Horse and donkey parasitology: differences and analogies for a correct diagnostic and management of major helminth infections

Francesco Buono[1] (iD), Vincenzo Veneziano[1] (iD), Fabrizia Veronesi[2] and Marcelo Beltrão Molento[3]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples 'Federico II', Naples, Italy; [2]Department of Veterinary Medicine, University of Perugia, Perugia, Italy and [3]Laboratory of Veterinary Clinical Parasitology, Department of Veterinary Medicine, Federal University of Parana, Curitiba, PR, Brazil

Abstract

In June 2022, at the XXXII Conference of the Italian Society of Parasitology, the parallels of the main endoparasitic infections of horses and donkeys were discussed. Although these 2 species are genetically different, they can be challenged by a similar range of parasites (i.e. small and large strongyles, and *Parascaris* spp.). Although equids can demonstrate some level of resilience to parasites, they have quite distinct helminth biodiversity, distribution and intensity among different geographical locations and breeds. Heavily infected donkeys may show fewer clinical signs than horses. Although parasite control is primarily provided to horses, we consider that there may be a risk of drug-resistance parasitic infection through passive infection in donkeys when sharing the same pasture areas. Knowing the possible lack of drug efficacy (<90 or 80%), it is advocated the use of selective treatment for both species based on fecal egg counts. Adult horses should receive treatment when the threshold exceeds 200–500 eggs per gram (EPG) of small strongyles. Moreover, considering that there are no precise indications in donkeys, a value >300 EPG may be a safe recommendation. We have highlighted the main points of the discussion including the dynamics of helminth infections between the 2 species.

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Introduction: early data and similarities

Equids (horses, donkeys, mules and hinnies) originated about 55 million years ago in North America from a browsing species named *Hyracotherium* (Librado and Orlando, 2021), which had the size of a large dog. All living species of equids belong to the genus *Equus*, composed of 2 lineages, caballine and non-caballine animals, that are split into 3 phylogenetic clades. The domestic horse (*Equus ferus caballus*) and the Przewalski's horse (*Equus ferus przewalskii*) belong to the first clade (caballine horses), while zebras and wild asses belong to the other 2 clades (non-caballine horses) (Cucchi *et al.*, 2017). The modern horse has been intensely raised and selected (kinship) for their athletic potential focusing on morphological parameters of body weight (BW), withers height and also sports performance (Brown-Douglas *et al.*, 2009; Schrurs *et al.*, 2022; Dall'Anese *et al.*, 2023).

It is commonly believed that donkeys are short horses with long ears; however, these 2 animal species are different not only in their physical features and behaviour but also in their genetic and physiological characteristics (Lizzaraga *et al.*, 2004). The chromosome number of horses and donkeys is 64 and 62, respectively, making their hybrids (mules and hinnies/asses) infertile animals. There are approximately 175 different breeds of donkeys worldwide (DAD-IS, 2017; https://www.fao.org/dad-is), characterized by their ability to survive in mountainous and semi-arid environments with scarce water availability (Burden and Thiemann, 2015). Donkeys can survive even having a water loss of 20–30% of their weight (Matthews and van Loon, 2019), whereas horses are less tolerant of water deprivation (Matthews *et al.*, 1997). Furthermore, even with the availability of water, the urine output of donkeys is lower than horses (Grosenbaugh *et al.*, 2011). Contrary to what happens in horses, donkeys have a greater ability to digest fibres of low-nutritional value. These animals are characterized by their lower energy requirement (about 50–75%) than that needed by horses of the same size (Smith and Burden, 2013).

Different responses to pain and fear by donkeys, compared to horses, led to the belief that donkeys could have superior tolerance to distress. For this reason, they are considered stoic animals that do not need regular veterinary care, vaccinations or anthelmintic treatments (Molento and Vilela, 2021). However, donkeys may respond to discomfort and pain more subtly than horses. As an example, sick donkeys (i.e. parasites and bacterial infections) often show dullness and depression but the clinical diagnosis of the disease is performed only at an advanced stage (Burden and Thiemann, 2015).

Parasitic infections are the most important limiting factors for equids' health and performance, as all common parasites of horses infect donkeys (Matthews and Burden, 2013). In comparison, scientific publications on parasites in horses are 20 times higher than those in donkeys

(Molento and Vilela, 2021). Figure 1 shows a map of the main parasite studies only in donkeys with their geographical location. In this review, the differences between the main helminthic diseases affecting donkeys and horses, and the different treatment and management strategies will be examined. Some recommendations will be also highlighted.

Helminth infections in horses and donkeys: head to head!

Horses and donkeys not only share similar parasites but also hold high helminth biodiversity (Gianfaldoni *et al.*, 2020; Sousa *et al.*, 2021). Both species can act as reservoirs for each other; however, in donkeys parasitized with a high helminth burden, clinical signs such as diarrhoea and poor body condition are less common than in horses (Matthews and Burden, 2013). Therefore, donkeys appear to be healthy even in the presence of a high-parasitic load (Maestrini *et al.*, 2020). The prevalence of major helminth infections in donkeys is reported in Table 1.

Intestinal strongyles: epidemiology, diagnostic and innovative research

As in horses and also in donkeys, the most common nematode parasites are intestinal strongyles (large and small strongyles) (Buono *et al.*, 2021; Dias de Castro *et al.*, 2022). In both species, parasitic infections are caused mainly (>90%) by small strongyles (Buono *et al.*, 2021; Jota Baptista *et al.*, 2021; Nielsen *et al.*, 2021). A recent meta-analysis reported that *Cylicocyclus nassatus*, *Cylicostephanus longibursatus* and *Cyathostomum catinatum* represent about 55% of the specimens in horses (Bellaw and Nielsen, 2020). The most common genera for donkeys were *Cylicostephanus* spp. and *Cylicocyclus* spp. (Maestrini *et al.*, 2020; Perrucci *et al.*, 2021).

In horses (Nielsen *et al.*, 2021) and donkeys (Perrucci *et al.*, 2021), small strongyle infections (cyathostominosis) are often asymptomatic. However, cyathostomins can cause colic, reduced nutrient absorption, diarrhoea and weight loss, and in some cases intestinal infarction and death in horses (Molento, 2005; Steuer *et al.*, 2018; Walshe *et al.*, 2021). In donkeys, clinical signs due to parasitic infections are somewhat rare (Getachew, 2006; Burden *et al.*, 2010). It is known that a high-parasitic fecal egg count (FEC) (>1000 eggs per gram – EPG) may cause quantitative and qualitative milk reduction in asymptomatic lactating jennies (Perrucci *et al.*, 2021). Small strongyles can be responsible for a clinical manifestation known as larval cyathostominosis in horses. This demonstration is caused by the synchronous emergence of the encysted larvae from the mucosa of the colon and caecum causing severe typhlocolitis. Although it is quite uncommon in both animal species, in the horse, the fatality rate of this syndrome can reach 50% (Love *et al.*, 1999). Even though larval cyathostominosis has been reported in working donkeys (Oryan *et al.*, 2015), the fatality rate of this syndrome is not known for the species. Matthews and Burden (2013) consider that the fatality would be lower than that for horses. Although horse foals (<1 year), yearlings (1–2 years) and young horses (3–5 years) are responsible for the majority of intestinal strongyle egg excretion (Lester *et al.*, 2018; Scala *et al.*, 2020), in donkeys this phenomenon still needs to be determined. Until recently, no differences in parasite FEC have been observed among age categories (da Costa *et al.*, 2018; Maestrini *et al.*, 2020; Buono *et al.*, 2021). Moreover, it has been reported that intestinal strongyle FEC is higher in donkeys than in horses (Mezgebu *et al.*, 2013). This trend is not quite implicit but perhaps it could be due to different reasons, such as the lower frequency of anthelmintic treatment in donkeys than in horses (Buono *et al.*, 2021). Another interesting observation is the

smaller amount of feces produced by donkeys when compared to an average-sized horse. Assuming a similar infection density, horses that produce a higher amount of feces (15–20 kg day^{-1}) than donkeys (6–10 kg day^{-1}) would have a dilution of eggs on EPG, showing a lower FEC than donkeys (M. Molento, personal observation). More data need to be gathered (i.e. performance, EPG, necropsy) to demonstrate this biological condition.

Large strongyles encompass *Strongylus* spp., *Triodontophorus* spp., *Craterostomum acuticaudatum*, *Oesophagodontus robustus* and *Bidentostomum ivaschkini* (Lichtenfels *et al.*, 2008). Of these, the most pathogenic are those belonging to genus *Strongylus* (Cav *et al.*, 2013). Three species of *Strongylus* spp. infect horses and donkeys (*Strongylus vulgaris*, *Strongylus equinus* and *Strongylus edentatus*). In donkeys, *Strongylus asini* has also been reported, whereas horses are less susceptible to this nematode (Malan *et al.*, 1982). *Strongylus asini* is morphologically more similar to *S. vulgaris* than *S. equinus* and *S. edentatus*; however, the internal transcribed spacer-2 sequence of *S. asini* proved to be more similar to *S. equinus* and *S. edentatus* (Hung *et al.*, 1996). In donkeys and zebras, *S. asini* develops in the lumen of the portal vein, having a comparable life cycle to *S. vulgaris* (Malan *et al.*, 1982). Generally, large strongyles are less abundant than small strongyles in horses and donkeys and these parasites are less common in farms that make constant use of macrocyclic lactones (MLs) [i.e. ivermectin (IVM), abamectin, moxidectin (MOX)]. It has been reported that the prevalence of *S. vulgaris* may increase if selective therapy is adopted in horses (Tydén *et al.*, 2019) and in donkeys (Sousa *et al.*, 2021).

In horses, the overwhelming majority of intestinal strongyle egg excretion is concentrated in certain animals. In general, 15–30% of adult horses shed approximately 80% of eggs (80:20 distribution rule) (Tzelos and Matthews, 2016; Lester *et al.*, 2018), and this pattern is known as overdispersion. In donkeys, this behaviour has been less investigated and the levels of egg excretion are quite different, as about 40% of adult donkeys can shed approximately 80% of eggs, suggesting an 80:40 distribution rule (Buono *et al.*, 2021). These differences in the overdispersion of intestinal strongyle eggs between horses and donkeys have important practical implications. In horses, the anthelmintic treatment of 20% of the animals is adequate to limit pasture contamination, whereas in donkeys the number of treated animals to obtain the same result would be considerably higher (Buono *et al.*, 2021).

As selective therapy and other on-farm management (i.e. reproduction) focuses on individual animal performance, it has been shown that invasion of the intestinal mucosa by parasites activates a defensive mechanism involving the solute-like carrier family 11a1 (SLC11A1) gene, also known as NRAMP1 (Pires *et al.*, 2021). The authors were looking to determine the DNA methylation profile of this gene in cyathostomin-infected horses correlating with FEC. First, they have shown that in the core of this gene, there were 2 cytosines adjacent to guanine (CpG) islands. The data presented a positive epigenetic correlation between the hypermethylation of island 2 of CpG of the gene and FEC. This information can help explain the differences in FEC detected among animals raised under similar conditions. Further research is being undertaken, looking to elucidate the host-specific aspects and the involvement of certain genes across different horse categories (foals, yearlings and adult horses) that may be related to host resilience to parasitic infections. In the future, epigenetic processes shall be used as biomarkers to identify and target animals for anthelmintic treatments, as well as other breeding purposes (Pires *et al.*, 2021). Epigenetic studies are still rare for horses and no such data have been produced for donkeys. As seen above, cyathostomin infections may be measured by individual FEC to be used in selective anthelmintic therapy. The
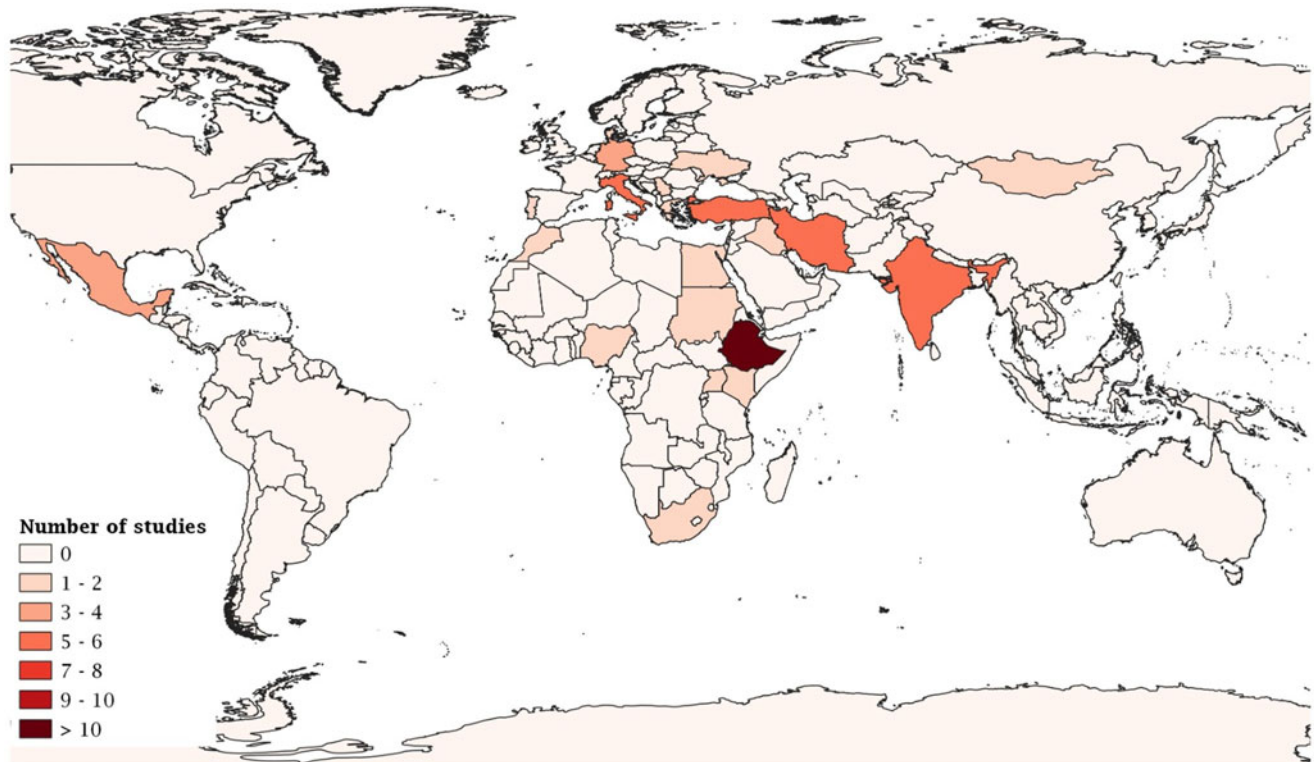
Figure 1. Choropleth map of the main epidemiological studies on major helminth infection in donkeys. Numbers in square brackets [*] represent the references reported in Table 1. **Africa**: Egypt [1–2], Ethiopia [3–38], Kenya [39–40], Morocco [41], Nigeria [42–43], Sudan [44–45], South Africa [46–47], Uganda [48–49]; **America**: Mexico [50–52]; **Asia**: India [53–57], Iran [58–62], Iraq [63], Mongolia [64]; **Europe**: Denmark [65], Germany [66–68], Italy [69–73], Macedonia and Thessalia-Greece [74], Portugal [75–76], Serbia [77], Turkey [78–82], Ukraine [83–84].

difference in FEC is a phenotypic condition that might be explained by the genetic effects of horses. A new research approach was used by Dias de Castro *et al.* (2022) that focused on genome-wide association studies (GWASs) using the Illumina Equine 70K BeadChip to correlate genomic variants and FEC of 90 thoroughbred horses. The GWAS used a panel containing 65 157 single-nucleotide polymorphism (SNP) markers. The analysis revealed 33, 21, 30, 21 and 19 genes related to FEC, packed cell volume, eosinophils, neutrophils and lymphocyte count, respectively. The data demonstrate a good correlation between important phenotypic health traits and the potential-specific SNP markers.

Molento and Vilela (2021) have proposed a susceptible–infected–recovered (SIR) predicted model for cyathostomins under limited (low host infection rate), moderate and severe (high host infection rate) infectivity scenarios for donkeys. The process data confirmed that once the infection level is low, disease recovery would be much faster than at the high-risk level. The larval numbers on pasture, parasite challenge and development in the host, parasite lifespan and the return of host susceptibility shall be considered when interpreting the complexity of the host–parasite model. The SIR model simulations should be taken into consideration when proposing herd management and health control programmes for donkeys and horses in distinct climatic zones (Molento and Vilela, 2021).

Considering the widespread anthelmintic resistance (AR), the use of selective therapy has been advocated to control parasitic infections, avoiding parasite selection. In horses, a cut-off of 200–500 EPG has been adopted (Nielsen *et al.*, 2014), whereas in donkeys there are no specific indications, and a value of 300 EPG has been suggested (Matthews and Burden, 2013). However, considering that donkeys with >1000 EPG are often asymptomatic this safe FEC limit could be increased. As drug

treatment is a human-made perturbation, the problem is that the lack of efficacy maintains the parasitic infection, increasing the risk of heavy parasitic infections of equids.

The diagnosis of intestinal strongyles is routinely based on copromicroscopic examination (Dias de Castro *et al.*, 2017) and fecal culture for larval identification (Bevilaqua *et al.*, 1993; Lichtenfels *et al.*, 2008; Madeira de Carvalho *et al.*, 2008; Santos *et al.*, 2016, 2018) to differentiate between large and small strongyles. However, larval cultures can result in false negative for *S. vulgaris* but real-time polymerase chain reaction (PCR) (Nielsen *et al.*, 2008), conventional PCR and other PCR-based methods (Gasser *et al.*, 1996; Hung *et al.*, 1999) are available for *S. vulgaris* diagnosis in horses and donkeys (AbouLaila *et al.*, 2020).

Parascaris *spp.*

Generally, equine ascarids refer to only 1 species: *Parascaris equorum*. However, in the literature other 2 species have been described: *Parascaris univalens* and *Parascaris trivalens*. These parasite species differ in having 1 (*P. univalens*), 2 (*Parascaris bivalens* also named *P. equorum*) (Goday and Pimpinelli, 1986) or 3 (*P. trivalens*) pairs of chromosomes (Li, 1937). Hybrids between *P. univalens* and *P. equorum* have been described; however, they are sterile (Goday and Pimpinelli, 1986). *Parascaris univalens* and *P. equorum* are quite similar morphologically and they differ only in their spicula, which appear distally truncated in *P. univalens* and rounded in *P. equorum* respectively (Biocca *et al.*, 1978). Different ascarid populations were karyotyped and identified as *P. univalens* suggesting that this ascarid species and not *P. equorum* is the predominant one in horses (Martin *et al.*, 2018, 2021*a*). There are few phylogenetic analyses for donkeys; however, a recent study on mitochondrial genes COX1 and NADH1 showed that *Parascaris* spp. isolated from donkeys formed a distinct clade

Table 1. Epidemiological studies of major helminth infections in donkeys

Country	Intestinal strongyles (%)	*Parascaris* spp. (%)	*Dictyocaulus arnfieldi* (%)	*Anoplocephala* spp. (%)	Reference
Africa					
Egypt	100	25.0			Attia *et al.* (2018) [1]
	15.85				AbouLaila *et al.* (2020) [2]
Ethiopia	92.8	17.1	2.6		Fikru *et al.* (2005) [3]
	100	+	+	+	Yoseph *et al.* (2005) [4]
	100	50.0	20.0	7.4	Ayele *et al.* (2006) [5]
	100	+	+	+	Getachew *et al.* (2008*a*) [6]
		51.1			Getachew *et al.* (2008*b*) [7]
	99.0	51.0		8.0	Getachew *et al.* (2010) [8]
	92.0	32.5			Ayele and Dinka (2010) [9]
	98.5	22.8	22.2	2.2	Abebew *et al.* (2011) [10]
	100	49.7	66.7	5.6	
	+	52.8	3.6		Ibrahim *et al.* (2011) [11]
	99.5	53.2	42.8	2.9	Zerihun *et al.* (2011) [12]
	82.7				Bogale *et al.* (2012) [13]
			35.3		Solomon *et al.* (2012) [14]
	87.8	42.3			Mezgebu *et al.* (2013) [15]
	70.8	10.4			Regassa and Yimer (2013) [16]
	65.1	13.7	22.2	23.1	Takele and Nibret (2013) [17]
	76.0	26.2			Tesfu *et al.* (2014) [18]
	84.8	20.5	21.8		Biniam and Abdisa (2015) [19]
	75.5	30.0			Seyoum *et al.* (2015) [20]
	100	10.2			Sheferaw and Alemu (2015) [21]
			35.3		Feye and Bekele (2016) [22]
	99.5	53.6			Naramo *et al.* (2016) [23]
	33.9	13.8			Wako *et al.* (2016) [24]
	80.1	41.8	44.0	1.6	Zeryhun and Tsegaw (2016) [25]
	50.5	19.2	5.0	5.0	Ambo *et al.* (2017) [26]
	98.2	21.3			Anteneh and Getachew (2017) [27]
	89.3				Dibala *et al.* (2017) [28]
	57.2	11.2		2.6	Getahun and Kassa (2017) [29]
	79.7	44.8			Mohamee *et al.* (2017) [30]
	36.7	46.0	26.5		Debere *et al.* (2018) [31]
	98.7	28.9	14.3	4.6	Sankuro *et al.* (2018) [32]
	79.3	6.8			Tedla and Abichu (2018) [33]
	66.0	34.8	3.5	16.1	Mathewos *et al.* (2021*a*) [34]
	59.1				Mathewos *et al.* (2021*b*) [35]
	53.0				Negash *et al.* (2021) [36]
	74.7	8.1		3.1	Adeba *et al.* (2022) [37]

(*Continued*)

Table 1. (*Continued.*)

Country	Intestinal strongyles (%)	*Parascaris* spp. (%)	*Dictyocaulus arnfieldi* (%)	*Anoplocephala* spp. (%)	Reference
	100	23.8		0.5	Fesseha *et al.* (2022) [38]
Kenya	87.5				Lewa *et al.* (1999) [39]
	44.7	5.3		0.4	Mulwa *et al.* (2020) [40]
Morocco			47.0		Pandey (1980) [41]
Nigeria	57.0				Ahmed *et al.* (2008) [42]
	78.3	40.3			Jajere *et al.* (2016) [43]
Sudan	29.0	4.6			Kheir and Kheir (1981) [44]
	35.8 large strongyles 36.7 small strongyles	10.7	70.5		Seri *et al.* (2004*a*) [45]
South Africa	+				Mushi *et al.* (2003) [46]
	96.0 97.1 95.2	9.6 0.6 33.9			Wells *et al.* (1998) [47]
Uganda	+	+	+	+	Saul *et al.* (1997) [48]
	42.3		0.4	15.4	Nakayima *et al.* (2017) [49]
America					
Mexico	80				Burden *et al.* (2010) [50]
	93.6				Valdéz-Cruz *et al.* (2013) [51]
	78.9	11.3			Villa-Mancera *et al.* (2021) [52]
Asia					
India		8.4			Kotwal *et al.* (2000) [53]
	54.8	29.2	3.6		Shrikhande *et al.* (2009) [54]
	76.1	14.9			Chitra *et al.* (2011) [55]
	55.3	23.8		1.0	Parsani *et al.* (2013) [56]
	40.7	74.0			Sathiyamoorthy *et al.* (2016) [57]
Iran	46.7	20.0		12.3	Hosseini *et al.* (2009) [58]
	67.3	19.2			Karimi Ghahfarrokhi *et al.* (2014) [59]
	100	15.5			Tavassoli *et al.* (2016) [60]
			31.8		Saadi *et al.* (2018) [61]
	96.4	10.1			Imani-Baran *et al.* (2020) [62]
Iraq	57.1	17.8	17.8		Wannas *et al.* (2012) [63]
Mongolia	6.4	6.4	64.5		Painer *et al.* (2011) [64]
Europe					
Denmark			87.5		Andersen and Fogh (1981) [65]
Germany		2.8			Gothe and Heil (1984) [66]
	48.0	2.2			Epe *et al.* (1993) [67]
	91.7	+	16.2	+	Beelitz *et al.* (1996) [68]
Italy	77.0	9.0	24.0		Giannetto *et al.* (2008) [69]
	93.8	18.0			Trentini *et al.* (2010) [70]
	98.9	15.4	46.1		Garippa *et al.* (2016) [71]
	100				Maestrini *et al.* (2020) [72]

(*Continued*)

Table 1. (Continued.)

Country	Intestinal strongyles (%)	Parascaris spp. (%)	Dictyocaulus arnfieldi (%)	Anoplocephala spp. (%)	Reference
	84.9	3.6	6.9	1.0	Buono et al. (2021) [73]
Macedonia and Thessalia-Greece	73.0 large strongyles 37.8 small strongyles				Sotiraki et al. (1997) [74]
Portugal	+				Couto et al. (2016) [75]
	+	+			Sousa et al. (2021) [76]
Serbia	33.3 Strongylus vulgaris	27.7	55.5	22.2	Živković et al. (2021) [77]
Turkey	94.7	2.6			Demir et al. (1995) [78]
			14.6		Ayaz, (2003) [79]
	72.7	2.7			Gül et al. (2003) [80]
	100	9.8			Uslu and Guçlu (2007) [81]
	96.7	22.5	9.6	6.4	Umur and Açici (2009) [82]
Ukraine	100				Kuzmina et al. (2007) [83]
	100				Kuzmina and Kuzmin (2008) [84]

+, positive animals; [*], see Fig. 1.

compared to those collected from mountain zebras and domestic horses (Peng et al., 2019). Furthermore, a recent whole-genome analysis performed in horses, donkeys and zebras showed that *P. univalens* found in horses belong to distinct clades than those reported in donkeys and zebras (Han et al., 2022).

Parascaris spp. in horses represents the most important parasite infecting young animals, causing coughing, nasal discharge, lethargy, poor appetite, diarrhoea and colic (Clayton and Duncan, 1978). In working equids, poor body condition has been associated with ascarid infection (Getachew et al., 2010; Seyoum et al., 2015). However, horse foals and donkeys bred under optimal farm management programmes may not show this pattern (Bellaw et al., 2016; Buono et al., 2021; Nielsen et al., 2021). In horses, the most important ongoing complication of this parasitosis is small intestine impaction and intussusception, which often requires surgery with a poor prognosis (Tatz et al., 2012), but there is no evidence of clinical complications in donkeys.

In horses, ascarid eggs are excreted mainly by foals and yearlings (<1.4 years old) (Hautala et al., 2019; Scala et al., 2021), whereas in donkeys a high prevalence of *Parascaris* spp. can be seen regardless of the age of the animals (Getachew et al., 2008b, 2010). These differences could be justified by the poor farming conditions of the donkeys that may not have developed an acceptable degree of immunity, associated with the lack of suitable nutrition (Getachew et al., 2010). A recent paper showed that even if ascarid eggs were reported in all age groups, a significantly higher prevalence was found in younger donkeys confirming that, as in horses, the main contaminators of roundworm eggs are juvenile animals (Buono et al., 2021).

The most common diagnostic method in horses and donkeys is by FEC. However, it is not possible to find eggs during the prepatent period and FEC may not correlate well with the worm burden (Nielsen et al., 2010). No statistical association has been seen between FEC and adult worms in horses (Fabiani et al., 2016). Transabdominal ultrasound may be used in routine diagnostics, but veterinarians must have sufficient practice to identify adult *Parascaris* spp. worms in foals (Nielsen et al., 2016).

The control of *Parascaris* spp. represents a key point in breeding farms and all foals under the age of 6 months should be considered potentially infected and at risk of developing acute clinical signs. Therefore, selective therapy is not recommended for juvenile animals if the stud has a history of *Parascaris* spp. infection. For this reason, anthelmintic treatments must be administered to horses and donkeys younger than 1 year of age. Moreover, it is important to evaluate the presence of other intestinal parasites (i.e. strongyles, *Strongyloides westeri*, Anoplocephalidae) and the efficacy of the anthelmintic against *Parascaris* spp. (Nielsen, 2016a). The widespread resistance of *Parascaris* spp. and small strongyles towards MLs and tetrahydropyrimidines (THPs)/benzimidazoles (BZDs), respectively, has complicated the pharmacological control of these helminths considering that in case of mixed parasitic infections, the efficacy of any active agents could be reduced (Molento et al., 2008; Morris et al., 2019).

Dictyocaulus arnfieldi

Dictyocaulus arnfieldi is the lungworm parasite of equids that infects the respiratory tract. Animals became infected by ingesting the infective larvae during grazing. The donkeys represent the competent host, and they are permissive of the entire life cycle of this nematode, whereas in horses, *D. arnfieldi* rarely develops into an adult, although the transmission between horses has been reported (Matthews, 2002).

Donkeys can be infected by a high number of adult helminths without showing clinical signs (Matthews and Burden, 2013) or only slight hyperpnoea and harsh lung sounds (Matthews, 2002). In donkeys, persistent infections are very common and patent infections can persist for at least 5 years. In this regard, donkeys can act as reservoirs and main pasture contaminators, representing an important risk factor for co-grazing horses (Beelitz et al., 1996). Horses may show signs of persistent cough, tachypnoea, chronic pneumonia and pulmonary oedema. Infections can easily develop secondary bacterial infections. In horses, patency is shorter than in donkeys which can range from 6 weeks to 8 months, in foals and older horses, respectively

(Matthews, 2002). Furthermore, young horses are at a major risk of infection than adult horses and are also characterized by a higher larval output (Jenkins *et al.*, 2020).

Considering the clinical signs that can occur in horses (light coughing), it is advisable to pay attention to those animals that share the same pasture with donkeys. It has been reported that donkeys co-grazing with horses are less infected than those that do not co-graze. Thus, the diagnosis of donkeys that share pasture with horses is essential for controlling this parasite and for reducing the risk of infection in horses (Buono *et al.*, 2021). In infected donkeys, the diagnosis is performed through the finding of first-stage larvae (L1), whether free or inside the eggs, in the feces by modified Baermann technique (Rode and Jorgensen, 1989). Considering that temperature fluctuations during the first 48 h following fecal collection could adversely affect the recovery of L1, it is mandatory to perform the parasitological examination of fresh samples (Rode and Jorgensen, 1989).

Horse lungworm infections do not often reach patency and fecal diagnosis is more difficult than in donkeys. Trans-tracheal aspirates can be useful for demonstrating the increase in eosinophil numbers and endoscopic examination may reveal the presence of larvae in the airways, lymphoid follicular hyperplasia and exudate in the trachea and bronchioles (Dixon *et al.*, 1995). Furthermore, an enzyme-linked immune sorbent assay (ELISA) test can demonstrate the presence of antibodies from 5 weeks after infection, and it could be useful during high-risk seasonal (late winter) infections (Tagesu, 2018). Resistance to MLs was determined in *Dictyocaulus viviparus* of cattle (Molento *et al.*, 2006) but no reports have confirmed the expected high efficacy in donkeys and horses.

Anoplocephala *spp.*

Tapeworm infection represents a serious worldwide parasitic disease of equids caused by the species *Anoplocephala perfoliata*, *Anoplocephala magna* that infect horses and donkeys and *Paranoplocephala mamillana* that occurs only in horses. These tapeworm species differ from each other in size, location and pathogenicity (Nielsen, 2016b). *Anoplocephala magna* and *P. mammillana* are less frequent and also have an uncertain or marginal pathogenic role (Gasser *et al.*, 2005). Some biological behaviour of *A. perfoliata* includes preferential adhesion sites, consisting of the caecum wall and the ileo–caeco–colic ostium. The parasite has the tendency to cluster with numerous specimens in this region causing serious clinical intestinal disorders (Edwards, 1986; Fogarty, 1994; Trotz-Williams *et al.*, 2008). The significant association between heavy parasitic burdens (greater than 100 specimens) of *A. perfoliata* and both medical (i.e. spasmodic) and surgical colics of the ileo–caecal–colic tract (i.e. ileo-caecal, caecal–caecal and caecal–colic intussusceptions, ileal impaction) has been demonstrated (Proudman and Edwards, 1993, 1998; Reinemeyer and Nielsen, 2009; Veronesi *et al.*, 2009; Pavone *et al.*, 2011) in horses. The pathogenesis and the clinical impact of this infection in donkeys are still limited. However, colic might be considered an occasional and exceptional onset of infection in both hosts. Animals in good health can tolerate a high-parasitic load, and tapeworm infections are usually suspected due to vague clinical signs – such as weight loss, the opacity of the coat and generic digestive disorders, such as constipation alternating with moderate diarrhoea.

Anoplocephalidae infections are considered a typical parasitosis of equids on pasture, although infections can occur in stabled animals, through the feeding of forage contaminated with oribatid mites, the intermediate hosts of the life cycle. In most of the developed countries, the prevalence of infection has been increasing over time, due to the absence of efficacy of the most used modern anthelmintics (i.e. IVM and MOX). The parasite also reduces the competitive pressure of further parasites of the digestive tract such as strongyles (Bello and Abell, 1999; Gasser *et al.*, 2005). The worldwide prevalence of infection in horses ranges from 18 and 82% with the highest positivity rates observed in humid and rainy areas that favours the development of the intermediate hosts (i.e. Sweden, UK, Germany, etc.) (Fogarty, 1994). Infection occurs in animals of all ages and patent infections can be described starting between the 16th and 20th weeks of life (Gasser *et al.*, 2005). However, there were some reports showing the highest prevalence and intensity of infection in animals younger than 3 years of age (Campigli *et al.*, 2009). In donkeys, epidemiological studies have reported high prevalences in Africa (>80%) (Matthews and Burden, 2013), and low in Europe (<10%) (Buono *et al.*, 2021).

Tapeworm infection in equids can be detected by direct parasitological methods based on qualitative tools. Differently from the other Cyclophyllidea, the search for proglottids in feces is of scant diagnostic value. The proglottids are rarely detectable since before they are released with feces they are broken down directly in the intestine (Nielsen, 2016b). The traditional techniques of concentration by flotation show an overall poor sensitivity (between 11 and 40%) for *A. perfoliata* infection. This is due to the inconstant egg output even in massive infestations (>100 specimens), and cannot be considered predictive of colic risk (Williamson *et al.*, 1998; Abbott and Barret, 2008). To improve the sensibility of detection, specific techniques have been developed in horses that can be used also in donkeys, i.e. Proudman's test (flotation concentration test) (Proudman and Edwards, 1992) and the Cornell–Wisconsin test (sediment concentration test) (Egwang and Slocombe, 1982). These techniques can attain sensitivities between 61 and 92% for parasitic loads greater than 20 specimens (Williamson *et al.*, 1998). Immuno-diagnostic assays have also been developed and validated in horses for the detection of antibodies (immunoglobulin G) stimulated by somatic or excretory/secretory antigens of *A. perfoliata* with a mean sensitivity of 68% and a specificity of 95% (Proudman and Trees, 1996a). A semi-quantitative capture antigen-ELISA test, using a purified 12/13 kDa antigen of *A. perfoliata*, is available in the UK and USA and can be used both for large-scale epidemiological screening and in the clinic as an early diagnostic test to prevent colic episodes (Proudman and Trees, 1996b). In addition, a commercially available ELISA test has also been validated for the detection of antibodies in saliva with a sensitivity of 83% and a specificity of 85% (Lightbody *et al.*, 2016, 2018). Such assay seems to be more useful than those conducted on sera to evaluate the efficacy of drug treatment, since post-treatment antibody levels in saliva decrease faster than in the blood. A coproantigen test in ELISA was also validated to detect the excreted/secreted proteins of *A. perfoliata*, showing a sensitivity of 74% and specificity of 92% (Kania and Reinemeyer, 2005) but is not currently available. Neither of these immunological tests has been validated in donkeys but several experiments have been conducted showing good sensibility (Getachew *et al.*, 2012).

Anthelmintics in horses and donkeys

Donkeys are characterized by the greater activity of some cytochrome p450 isoenzymes than horses, giving them a greater ability to metabolize certain drugs (Peck *et al.*, 1997). For this reason, there may be differences in the drugs' disposition and availability, which may require higher concentrations or shorter dosing intervals than those used in horses to obtain effective drug concentrations for optimum parasite control (Horspool *et al.*, 1994; Mealey *et al.*, 1997; Grosenbaugh *et al.*, 2011).

The most common anthelmintic drugs used in equids are BZDs, THPs, MLs and praziquantel (PZQ) (Gokbulut and McKellar, 2018). Only a few drugs are registered for use in donkeys (none exclusive). Thus, extra-label administration of products registered for horses or ruminants is the norm. Although there is less than a hand-full data on the pharmacokinetics and pharmacodynamics of anthelmintic drugs in donkeys and mules (Gokbulut *et al.*, 2014, 2016*a*), anthelmintic treatment is performed using the same doses and regimens suggested for horses without considering species, breed, nutritional status or individual (height and weight) differences (Lizzaraga *et al.*, 2004; Veneziano *et al.*, 2011, 2013) leading to a reduction in effectiveness (Molento *et al.*, 2008), and an increased risk of toxic signs due to overdosing (Grosenbaugh *et al.*, 2011). Therefore, there is a great need for more data on the donkey as well as new horse anthelmintic drugs and treatment strategies to support welfare (Senior, 2013).

Tetrahydropyrimidines

Pyrantel (PYR) is the only molecule of its class, licensed for horses and is available as salt PYR pamoate (syn. embonate), insoluble in water and as PYR tartrate (soluble in water). PYR pamoate is available as paste or granule formulations, and it is poorly absorbed in the gastrointestinal (GI) tract, which increases its persistence in the intestine (Bjorn *et al.*, 1996). The mechanism of action of THP is by binding to nicotinic acetylcholine receptors of the nematode muscle cells causing spastic paralysis and subsequent elimination from the host (Martin and Robertson, 2007).

A pharmacokinetic study showed that PYR pamoate administered to horses at 13.2 mg kg^{-1} BW was poorly absorbed and the plasma concentration of the parent drug was very low (C_{max} = 0.09 ± 0.02). Moreover, its persistence in feces was 48 h and the highest dry fecal concentration (1.034 mg g^{-1}) was detected at 24 h (Gokbulut *et al.*, 2001*a*). In donkeys, PYR pamoate paste formulation at 6.94 mg kg^{-1} BW showed a lower C_{max} and a smaller area under the curve (AUC) than the granule formulation administered at the same dose (Gokbulut *et al.*, 2014) (Table 2). This difference in plasma levels can be attributed to lower intestinal absorption of the paste rather than granule formulation. The pharmacokinetic parameters of PYR in donkeys are quite different from those reported in horses, showing in the latter a lower concentration and a short mean residence time (MRT) than in donkeys and, consequently a lower bioavailability (Gokbulut *et al.*, 2014). These differences were attributed to a different diet as donkeys were fed with hay while horses were kept in grass pastures. The process promoted a decrease in the gut transit time resulting in a lower bioavailability of PYR in horses (Gokbulut *et al.*, 2001*a*). Moreover, residues of PYR were found in the feces of donkeys and horses for up to 120 h (Gokbulut *et al.*, 2014) and 48 h (Gokbulut *et al.*, 2001*a*), respectively.

In equids, PYR is licensed at a dosage of 6.6 mg kg^{-1} BW (Gokbulut and McKellar, 2018) and it is highly effective against adults of small strongyles, *S. vulgaris* and *Parascaris* spp. but shows moderate activity against *S. edentatus* and *Oxyuris equi* (Mirck, 1985). Moreover, a high dose, twice the nematocidal dose of PYR (13.2 mg kg^{-1} BW) was effective in controlling *A. perfoliata* infection (Slocombe, 1979; Höglund *et al.*, 1998). In donkeys, PYR showed high efficacy against cyathostomins and *S. vulgaris* (Napoli *et al.*, 2013; Gokbulut *et al.*, 2014; Buono *et al.*, 2018). Although it has been reported that PYR-administered high dose used for intestinal strongyles was effective against tapeworms in horses (Höglund *et al.*, 1998), there are still no studies on the effectiveness of PYR pamoate against cestodes of donkeys.

Benzimidazoles and pro-benzimidazoles

The first anthelmintic drug belonging to the BZDs class licensed in 1961 for horses was thiabendazole (Drudge *et al.*, 1981). BZDs are characterized by poor water solubility and are administered as paste or drench formulations in horses (Gokbulut and McKellar, 2018). BZDs bind to the *β*-tubulin of microtubules, preventing their polymerization, destroying the cellular structure causing the death of the parasite (Martin, 1997).

Fenbendazole (FBZ) represents the most common BZD used for horses that belong to the methylcarbamate group. The pharmacokinetics of FBZ is different in donkeys and horses and influencing its efficacy. FBZ is poorly absorbed from the GI tract of horses, and this would explain why higher concentrations are needed to be effective against migrating larvae and encysted larval stages of cyathostomins (McKellar *et al.*, 2002). Following oral administration in donkeys, FBZ and its metabolites, FBZ sulphoxide (oxfendazole – FBZSO) and FBZ sulphone (FBZSO$_2$) were not detected in plasma probably due to the lower absorption and greater fecal excretion when compared to horses (Gokbulut *et al.*, 2006). Furthermore, FBZ showed a longer gut transit time in donkeys than in horses (Gokbulut *et al.*, 2006) (Table 2).

In horses, FBZ is licensed at a dosage of 7.5 mg kg^{-1} BW and it is effective against adult large and small strongyles, *O. equi*. FBZ appears to be the best option for controlling *P. equorum* in foals, characterized by large ascarid burdens (Reinemeyer and Nielsen, 2017). Moreover, in horses, a 5-day regimen of FBZ at 10 mg kg^{-1} BW was effective for controlling the larval stage of small strongyles in enteric mucosa (Duncan *et al.*, 1998). However, a reduced efficacy against early third-stage larvae (EL3), late L3 (LL3) and L4 were reported probably due to the presence of BZD-resistant cyathostomin populations (Reinemeyer *et al.*, 2015; Bellaw *et al.*, 2018). FBZ is registered for use in donkeys, and it seems to have the same spectra of action as in the horse (Gokbulut and McKellar, 2018).

In donkeys, following oral administration of FBZ, 10 mg kg^{-1} BW, the drug was not detected in plasma probably due to the lower absorption and greater fecal excretion of the drug faster than observed in horses (Gokbulut *et al.*, 2006). The lack of absorption of FBZ supports its ineffectiveness against *D. arnfieldi* even when administered at high doses (50 mg kg^{-1} BW) (Taylor and Craig, 1993).

Mebendazole (MBZ) is an anthelmintic drug belonging to the methylcarbamate group and pharmacokinetic studies have shown that the peak plasma concentration and AUC were lower than those of albendazole (ABZ); however, $T_{1/2}$ and MRT were longer than FBZSO and ABZ when administered at 10 mg kg^{-1} BW (Gokbulut *et al.*, 2006, 2016*a*) (Table 2).

The suggested dose of MBZ is 5–10 mg kg^{-1} BW for horses (McKellar and Scott, 1990), and when administered at 8.8 mg kg^{-1} BW it was effective against large and small strongyles (Colglazier *et al.*, 1977). In donkeys, MBZ oral paste administered at 10 and 20 mg kg^{-1} BW was effective against cyathostomins. Moreover, at 10 mg kg^{-1} BW, there was no residue found in the milk of donkeys, apart from what was observed when MBZ was administered at 20 mg kg^{-1} BW. The data suggest that MBZ at 10 mg kg^{-1} BW proved to be effective for controlling cyathostomins in donkeys and its milk-withdrawal period is zero (Gokbulut *et al.*, 2016*a*). Furthermore, MBZ administered at 20 mg kg^{-1} BW for 5 consecutive days was effective in treating *D. arnfieldi* infection in donkeys (McKellar and Scott, 1990).

ABZ belongs to the methylcarbamate group, and it is recommended orally in horses at a dosage of 5 mg kg^{-1} BW. The pharmacokinetics parameters of ABZ and its metabolites (albendazole sulphoxide – ABZSO and albendazole sulphone – ABZSO$_2$) are shown in Table 2. In experimentally infected ponies, ABZ administered at 50 mg kg^{-1} BW twice a day for 2 days was effective in controlling *S. vulgaris* larvae in the cranial mesenteric artery

Table 2. Comparative studies on pharmacokinetic parameters of THPs and BZDs in horses and donkeys

Species	Anthelmintic drug	Abbreviation	C_{max} (µg mL^{-1})	T_{max} (h)	AUC_{last} (µg h mL^{-1})	$AUMC_{last}$ (µg h^2 mL^{-1})	MRT (h)	$T_{1/2}$ (h)	Reference
Horse									
	Pyrantel paste (13.3 mg kg^{-1})	PYR	0.09 ± 0.03	7.50 ± 1.41	1.06 ± 0.24	–	11.99 ± 1.30	13.43 ± 1.38	Gokbulut et al. (2001a)
	Fenbendazole	FBZ	0.04 ± 0.01	8.00 ± 2.70	0.61 ± 0.11	9.33 ± 2.89	14.21 ± 1.74	–	McKellar et al. (2002)
		FBZSO	0.01 ± 0.00	9.50 ± 3.52	0.17 ± 0.02	2.26 ± 0.46	12.90 ± 1.33	–	
		FBZSO$_2$	0.06 ± 0.01	10.50 ± 3.20	1.12 ± 0.19	12.54 ± 2.43	16.50 ± 1.00	–	
		FBZ	0.11 ± 0.05	10.00 ± 7.38	–	–	–	–	Marriner and Bogan (1985)
		FBZSO$_2$	0.16 ± 0.08	12.00 ± 6.20	–	–	–	–	
	Netobimin	ABZSO	0.53 ± 0.14	10.50 ± 3.66	8.63 ± 1.01	129.12 ± 20.10	15.08 ± 2.50	5.97 ± 1.59	Gokbulut et al. (2009)
		ABZSO$_2$	0.36 ± 0.09	19.50 ± 3.96	8.21 ± 2.87	177.55 ± 74.17	21.33 ± 2.31	7.44 ± 1.06	
Donkey									
	Pyrantel paste (6.94 mg kg^{-1})	PYR	0.09 ± 0.02	14.86 ± 5.52	2.65 ± 0.81	–	24.80 ± 5.54	12.39 ± 5.35	Gokbulut et al. (2014)
	Pyrantel granule (6.94 mg kg^{-1})	PYR	0.21 ± 0.07	14.00 ± 9.45	5.60 ± 0.59	–	25.44 ± 10.68	14.86 ± 5.59	
	FBZSO administered	FBZSO	0.49 ± 2.89	5.67 ± 2.89	5.17 ± 0.82	58.12 ± 14.18	10.95 ± 1.92	4.49 ± 0.74	Gokbulut et al. (2006)
		FBZSO$_2$	0.60 ± 0.09	8.00 ± 2.53	10.33 ± 0.87	161.00 ± 22.99	15.38 ± 1.50	7.53 ± 0.35	
	Albendazole	ABZSO	0.08 ± 0.01	5.71 ± 0.87	0.84 ± 0.14	8.27 ± 1.86	9.15 ± 1.20	6.65 ± 3.22	
		ABZSO$_2$	0.04 ± 0.01	8.00 ± 2.31	0.05 ± 0.11	5.68 ± 1.93	9.98 ± 1.58	7.44 ± 1.19	
	Mebendazole (10 mg kg^{-1} BW)	MBZ	0.04 ± 0.01	7.33 ± 3.93	0.78 ± 0.35	19.49 ± 7.86	20.34 ± 7.59	11.97 ± 4.38	Gokbulut et al. (2016a)
	Mebendazole (20 mg kg^{-1} BW)	MBZ	0.07 ± 0.01	8.00 ± 0.00	1.42 ± 0.20	33.78 ± 10.38	23.43 ± 5.55	13.13 ± 3.85	

Adapted from Gokbulut and McKellar (2018).

C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentration; AUC_{last}, area under the (zero moment) curve; $AUMC_{last}$, area under the first moment curve; MRT, mean residence time; $T_{1/2}$, terminal half-life; PYR, pyrantel; FBZ, fenbendazole; FBZSO, fenbendazole sulphoxide; FBZSO$_2$, fenbendazole sulphone; ABZSO, albendazole sulphoxide; ABZSO$_2$, albendazole sulphone; MBZ, mebendazole.

showing few toxic signs in 3 of 11 ponies (Georgi *et al.*, 1980). Furthermore, when administered at 50 mg kg^{-1} BW twice a day for 4 days and at 25 mg kg^{-1} BW 3 times a day for 5 days resulted in a faster larval kill but more toxic symptoms and death occurred in 3 of 6 ponies (Georgi *et al.*, 1980). An extra-label pellet formulation of ABZ licensed for ruminants and administered to horses at a dose of 7.5 mg kg^{-1} BW showed a reduced efficacy against small strongyles. However, considering that there are no precise indications on the evaluation of the formulation used, it is not possible to state the certain presence of AR (Salas-Romero *et al.*, 2017). ABZ suspension (25 mg mL^{-1}) was administered in a single oral dose at 10 mg kg^{-1} BW and 2 oral doses 14 days apart at 10 mg kg^{-1} BW in donkeys and both doses showed high efficacy against adult stages of large and small strongyles until the end of the study period (Imam *et al.*, 2010).

Macrocyclic lactones

MLs (avermectins and milbemycins) are a class of natural and semisynthetic drugs of which IVM, MOX, doramectin (DRM) and eprinomectin (EPM) are the most commonly administered in equids (Gokbulut and McKellar, 2018). MLs are characterized by a high activity against endo- (i.e. nematodes) and ectoparasites (i.e. mites, flies, lice) in humans and animals and thus are also defined as endectocides (Gokbulut and McKellar, 2018). Apart from that, although IVM and MOX have a similar mechanism of action by binding to glutamate and gammabutyric acid-gated chloride channels, these drugs have profound pharmacological differences (Dent *et al.*, 1997; Feng *et al.*, 2002) and must be further studied in donkeys.

In horses, the kinetic of IVM was evaluated in several studies showing significant differences probably due to the distinct

methods of application. A larger AUC and a longer MRT in donkeys than in horses were reported (Gokbulut *et al.*, 2005). These data suggest that in the GI tract of donkeys, IVM had a longer persistence and greater absorption. However, Marriner *et al.* (1987) reported a larger AUC and a higher $C_{\max}$ in horses than in donkeys and these differences could be due also to diet, breed and anatomical differences between horses and donkeys (Table 3). No pharmacokinetic data of MOX are available in donkeys.

In horses, IVM and MOX are registered at 200 and 400 µg kg^{-1} BW, respectively. Both IVM and MOX, only when used orally in combination with PZQ, are active against tapeworms (Gokbulut and McKellar, 2018). Although MLs are highly effective in controlling intestinal strongyles they are not effective to control roundworms (Veronesi *et al.*, 2010). In donkeys, off-label IVM administered at 200 µg kg^{-1} BW was highly effective for controlling small strongyles (Papini *et al.*, 2020). Even though IVM (200 µg kg^{-1} BW) and MOX (400 µg kg^{-1} BW) were effective for controlling intestinal strongyle infection 14 days post-treatment, a shorter egg reappearance period (ERP) was reported for both active drugs in Italy (Buono *et al.*, 2018).

IVM and MOX administered at 200 and 400 µg kg^{-1} BW, respectively, are the drugs of choice for the treatment of *D. arnfieldi* in horses and donkeys (Lyons *et al.*, 1985; Coles *et al.*, 1998; Matthews and Burden, 2013).

DRM following oral administration at 200 µg kg^{-1} BW showed a high persistence and bioavailability than IVM (Gokbulut *et al.*, 2005) in donkeys, and it also showed a larger AUC and a longer MRT than in horses (Table 3). Furthermore, in horses, DRM administered orally at 200 µg kg^{-1} BW showed a faster absorption than the injectable 1% formulation administered at the same dose by intramuscular route (Pérez *et al.*, 2010).

In horses, DRM administered both orally and intramuscularly at 200 µg kg^{-1} BW was effective for controlling intestinal strongyles (Pérez *et al.*, 2010). In naturally infected horses, DRM following oral administration at 200 µg kg^{-1} BW was effective against small strongyles with an ERP of 10 weeks (Cirak *et al.*, 2007). In donkeys, DRM administered at 1 mL per 50 kg by subcutaneous injection was effective for controlling small strongyles until day 28 post-treatment (Elmeligy *et al.*, 2021).

EPM is the last licensed drug belonging to avermectins (Gokbulut and McKellar, 2018) that is 2 or 3 times more effective than IVM and yields zero milk-withdrawal time. Therefore, EPM can be used safely in lactating animals (Shoop *et al.*, 1996). The pharmacokinetic parameters of EPM in horses and donkeys are shown in Table 3 and the low disposition rate of EPM in the milk of animals allows the use of the drug in milk-producing horses (Gokbulut *et al.*, 2016b) and donkeys (Gokbulut *et al.*, 2011). In donkeys, EPM following pour-on administration (bovine dose – 500 µg kg^{-1} BW) was effective in eliminating *D. arnfieldi* larvae for 28 days (Veneziano *et al.*, 2011). Moreover, following topical administration, EPM showed high efficacy against large and small strongyles (Gokbulut *et al.*, 2011).

Praziquantel

PZQ is an isoquinoline and it is licensed both in human and animal medicine for controlling cestodes and trematodes (Gokbulut and McKellar, 2018). PZQ acts by binding to the parasites' glutathione S-transferase (McTigue *et al.*, 1995), altering the concentration of intracellular calcium, causing muscle contractions and tegument rupture (Harnett, 1988). There are no data on the pharmacokinetics of PZQ in horses and donkeys but, it is quickly and widely absorbed following administration in humans (Leopold *et al.*, 1978).

In horses, PZQ is registered at 1.0 mg kg^{-1} BW and a PZQ paste of 9% was effective for controlling *A. perfoliata*, *A. magna* and *A. mamillana* (Slocombe *et al.*, 2007). Moreover, following PZQ administration, the prevalence of cestodes was reduced by 96% until 10 weeks post-treatment (Lyons *et al.*, 2017). Similarly, PZQ paste administered orally at 1 mg kg^{-1} BW was effective for controlling *A. perfoliata* in donkeys (Getachew *et al.*, 2013). In equids PZQ is licensed in association with the MLs (IVM and MOX); however, anecdotal data suggest that PZQ should be poorly tolerated in donkeys (Matthews and Burden, 2013). PYR pamoate paste administered at a dose of 13.2 mg kg^{-1} BW was safe and highly efficacious for controlling *Anoplocephala* spp. infection in horses (Marchiondo *et al.*, 2006) and, although there are no safe dosage studies in donkeys, it would be safer to administer PYR (at a double dose of 13.2 mg kg^{-1} BW) than PZQ for controlling *Anoplocephala* spp.

AR in equids: horses *vs* donkeys

Resistance is the ability of a worm population to survive treatments generally effective against the same species and stage of infection (Sangster, 1999). Drug resistance was first reported in horses in the 1960s against phenothiazine in small strongyles (Gibson, 1960). Resistance to anthelmintics persists for many years and is transmitted to parasite populations being a genetic-based trait. In horses, the overuse of anthelmintics has led to the development of resistance, especially against small strongyles and *Parascaris* spp. (Molento, 2005; Peregrine *et al.*, 2014). The FEC reduction test is the *in vivo* test for determining the effectiveness of anthelmintic treatment against intestinal strongyles and *Parascaris* spp. in horses and donkeys and it is based on the percentage of reduction of eggs in the faces after 14 days post-treatment (Nielsen *et al.*, 2019). The first report of cyathostomin resistance to PYR in horses was published in 1996 (Chapman *et al.*, 1996) and nowadays, resistance to this drug class is very common (Zanet *et al.*, 2021; Nielsen, 2022). In horses, resistance to THP was evaluated since 2000 in 37 studies and reported in 34 (92%) (Nielsen, 2022).

Resistance to the BZDs has been commonly reported in the equine industry worldwide (Matthews, 2014) and in wild equids (Kuzmina *et al.*, 2020), showing that resistance against this anthelmintic drug class is not always associated with the intensity of anthelmintic treatments. In horse strongyle infection, resistance to the BZDs has been associated with the polymorphisms of codons 167, 168 and 200 of isotype 1 β-tubulin (Ishii *et al.*, 2017; Özben *et al.*, 2022). In horses, since 2000, AR against BZDs has been evaluated in 58 studies and it was reported in all of them (Nielsen, 2022). Moreover, in horses, multiple resistance to different anthelmintic drug classes has been reported (Flores *et al.*, 2020) and it is quite common to find a population of small strongyles resistant both to BZD and THP (Canever *et al.*, 2013). Another minor mutation was described by Ishii *et al.* (2017) at codon 172 that deserves further research.

Although MLs are the most commonly administered anthelmintic drugs in horses (Tzelos *et al.*, 2019) as in donkeys (Buono *et al.*, 2021), drug resistance is not so common and it may develop in a more complex way – as more genes are involved. However, in the last few years, several studies have reported a reduced efficacy of MLs against small strongyles in horses, both as a reduced efficacy at 14 days post-treatment and as a shortened ERP (Molento *et al.*, 2008, 2012; Tzelos *et al.*, 2017; Nielsen *et al.*, 2020; Abbas *et al.*, 2021) that it was postulated representing the first sign of emerging resistance (Sangster, 1999). For this reason, in the next few years, an increased numbers of reports of AR to MLs are expected worldwide. A recent study showed that a shortened ERP cannot be explained only by the survival of 4th-stage larvae but probably could be associated also with other factors

Table 3. Comparative studies on pharmacokinetic parameters of MLs in horses and donkeys

Species	Anthelmintic drug	Route	C_{max} (ng mL^{-1})	T_{max} (h)	AUC$_{last}$ (ng h mL^{-1})	MRT (h)	$T_{1/2}$ (h)	Reference
Horse								
	Ivermectin (200 µg kg^{-1})	P.O.	82.3	3.1	4821.6	–	66.3	Marriner *et al.* (1987)
			46.3	7.0	2646	–	–	Scott (1997)
			44.0	2.2	3184	114.7	102	Pérez *et al.* (1999)
			21.4	7.9	1106.4	55.2	51.6	Gokbulut *et al.* (2001*b*)
			51.3	3.6	3290.4	100.8	69.4	Pérez *et al.* (2003)
			61.3	4.1	3959	176.2	156.7	Gokbulut *et al.* (2010)
			25.8	5.5	1941.8	117.6	88.1	Gokbulut *et al.* (2016*b*)
		P.O. paste	30.1	7.92	2736	122.4	131.52	Saumell *et al.* (2017)
		P.O. sol.	33.7	12	3312	124.8	151.68	
	Doramectin (200 µg kg^{-1})	P.O.	21.3	8.0	1279.2	72.0	93.8	Gokbulut *et al.* (2001*b*)
			51.6	4.8	4286.4	185.3	124.3	Pérez *et al.* (2010)
	Eprinomectin (500 µg kg^{-1})	T.	17.7	43.2	424.6	70.3	32.9	Gokbulut *et al.* (2016*b*)
Donkey								
	Ivermectin (200 µg kg^{-1})	P.O.	23.6	24	2863.2	156.0	177.6	Gokbulut *et al.* (2005)
	Ivermectin (300 µg kg^{-1})		43.2	8.0	1811	–	–	Scott (1997)
	Doramectin (200 µg kg^{-1})	P.O.	33.9	24.0	5493.6	218.4	266.4	Gokbulut *et al.* (2005)
	Eprinomectin (500 µg kg^{-1})	T.	6.1	113.0	1761.4	215.5	113.44	Gokbulut *et al.* (2011)
			14.2	81.1	3106.4	214.8	151.9	Gokbulut *et al.* (2013)

Adapted from Gokbulut and McKellar (2018).

C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentration; AUC$_{last}$, area under the (zero moment) curve from time 0 to the last detectable concentration; MRT, mean residence time; $T_{1/2}$, terminal half-life; P.O., oral route/*per os*; T., topical.

Table 4. Studies in donkeys reporting anthelmintic efficacy – FECRT (14 days post-treatment)

Country	Reference	Categories of parasites	PYR	ABZ[a]	MBZ	FBZ	IVM	MOX	EPM[a]	DRM[a]	PZQ[a]
Bulgaria	Binev et al. (2005)	Large and small strongyles					96% (SC)-SR				
Egypt	Elmeligy et al. (2021)	Strongylus spp.								100% (SC)-S	
Ethiopia	Fesseha et al. (2020)	Intestinal strongyles Parascaris equorum				<94%-SR	100%-S 100%-S				
	Getachew et al. (2013)	Anoplocephala perfoliata									100%-S
Germany	Becher and Pfister (2010)	Intestinal strongyles						100%-S			
Italy	Buono et al. (2018)	Small strongyles	99.3%-S 86.3%-SR			99.8%-S 83.9%-R	100%-S 100%-S	100%-S 100%-S			
	Gokbulut et al. (2011)	Large and small strongyles							100%-S		
	Gokbulut et al. (2014)	Large and small strongyles	98.5%-S (paste) 97.3%-S (granule)								
	Gokbulut et al. (2016a)	Small strongyles			99.7%-S 99.3%-S						
	Napoli et al. (2013)	Small strongyles D. arnfieldi	93%-S 100%-S						99.6%-S 100%-S		
	Papini et al. (2020)	Small strongyles					100%-S				
	Veneziano et al. (2011)	D. arnfieldi							100%-S		
	Veneziano et al. (2015)	Small strongyles						99.7%-S			
Nigeria	Okaiyeto et al. (2022)	Intestinal strongyles					76%-R				
Romania	Cernea et al. (2015)	Intestinal strongyles		86%-R							
Spain	Arias et al. (2013)	Intestinal strongyles					100%-S				
Sudan	Seri et al. (2004b)	Large and small strongyles P. equorum Large and small strongyles								100% (IM)-S 100% (IM)-S 99.2% (SC)-S	

	Seri *et al.* (2005)	Large and small strongyles *P. equorum*		100% (IM)-S 100% (IM)-S	
	Imam *et al.* (2010)	Large and small strongyles *P. equorum*	100%-S 100%-S	100%-S 100%-S	
	Fangama *et al.* (2013)	Intestinal strongyles		100% (SC)-S	90.9% (SC)-R 78.5% (IM)-R
UK	Trawford *et al.* (2005)	Small strongyles			87%-R (inj. for. PO) 31%-R (inj. for. PO)
	Lawson *et al.* (2015)	Small strongyles	72%-R		
Ukraine	Kuzmina *et al.* (2020)	Small strongyles		45.2%-R	

PYR, pyrantel pamoate; ABZ, albendazole; MBZ, mebendazole; FBZ, fenbendazole; IVM, ivermectin; MOX, moxidectin; EPM, eprinomectin; DRM, doramectin; PZQ, praziquantel; SC, subcutaneous administration; IM, intramuscular administration; inj. for., injectable formulation; PO, *per os*.

According to American Association of Equine Practitioners Parasite Control Guidelines cut-off (Nielsen *et al.*, 2019), the cut-off values used to interpret the results of FECRT were the following: PYR susceptible (S) >90%, suspected resistance (SR) 85–90%, resistant (R) <85%; FBZ susceptible (S) >95%, suspected resistance (SR) 90–95%, resistant (R) <90%; IVM/MOX susceptible (S) >98%, suspected resistance (SR) 95–98%, resistant (R) <95%.

[a]For these anthelmintic drugs, cut-off values are not suggested in AAEP Guidelines.

such as the selection of species or strains that accelerate their life cycle. Thus, a shortened ERP could not indicate the development of drug resistance (Nielsen *et al.*, 2022).

Since 2000, resistance against MLs in horses has been evaluated in 57 studies and reported in 13 (23%) (Nielsen, 2022). In several nematode species, MLs resistance is associated with a group of genes that encode adenosine triphosphate-binding cassette transporters (Tydén *et al.*, 2014; Raza *et al.*, 2015).

In donkeys, drug resistance has not been reported as commonly as in horses and few clinical trials have been performed for evaluating the efficacy of the most common anthelmintic drugs (FBZ, PYR and MLs) (Matthews and Burden, 2013). Resistance to PYR was reported in 2 donkey farms in the UK (Lawson *et al.*, 2015); furthermore, in 1 of these farms, a suspected resistance to MOX was reported 10 years earlier after continuous use of the cattle formulation (Trawford *et al.*, 2005). However, considering that donkeys were treated orally using an injectable formulation licensed for cattle, the data reported for MOX probably did not confirm the presence of AR. Recently, a population of small strongyles resistant to FBZ and PYR was reported in an Italian donkey farm, also associated with a reduction of ERP for IVM and MOX (Buono *et al.*, 2018).

In horses, the first report of MLs resistance in *Parascaris* spp. was described by Boersema *et al.* (2002) in the Netherlands. Nowadays, AR to MLs have been reported in 29 out of 29 studies worldwide, while resistance against THP and BZDs was reported in 4 out of 16 and 3 out of 13 studies, respectively (Nielsen, 2022). The sporadic resistance of *Parascaris* spp. against BZDs and THP should be due to the limited studies on the effectiveness of these anthelmintic drug classes. Thus, drug resistance should be more common than those reported in the literature, as also suggested in some studies (Martin *et al.*, 2018, 2021*b*; Hautala *et al.*, 2019).

AR of *P. univalens* against BZDs is not associated with SNP suggesting that the mechanism of AR in this parasite is different from those reported for intestinal strongyles (Martin *et al.*, 2021*b*). The lack of efficacy of IVM, MOX and PYR has been reported also in donkeys (Matthews and Burden, 2013). Studies on anthelmintic efficacy in donkeys are reported in Table 4.

Conclusions

The host–parasite relationship is a complex process in equids that still needs much attention. Although donkeys and horses are closely related species and share practically the same parasite fauna, they are divergent in their physiological and pharmacological (absorption and distribution of drugs) characteristics. Some features such as breed, geographic location/climate, parasite challenge and intensity, immune response and control methods including chemical use shall be regarded as a priority to help solve important parasitic infections in both species. Target selective treatment can also be adopted, looking mainly for clinical signs, performance (body growth and withers height) and to FEC. Once adopted, the selective strategy may help preserve drug effectiveness and the welfare of both horses and donkeys. Horses and donkeys are 'different cousins'; for this reason, precise farm management and parasite control programme must take these differences into account.

Author's contribution

F. B. conceived and designed the study and wrote the first draft of the manuscript. V. V., F. V. and M. B. M. conceived and designed the study and wrote and reviewed the manuscript.

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