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Original papers

Anthelmintic resistance in strongylids (Nematoda: Strongylidae) parasitizing wild and domestic equids in the Askania Nova Biosphere Reserve, Ukraine

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ABSTRACT. Resistance of strongylids in domestic horses to benzimidazole anthelmintics (BZ) has been detected worldwide; however, information on the presence of BZ-resistance in wild equids has not been published to date. The purpose of this study was to analyze the manifestations of the BZ resistance in strongylids in domestic and wild equids kept in the Askania Nova Biosphere Reserve, Ukraine. Four species of equids: domestic horses and Shetland ponies (*Equus caballus*), donkeys (*E. asinus*), plains zebras (*E. burchelli*) and Grévy's zebras (*E. grevyi*) kept under semi-free conditions were examined using the Fecal Egg Count Reduction Test (FECRT) in order to detect the presence of resistance to the BZ anthelmintics. Analysis of long-term data (2009–2019) revealed a decrease in the efficacy of BZ drugs against strongylids in these four species of equids from 97.6% in 2009 to <75% in 2019. The efficacy of anthelmintic treatments was low in all species of equids: in plains zebras – 69.4%, Grévy's zebras – 72.7%, horses – 66.4%, ponies – 61.1% and donkeys – 45.2%. Ten species of cyathostomins (*Cyathostomum catinatum*, *Cylicocyclus nassatus*, *C. ashworthi*, *C. leptostomus*, *Cylicostephanus calicatus*, *C. goldi*, *C. longibursatus*, *C. minutus*, *Coronocyclus labiatus*, *C. labratus*) were found after horse deworming with albendazole. Our results are the first detection of BZ-resistance in strongylids of wild equids kept under semi-free conditions in the Reserve.

Keywords: benzimidazole resistance, cyathostomins, FECRT, zebra, donkey, equids

Introduction

Anthelmintic resistance in nematodes parasitic in livestock, including domestic horses, is widespread throughout the world [1–5]. In domestic horses, small strongylids or cyathostomins (subfamily Cyathostominae of the Strongylidae) are designated as the most important group of parasites [3,6–9]; currently, the resistance of cyathostomins to benzimidazole anthelmintics (BZ) is a significant problem worldwide [1,2,4,5,9–13]. Cases of resistance to other groups of anthelmintics, such as pyrantel or macrocyclic lactones, were documented in various countries, as well [2,3,5,13–20]. However, pyrantel and macrocyclic lactones are still widely used because the efficacy of these drugs is rather high.

In Europe, BZ-resistance in cyathostomins of domestic horses was detected in many countries, including Ukraine [1,3,4,11,12,21–25]. Various tests such as the Fecal Egg Count Reduction Test (FECRT), Egg Hatch Assay (EHA), Larval Development Assay (LDA), Larval Migration Inhibition Assay (LMIA), several molecular genetic tests, and others are applicable to demonstrate the anthelmintic resistance in parasitic nematodes of livestock [8,9,25–29]. However, the FECRT is still the „gold standard" for documenting anthelmintic resistance. This method is based on estimating the anthelmintic efficacy by comparing the number of parasites' eggs (eggs per gram of feces, EPG) in horse feces before and after treatment [26,27]. The

FECRT is easy to use for equids kept under various horse-management systems; thus, currently, this test is used to monitor the anthelmintic resistance in domestic horses worldwide [5,8,9,25]. To the best of our knowledge, there are no published data on the FECRT applied for detection of the anthelmintic resistance in wild equids, such as zebras, wild Przewalski's horses, kulans, etc.

There are seven species of wild equids within the genus *Equus* in the world: three species of zebras (*Equus grevyi* Oustalet, 1882; *Equus zebra* L., 1758 and *Equus burchelli* Gray, 1825), one species of African wild ass (*Equus africanus* Heuglin et Fitzinger, 1866), two species of Asian wild asses (*Equus hemionus* Pallas, 1775 and *Equus kiang* Moorcroft, 1841) and one species of Asian wild horse (*Equus ferus przewalskii* (Groves, 1986)). Most of them are free-ranging in regions of Africa and Asia [30]. Several populations of wild equids are kept in zoos, natural reserves and sanctuaries throughout the world. In Ukraine, six species of equids are kept under semi-free conditions in the Askania Nova Biosphere Reserve: wild Przewalski's horses, donkeys (*E. asinus* L., 1758), Turkmenian kulans (*E. hemionus kulan* Groves et Mazak, 1967), plains zebras (*E. burchelli*), Grévy's zebras (*E. grevyi*), domestic horses and Shetland ponies (*Equus caballus* L., 1758). All these equids graze together in large enclosures (total area is 2,330 ha) during pasture season, from April till October; in winter, from November till March, some species (plains and Grévy's zebras, ponies and donkeys) are moved to winter barns where they are kept in separate stables and fed hay and grain with ad lib fresh water.

Since 1984, regular parasitological examinations of equids have been conducted in the Reserve twice a year, in spring and autumn. However, specific studies on the efficacy of anthelmintic treatments as well as addressing the presence/absence of anthelmintic resistance in gastrointestinal parasites have not been carried out in the Reserve till now. The purpose our study was to investigate the possibility to detect of BZ-resistance in strongylid nematodes parasitizing four species of equids kept in the Askania Nova Biosphere Reserve using the FECRT. Identification of the strongylid species resistant to benzimidazole anthelmintics was also of the interest in this study.

Materials and Methods

This study was performed on April 2019 in the Askania Nova Biosphere Reserve in southern Ukraine (46°27'07"N; 33°52'51"E). Four species of equids: domestic horses and Shetland ponies, donkeys, plains and Grévy's zebras were included in the study. Each animal had its own number and name; therefore every individual animal was traced during the entire period of the study.

Routine parasitological monitoring of all individual ungulates kept in the Reserve was performed twice a year from 1984. Starting in 2004 the McMaster technique with a sensitivity of 25 eggs per gram of feces (EPG) [31] was used in the Reserve. All EPG-value were recorded in the Parasitological Examination Protocols by authorized veterinarians; however, analysis of these data has not been performed until now. According to the records of parasitological examinations, only the most infected animals (with EPG>150) were treated with anthelmintics, mostly with BZ drugs (albendazole) or macrocyclic lactones (aversectin C, ivermectin). Regular anthelmintic treatments were carried out twice a year – in spring (March–April) before the beginning of pasture season and in the autumn (November) at the end of pasture season.

In April 2019, the FECRT was performed in the four equid species (plains and Grévy's zebras, donkeys, working horses and ponies) which were kept in the winter barns before they were turned-out to the steppe pastures. These animals have not been treated with any anthelmintics for four months prior to this study. The equids were examined for the presence of strongylid eggs individually (EPG-value); the most infected animals with EPG>150 (n=90) were selected for this study and dewormed with „Albendazole-10%" (ZooVetPromSnab, Ukraine) at 0.75 g per 10 kg of body weight. Since deworming of wild equids was possible only by adding the anthelmintic mixed with food (oat grain) to the feeders, the dosage of the drug for every animal was increased by 10% to avoid under-dosing due to spreading of the drug in the feeder. As best as could be determined, the entire dose of albendazole was consumed by each animal.

Fecal egg count examinations (EPG-value) using the McMaster technique [31] were performed on the day before anthelmintic treatment (FEC-pre) and on the 14th day after treatment (FEC-post). Every animal was sampled individually. The FECRT was performed according to the WAAVP protocol

[26,27] using the following formula [32]:

$$\text{FECR (in \%)} = \frac{\text{FEC-pre} - \text{FEC-post}}{\text{FEC-pre}} \times 100\%$$

Additionally, multi-year data of EPG-values collected individually from the four equid species during 2009–2019 were re-analyzed using the FECRT routine. Totally 1,630 fecal samples from 192 animals were included into this analysis, of which 524 samples were from plains zebras, 144 samples from Grévy's zebras, 276 samples from donkeys, 306 samples from ponies, and 380 samples from working horses and ponies.

To identify the strongylid species resistant to BZ drugs, nematodes were collected individually from fresh feces from each horse at 24, 36 and 48 hours post-treatment as described previously [33,34]. All nematodes expelled in fresh feces were collected manually, fixed in 70% non-denatured ethanol and identified under a light microscope using morphological criteria [35]. Due to the impossibility to collect feces from wild zebras and donkeys after their turn-out to the steppe enclosures, only strongylids from domestic horses and Shetland ponies were collected and identified.

The data obtained were analyzed using the Paleontological Statistics Software (PAST) [36]. The prevalence frequency distribution for collected strongylid species was determined according to Bucknell et al. [37]. The proportion of each species in the strongylid community was calculated as the number of specimens of the particular species in relation to the total number of strongylids collected from each sample.

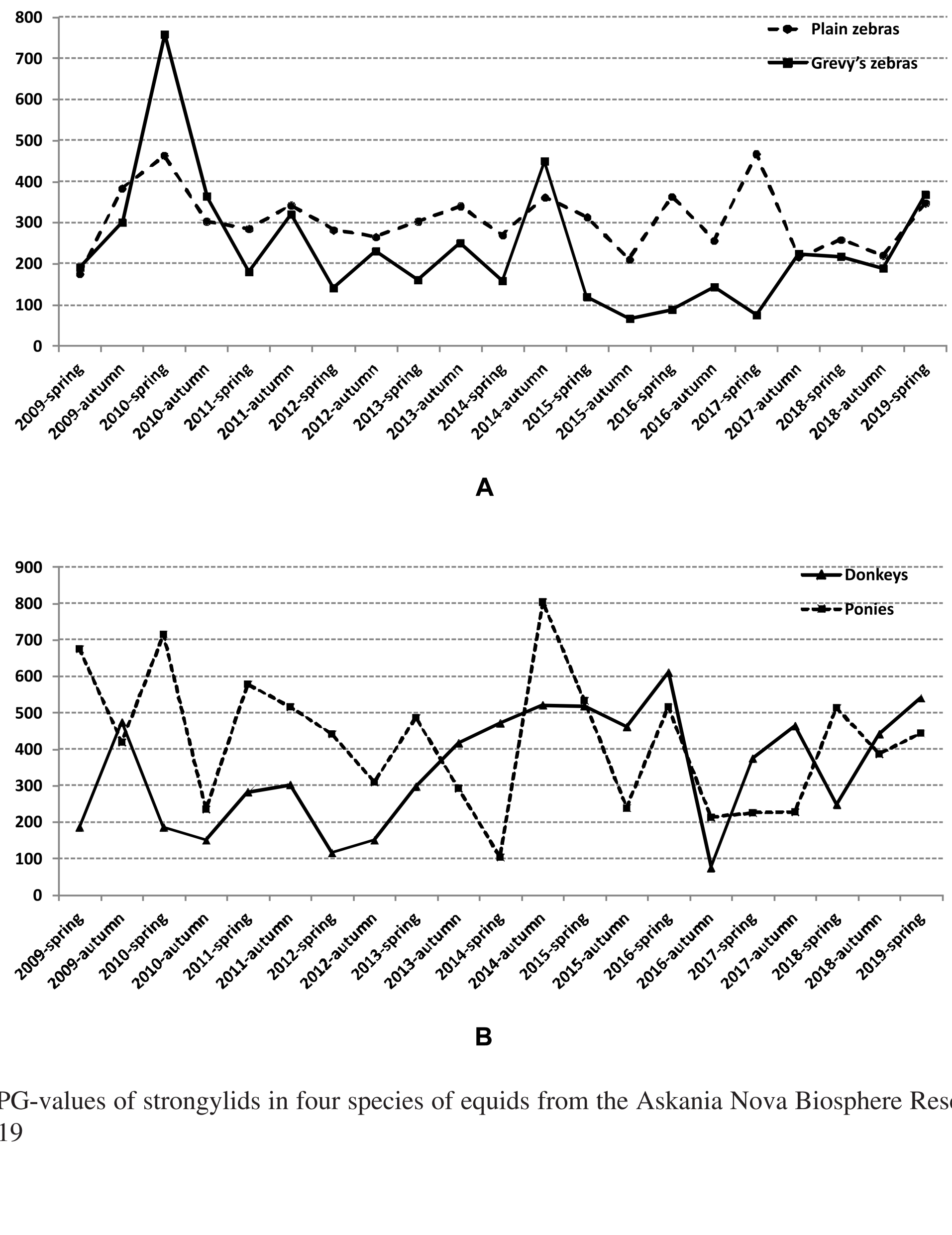

Figure 1. The EPG-values of strongylids in four species of equids from the Askania Nova Biosphere Reserve studied during 2009–2019

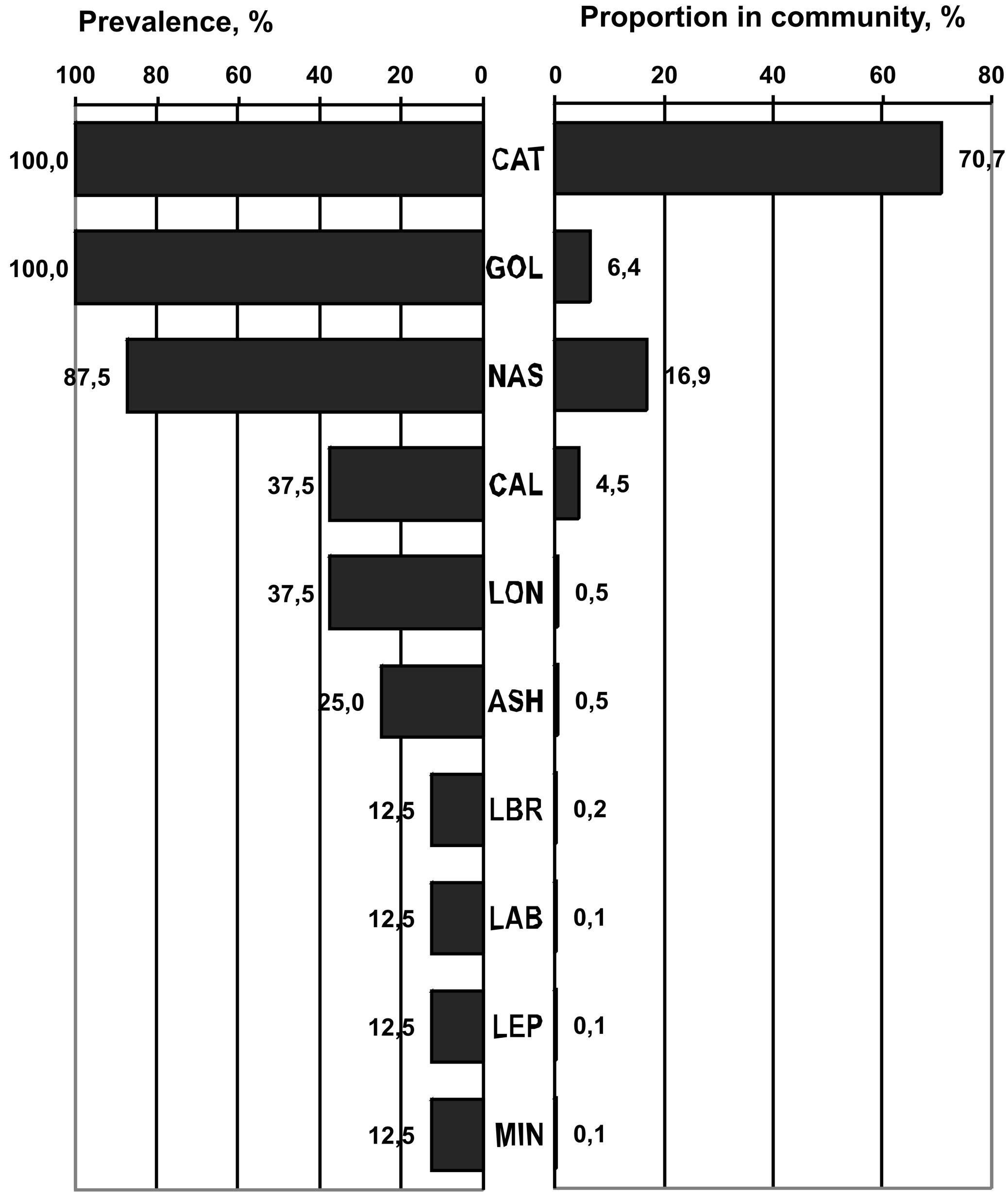

Figure 2. Prevalence and proportion in the community of ten cyathostomin species found in horses with approved BZ-resistance. CAT – *Cyathostomum catinatum*, NAS – *Cylicocyclus nassatus*, ASH – *C. ashworthi*, LEP – *C. leptostomus*, CAL – *Cylicostephanus calicatus*, GOL – *C. goldi*, LON – *C. longibursatus*, MIN – *C. minutus*, COR – *Coronocyclus labiatus*, LAB – *C. labratus*.

Results

All wild and domestic equids were found to be infected with intestinal strongylids during the entire observation period (2009–2019). The EPG-values varied widely, from 25 EPG to 2500 EPG (Tables 1 and 2). Despite the wide variations in the EPG values, the mean infection levels for most equids were within the ranges of the average infection level (from 200 to 500 EPG); only Shetland ponies had higher levels of strongylid infection (>500 EPG) (Fig. 1). Statistically significant differences between EPG-values in spring and in autumn were not observed in any of the equid species (Mann–Whitney test, p>0.05).

Grévy's zebras were significantly less infected with strongylids than others equids (Mann–Whitney test, p<0.05); while statistically significant differences in strongylid EGP-values were not observed between plains zebras, donkeys and ponies (Mann–Whitney test, p>0.05).

The analysis of the long-term data (2009–2019) collected individually from wild and domestic equids revealed a decrease of the efficacy the BZ drugs both in wild zebras and in domestic equids (Tables 1 and 2). The first evidence of BZ-resistance was detected in 2009 in plains zebras dewormed with „Vermitan„ (albendazole, 10%); the efficacy of their treatment was less than 90%. In Grévy's zebras, the first evidence of decreased treatment efficacy to less than 90% was detected in 2010 (Table 1). In donkeys, the decrease of treatment efficacy to less than 90% was detected in 2011, in ponies – in 2012 (Table 2). Working horses were not treated regularly with any anthelmintics from 2012; however, the FECRT performed in 2019 showed that efficacy of albendazole was only 66.4% which is evidence of BZ resistance in strongylids parasitizing these working horses.

The results of FECRT based on multi-year data (2009–2019) demonstrated a steady gradual decline in BZ treatment efficacy in all equid species (Tables 1 and 2). In 2019, the FECRT was 69.4% in plains zebras, 72.7% in Grévy's zebras, 66.4% in domestic horses, 61.1% in ponies, and 45.2% in donkeys.

Ten species of small strongylids or cyathostomins (subfamily Cyathostominae) from four genera were found in horses and ponies with approved BZ-resistance: *Cyathostomum catinatum*, *Cylicocyclus nassatus*, *C. ashworthi, C. leptostomus*, *Cylicostephanus calicatus*, *C. goldi*, *C. longibursatus*, *C. minutus*, *Coronocyclus labiatus*, and *C. labratus* (Fig. 2); species of large strongylids (subfamily Strongylinae) were not found in the fecal samples. From 3 to 6 cyathostomin species were found to parasitize one host.

Distribution of these ten cyathostomin species between ten prevalence classes showed a bimodal structure of the strongylid community with dominant and rare species similar to the „core – satellite mode" according to Hanski [38] (Fig. 3).

Discussion

The results of this study present the first findings of BZ-resistance in small strongylids (cyathostomins) parasitic in wild equids. So far, anthelmintic resistance of cyathostomins has been reported mostly in domestic horses [1–5,10–12,21]; there are few reports on anthelmintic resistance in cyathostomins from donkeys [39,40,41]. Data on the occurrence of anthelmintic resistance of nematodes in wild equids (zebras, wild Przewalski's horses, kulans, etc.) have not been published until now.

Our study used the FECRT, which is considered to be the most accurate and most widely used *in vivo* technique for detecting anthelmintic resistance in equine nematode populations [8,9,26,27]. The FECRT guidelines have been elaborated only for domestic animals including the domestic horses [26,27]. Furthermore, in the FECRT guidelines, there is no exact information how to administer the anthelmintic drugs to horses, particularly, whether the anthelmintic drugs can be mixed with food. For wild equids kept in zoos or natural reserves, it is difficult to administer anthelmintics orally using a deworming syringe; thus mixing of the anthelmintics

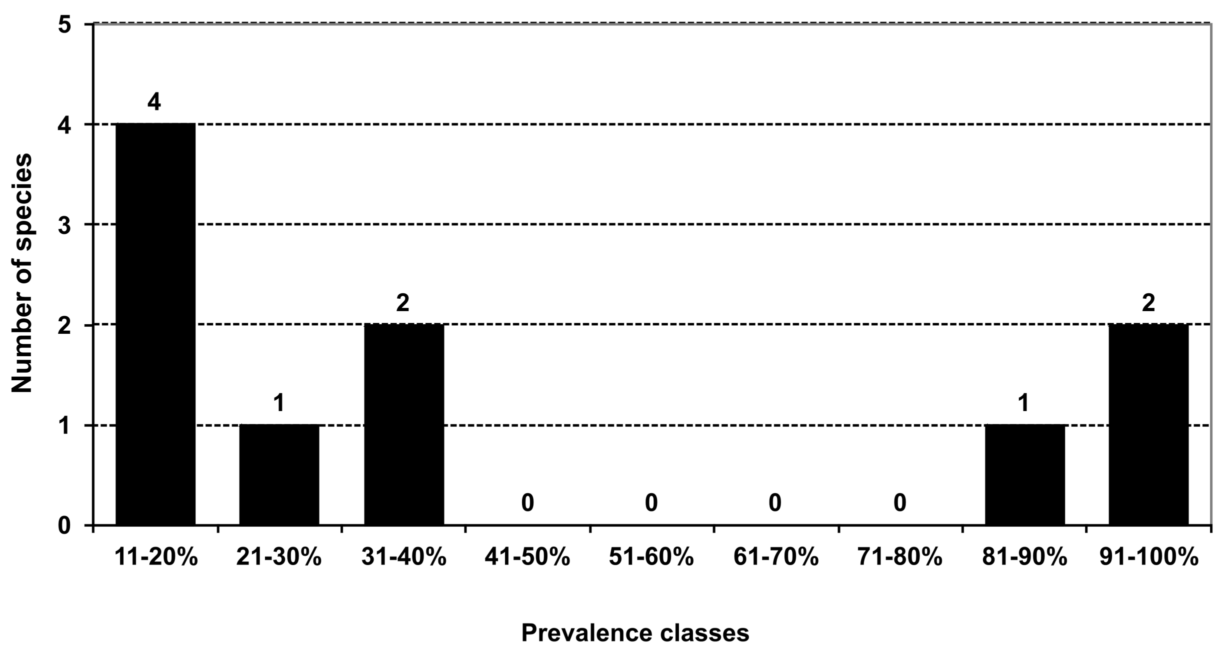

Figure 3. Prevalence–frequency distribution of ten cyathostomin species found in horses with approved BZ-resistance

Table 1. Strongylid EPG-values and efficacy of treatments of the plains zebras (*Equus burchelli*) and Grévy's zebras (*E. grevyi*) from the Askania Nova Biosphere Reserve with benzimidazole drug (albendazole)

Year	Plain zebras				Grévy's zebras			
	spring		autumn		spring		autumn	
	EPG[1]	FECRT[2]	EPG	FECRT	EPG	FECRT	EPG	FECRT
2009	175 (25–450)	92.4 %	383 (75–725)	87.4%	191 (175–200)	91.7%	300 (100–500)	ND[4]
2010	463 (50–2000)	96.4%	346 (50–700)	Univerm[3] =100%	758 (600–975)	ND	365 (75–775)	88.3%
2011	285 (50–775)	Univerm =100%	369 (25–1750)	97.7%	180 (25–475)	Univerm =100%	320 (50–650)	87.8%
2012	304 (50–600)	ND	281 (25–800)	Univerm =100%	175 (125–275)	ND	230 (75–375)	Univerm =100%
2013	322 (25–825)	ND	360 (25–625)	88.6%	160 (50–300)	ND	250 (100–475)	78.2%
2014	307 (25–875)	89.3%	361 (25–1600)	86.4%	190 (25–425)	80.3%	450 (50–825)	85.5%
2015	332 (25–1425)	77.6%	223 (50–375)	78.2%	150 (75–325)	79.3%	67 (25–125)	ND
2016	386 (50–925)	Univerm =98.6%	270 (25–1500)	ND	117 (50–225)	Univerm =100%	144 (25–275)	ND
2017	520 (75–1250)	69.4%	271 (25–725)	72.6%	75 (25–175)	ND	225 (50–400)	77.2%
2018	258 (25–625)	71.5%	220 (25–675)	ND	218 (25–350)	65.2%	206 (75–325)	ND
2019	388 (75–1325)	69.4%	—	—	368 (75–800)	72.7%	—	—

[1]EPG – eggs per 1 gram of feces: average (min–max); [2]FECRT (in %) – efficacy of anthelmintic treatment re-calculated using the Fecal Egg Count Reduction Test formula (Kaplan, Nielsen 2010); [3]Univerm –treatment with the macrocyclic lactone drug "Univerm" (0.2% aversectin C); [4]ND – data were not collected after deworming.

with food [42] is the only the way to administer them to wild animals. In the present study, we modified the method of administration of the albendazole (in powder form) to wild equids by mixing it with a small amount of oat grain and adding 10% of the dosage to prevent under-dosing. Previously, this method was used in the Reserve for the administration of the marcocyclic lactone drugs to wild equids [34,43,44]. Since all the animals included in the study consumed the entire dose of the anthelmintic, we believe that our method of the anthelmintic administration for wild equids was effective to prevent the drug from being under-dosed. Therefore, the results of FECRT can be considered as reliable and can be used for future studies on the anthelmintic resistance in wild equids.

Frequent dewormings of the hosts with the same group of anthelmintic drugs and under-dosing of anthelmintics are among the main causes contributing to the widespread development of anthelmintic resistance in parasitic nematodes including horse strongylids [1,3,5,9,45–50]. As the result, the exposure of the nematode populations to anthelmintics increases the chance of mutations that ensure their resistance to the drugs [4,5,25,28, 48,50], or selection of nematode populations with shortened egg reappearance periods [18,20]. Frequent dewormings of horses, 4–6 times a year or more often, has been shown to result in the rapid development of anthelmintic resistance in cyathostomins [1,2]. Our previous studies in Ukraine have shown that frequent dewormings of horses with benzimidazoles also predisposes to the destruction of the strongylid community structure and to reduction of the species diversity in cyathostomins [23]. Several researchers recommend selective dewormings of horses, not more than twice a year, to prevent the development of anthelmintic resistance in strongylids [6,15,50–53]. However,

Table 2. Strongylid EPG-values and efficacy of treatments of donkeys (*Equus asinus*), working horses and ponies (*E. caballus*) from the Askania Nova Biosphere Reserve with benzimidazole drug (albendazole)

Year	Donkeys				Ponies				Working horses			
	spring		autumn		spring		autumn		spring		autumn	
	EPG[1]	FECRT[2]	EPG	FECRT	EPG	FECRT	EPG	FECRT	EPG	FECRT	EPG	FECRT
2009	186 (25–475)	ND[3]	425 (100–825)	ND	300 (50–800)	ND	418 (25–2075)	92.8%	531 (25–1575)	Univerm[4] =100%	385 (25–1250)	97.6%
2010	176 (25–325)	92.3%	150 (50–200)	Univerm =100%	715 (50–1575)	Univerm =100%	235 (25–1375)	ND	580 (100–1250)	97.2%	—	—
2011	282 (25–775)	86.5%	303 (75–575)	88.2%	578 (250–1050)	Univerm =100%	516 (150–800)	94.7%	869 (75–1275)	96.8%	100 (25–250)	ND
2012	115 (50–250)	ND	150 (25–275)	Univerm =100%	441 (50–1250)	84.3%	310 (50–1325)	Univerm =100%	815 (125–1675)	Ivomec[5]	—	—
2013	297 (75–900)	ND	416 (50–925)	ND	485 (50–1525)	ND	292 (25–775)	ND	—	—	223 (25–450)	ND
2014	470 (25–950)	ND	521 (50–1450)	ND	103 (25–250)	ND	805 (50–2500)	ND	300 (50–775)	ND	—	—
2015	518 (75–1725)	ND	461 (250–850)	82.5%	533 (175–1425)	78.7%	239 (25–1075)	76.4%	—	—	—	—
2016	610 (75–1475)	Univerm =100%	75 (50–100)	ND	515 (50–2450)	Univerm =100%	212 (100–475)	77.1%	—	—	—	—
2017	372 (50–925)	68.4%	464 (50–1000)	ND	362 (50–775)	ND	227 (25–775)	ND	1065 (25–2225)	ND	—	—
2018	247 (25–750)	58.8%	441 (75–1300)	ND	513 (50–1600)	68.1%	386 (25–1775)	ND	—	—	—	—
2019	540 (50–1175)	45.2%	—	—	442 (25–1475)	61.1%	—	—	656 (25–1150)	66.4%	—	—

[1]EPG – eggs per 1 gram of feces: average (min–max); [2]FECRT (in %) – efficacy of anthelmintic treatment re-calculated using the Fecal Egg Count Reduction Test formula (Kaplan, Nielsen 2010); [3]ND – data were not collected after deworming; [4]Univerm – treatment with the macrocyclic lactone drug “Univerm” (0.2% aversectin C); [5]Ivomec – treatment with the macrocyclic lactone drug „Ivomec” pour-on.

other authors [54] reported on the development of anthelmintic resistance even under programs using few treatments.

Analysis of multi-year (2009–2019) EPG-value data collected from equids in the Askania Nova Reserve showed that though all the equids were treated twice a year or less, the first signs of BZ-resistance appeared in 2010–2012. Working horses were dewormed once a year or less; according to the veterinary records, the working horses included in our study have not been treated with any anthelmintics since 2014. However, the results of the FECRT performed in 2019 revealed the presence of BZ-resistance in all equid species including the working horses (Tables 1 and 2).

We assume that the under-dosing of anthelmintics is the main factor responsible for the development and spreading of BZ-resistance in strongylids in all equids kept in the Askania Nova Reserve, especially in wild ones. During the deworming of wild equids (zebras, wild Przewalski's horses, and kulans) it is almost impossible to control the dosage of anthelmintics precisely; usually anthelmintics are given to the animals mixed with food (oats or crushed barley grain). However, most wild equids, especially zebras, reluctantly eat food with the anthelmintics; therefore, it is very probable that they receive a lower dosage of the drugs. Analysis of the ten-year data (2009–2019) performed in our study using the FECRT showed that treatment efficacy has steadily decreased over last ten years, from >93–97% in 2009–2010 to less than 70–73% in 2019 for all species of equids; in domestic equids (donkeys and ponies) the efficacy was the lowest (Tables 1 and 2).

Traditionally, dewormings of the equids in the Askania Nova Reserve are carried out using the BZ drugs (albendazole), because these drugs are not expensive and are available in Ukraine. Other pharmacological groups of anthelmintics (pyrantel or macrocyclic lactones) are more expensive; therefore, as we have previously observed [23], most state horse farms and private horse facilities use mainly benzimidazoles. Therefore, in our opinion, the long-term under-dosing of the BZ anthelmintics, as well as the lack of anthelmintic rotation, are the most apparent reasons for the development of BZ-resistance in strongylids in domestic and wild equids in the Askania Nova Biosphere Reserve.

Many parasitologists consider that the levels of "refugia" is the most important factor slowing down the development and selection of parasite populations resistant to anthelmintics [5,9,11,32, 55–58]. In the refugia, the parasite subpopulations from either the stages within the host or free-living stages on pasture that are not exposed to dewormings provide a pool of genes susceptible to anthelmintics and, therefore, diluting the frequency of resistant genes [9,49,55,58]. The results obtained in our study do not support this concept; moreover, they can be an example which contradicts this idea. The results of the FECRT in working horses showed that despite extremely rare anthelmintic treatments of these horses and, therefore, very high level of refugia in their parasites, these working horses were infected with BZ-resistant strongylids (FECRT= 66.4%). In other words, the free-living larval stages of BZ-resistant strongylids accumulated on pasture grass and, thereafter, transmitted to rarely-treated working horses when they grazed on the pastures. Moreover, the results of molecular studies of six cyathostomin species collected in 2007 from never-treated wild Przewalski's horses from the Askania Nova Reserve revealed the presence of the single nucleotide polymorphism in β-tubulin isotype 1 codon 167 in two species (*C. ashworthi* and *C. longibursatus*) that indicated the presence of BZ-resistance [29]. As those wild Przewalski's horses were kept free in steppe enclosures and had never been treated with any anthelmintics, they could have been infected by BZ-resistant strongylids only by grazing the same pasture with zebras, ponies or donkeys regularly treated with albendazole twice a year. Thereby, our data showed that the refugia might slow down the development of resistance in the strongylid on horse farms, as it was assumed by Kaplan and Nielsen [32]; however, in case of the wild and domestic equids kept in the Askania Nova Reserve, refugia does not prevent the spreading of BZ-resistance in strongylids. More studies including molecular examinations of the resistant species of strongylids are necessary to understand the mechanism of BZ-resistance spreading in strongylid populations from different equid species.

In the present study, the examination of the species diversity of the BZ-resistant strongylids was possible only for domestic horses (working horses and ponies), since after deworming all other equids (donkeys, plains and Grévy's zebras) were released into large steppe enclosures; thus, collecting fecal samples from individual animals was impossible. Results of our previous studies on the strongylid communities in plains and Grévy's zebras from the

Askania Nova Reserve showed that the species compositions of the strongylid communities in both species of zebras were similar to those of domestic horses and ponies [34,43]. Therefore, we assume that BZ-resistant species of cyathostomins parasitized domestic horses do parasitize the plains and Grévy's zebras as well.

Ten species of cyathostomins were found in horses and ponies with approved BZ-resistance in the present study (Fig. 2). These species also composed the "core" of the strongylid community in ponies and domestic horses in the Askania-Nova Reserve [43]. Also, the same species were reported to be BZ-resistant in domestic horses in Ukraine [23], as well as in other countries [1,3,59,60]. Conducting of the critical test (necropsy) is necessary to find out which cyathostomin species can survive albendazole treatments of their hosts and are BZ-resistant. Since necropsy of equids in the Askania Nova Reserve for research purposes is impossible, further studies with using of various anthelmintics (such as ivermectins) are necessary to determine the BZ-resistant strongylid species.

Parasitic nematodes are known to be predisposed to the development of anthelmintic resistance due to their considerable population sizes, high level of genetic diversity and relatively rapid generation rates [61]. Since BZ-resistant species of Cyathostominae are the most abundant in the parasite communities [3,23,33,34,43,60], they may possess wider genetic variability and plasticity allowing them to develop resistance rapidly. At the same time, rare strongylid species are constantly removed from the strongylid community after regular dewormings [23,34,43]. Analysis of the prevalence–frequency distribution of cyathostomin species in the present study (Fig. 3) showed that the parasite community structure was bimodal (corresponding to the "core – satellite model" according to Hanski [38], typical for strongylid communities in equids underwent frequent dewormings [23,34,37,43].

Two more species of wild equids, the Przewalski's horses and Turkmenian kulans, are kept in the Askania Nova Biosphere Reserve. Both these species are kept free in large steppe enclosures (total area is 2,330 ha) and are never treated with any anthelmintics. First attempts to study the strongylid community of wild Przewalski's horses *in vivo* were performed in the Reserve in 2007 [44]. Molecular examinations of the cyathostomin specimens collected from those horses revealed different haplotypes in *Cylicocyclus nassatus* from different equid hosts [62], as well as the emergence of the first signs of BZ-resistance in two other cyathostomin species [29]. The results obtained in this study revealed the spreading of BZ-resistant populations of cyathostomins in four species of equids regularly treated with anthelmintics. Further studies on the spread of the BZ-resistance between the cyathostomin populations in the wild Przewalski's horses and Turkmenian kulans as well as the analysis of genetic variability of the nematodes are necessary to explore the molecular mechanisms of anthelmintic resistance in this group of parasites. Joint grazing of several species of wild and domestic equids on the same pastures in the Askania Nova Reserve and, consequently, free exchange of the parasites between them provide an opportunity to analyze the genes associated with the development of anthelmintic resistance in cyathostomins parasitizing different species of equids.

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Compliance with ethical standards. All animals included in this study were clinically healthy and were not subjected to any kind of restraint. Anthelmintic products administered orally according to label instructions; fecal samples were collected following standard procedures used in the equine veterinary industry.

Conflict of interest. The authors declare that they have no conflicts of interest.

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