently been

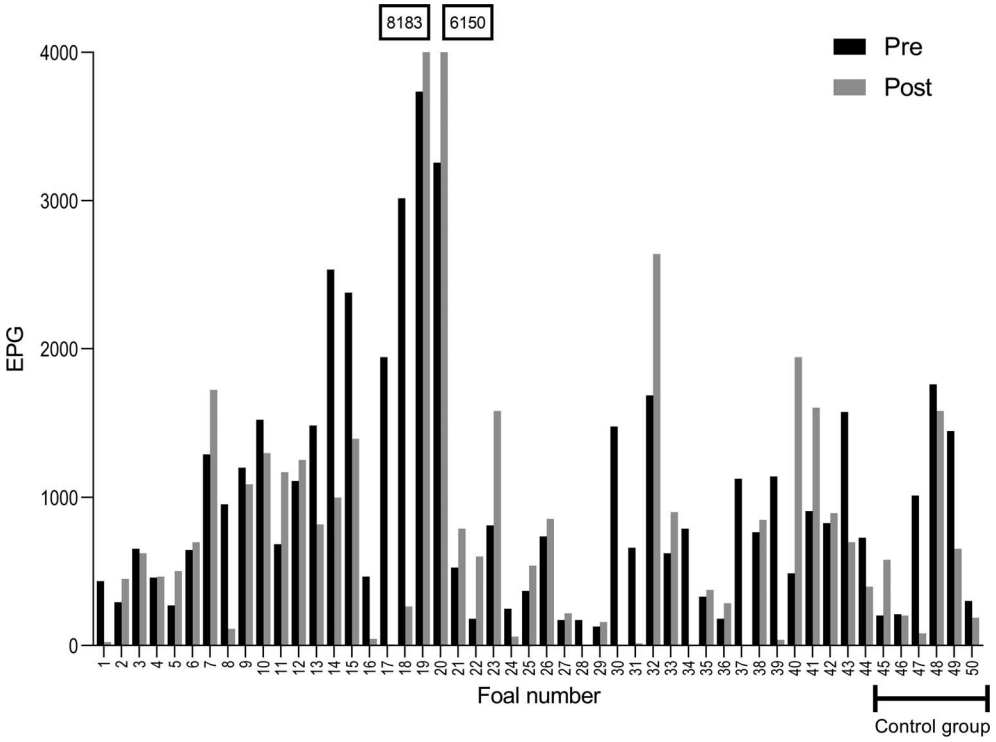

Figure 1. Individual pre- (black bars) and post- (gray bars) treatment fecal egg counts for *Parascaris univalens*. Foal numbers 1–44 were treated by subcutaneous injection of ivermectin, whereas foal numbers 45–50 were included in an untreated control group. For information on the farms to which the individual foals belong, see Table II.

recommended by Morris et al. (2019) to include an untreated control group for the evaluation of treatment efficacy against *Parascaris* spp. to account for natural changes in egg excretion. However, the mean FEC in our control group was reduced at the second sampling compared with an increase of mean FEC of the foals that received treatment on 5 farms. This resulted in an even lower efficacy of ivermectin treatment using the FECR (Presidente, 1985) compared with when calculated by the eggCounts package in R. Taken together, both FECR methods reveal poor treatment efficacy of subcutaneous injection of ivermectin against *P. univalens* in Icelandic foals. Even though resistance to ivermectin in *Parascaris* spp. is widespread across the world (Boersema et al., 2002; Lyons et al., 2008; Armstrong et al., 2014; Cooper et al., 2020), the low efficacy of ivermectin in Iceland was somewhat surprising since neither farmers nor veterinarians included in this study have observed any severe clinical signs of *Parascaris* spp. infection.

Table III. *Parascaris univalens* egg reduction on each farm using 2 different methods of calculating the fecal egg count reduction (FECR).

	FECR %	
Farm	FECR (R)*	FECR (P)†
1	0.11	−66.67
2	33.64	0.39
3	0.02	−83.61
4	0.03	−132.05
5	80.78	71.12
6	5.98	−42.11
7	56.35	34.43
8	0.05	−83.86
C‡	33.24	—

* FECR calculated by the R-package "eggCounts".
† FECR calculated by the Presidente formula: $\text{FECRT} = 100 \times (1 - [T_{post}/T_{pre}] \times (C_{pre}/C_{post}])$.
‡ C, untreated control foals on farm 1.

Because of the restriction of the importation of horses into Iceland since well before the introduction of anthelmintic drugs on the market, we can assume that no ivermectin resistance alleles have been imported. According to Doyle and Cotton (2019), resistance-conferring alleles are believed to arise de novo in a population and then be selected from a low starting allele frequency in response to drug treatment. Despite the less intense use of anthelmintic drugs in Iceland compared with other countries (Eydal and Gunnarsson, 1994), resistance alleles seem to have been favored and are now inherited in the *P. univalens* population. Since foals from different farms roam the highlands with their herds during the grazing season, extensive transmission of resistance alleles between geographically distant farms can occur. This study shows that the spread of an ivermectin-resistant phenotype, and thereby most likely resistance alleles, occurs in the Icelandic *P. univalens* population.

Foals in Iceland are normally dewormed with a single treatment in the autumn, in comparison with more intense recommendations in other countries, with 2–3 deworming occasions during the first year (ESCCAP, 2019). According to a simulation model developed by Leathwick et al. (2017), the timing of a single anthelmintic treatment against *Parascaris* spp. has considerable influence on the development of resistance, which arises more rapidly when foals receive a single treatment at 3–4 mo of age rather than earlier or later. It could thus be speculated that the single treatment in the autumn, often at the age of 3–4 mo, might have driven the development of resistance since it has given an advantage to resistant-genotype worms to survive treatment and continue passing eggs into the environment.

Table IV. Parasite management and deworming routines.

Farm	Number of horses	Number of foals	Separate summer/winter pasture	Sharing summer pasture with other farms	Mixed grazing with sheep	Deworming routines applied	Time of deworming
1	120	47	Yes	Yes	Yes	Routinely once/yr	Oct–Nov
2	130	13	Yes	Yes	Yes	Routinely once/yr	Sep–Nov
3	45	4	Yes	No	No	Routinely once/yr	Oct
4	80	9	Yes	Yes	No	Routinely twice/yr	May and Oct
5	110	9	Yes	Yes	No	Routinely once/yr	Oct
6	40	9	Yes	No	No	Routinely twice/yr	Apr and Oct–Nov
7	100	12	Yes	Yes	No	Routinely once/yr	Oct
8	80	7	Yes	Yes	Yes	Routinely twice/yr	Apr and Oct
C *	See farm 1						

* C, untreated control foals on farm 1.

In this study we evaluated the efficacy of subcutaneous injection of ivermectin, since this formula is widely used for off-label treatment of horses in Iceland rather than the oral paste (Paulrud et al., 1997). When ivermectin was approved for use in horses in the 1980s it was initially introduced as a sterile solution for intramuscular administration, showing a 100% elimination of adult *Parascaris* spp. worms and 98.5% elimination of immature worms at doses of 0.2 and 0.3 mg/kg. However, the parenteral formula was later withdrawn after association with adverse reactions such as inflammation and infections with *Clostridium* spp. at the injection site (Yazwinski et al., 1982; Campbell et al., 1989). Several studies have observed that the route of administration considerably affects the deposition of ivermectin, where parenteral administration has a more prolonged availability and persistent concentration compared with oral administration of paste (Marriner et al., 1987; Perez et al., 2002; Saumell et al., 2017). However, Saumell et al. (2017) noted a lower efficacy against small strongyles after intramuscular injection in comparison with oral paste, suggesting that worms located in the lumen of the large intestine receive a higher drug concentration after oral administration. The foals in this study likely received some additional ivermectin through the oral route since the mares were treated the same day and macrocyclic lactones are excreted to some extent in the milk (Campillo et al., 2013; Gokbulut et al., 2013). Thus, the parenteral administration of ivermectin in this study should not be the reason for the low efficacy of the drug.

We have also shown that the ascarid species infecting horses in the northern and western parts of Iceland is *P. univalens*. This is in accordance with previous work where *P. univalens* has been detected by cytological tests in the USA (Nielsen et al., 2014), Switzerland (Jabbar et al., 2014), and Sweden (Martin et al., 2018), as well as the south of Iceland (Martin et al., 2020). Since the importation of horses has been officially prohibited for more than 100 yr and there is no evidence of importation since the settlement of the island, it is likely that *P. univalens* has been the major species infecting Icelandic horses for a long time. This is further supported by a population study based on genetic mapping of *Parascaris* spp. from 6 countries, including Iceland, showing that the global population of *Parascaris* is genetically homogenous (Tydén et al., 2016). Taken together, these findings support the hypothesis that *P. univalens* is the dominating species infecting horses worldwide.

In summary, we confirmed that the species of equine ascarids present in Iceland is *P. univalens*. This study further provides evidence for treatment failure of ivermectin against *Parascaris* spp. infection in foals. Since Icelandic horses have been isolated on the island since before the introduction of ivermectin, this implies that the resistance phenotype has developed independently in the Icelandic *P. univalens* population. This population would therefore be valuable in further studies into the genetic background of ivermectin resistance. The actual clinical impact of ivermectin resistance is unknown but another drug of choice should be considered to treat *Parascaris* infection in foals in Iceland.

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