

Research Paper

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Helminth infections in Italian donkeys: *Strongylus vulgaris* more common than *Dictyocaulus arnfieldi*

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Abstract

Donkeys have been used as working animals for transport and farm activities worldwide. Recently, in European countries, there has been an increasing interest in donkeys due to their use as pets, onotherapy or milk production. During 2014–2016, a countrywide survey was conducted to determine prevalence and risk factors of principal helminth infections in 1775 donkeys in 77 Italian farms. A questionnaire on management and parasite control practices was filled out for each farm. Faecal samples were examined using a modified McMaster technique, a centrifugation/flotation method and a sedimentation technique. Pooled coprocultures were performed for differentiation of strongylid eggs. Strongyles were the most common parasites detected (84.9%), followed by *Dictyocaulus arnfieldi* (6.9%), *Oxyuris equi* (5.8%), *Parascaris* spp. (3.6%), *Anoplocephala* spp. (1.0%), *Strongyloides westeri* (0.3%). Coprocultures revealed an omnipresence of cyathostomins (100%), followed by *Strongylus vulgaris* (31.0%), *Poteriostomum* spp. (25.0%), *Triodontophorus* spp. (9.0%), *Strongylus edentatus* (7.0%), *Strongylus equinus* (5.0%). Logistic regression analysis identified breed, co-pasture with horses, living area, herd size and number of treatments as significantly associated with strongyles. Sex, age, living area and herd size were significantly associated with *Parascaris* spp. *Dictyocaulus arnfieldi* was significantly associated with sex, grass, co-pasture with horses, living area and herd size. *Strongylus vulgaris* was significantly associated with living area and herd size. The mean number of anthelmintic treatments/year was 1.4; most of the donkeys (71.8%) were dewormed using an ivermectin drug. It is important to design parasite programs to specifically address both *D. arnfieldi* and *S. vulgaris* in donkeys, and this is especially important if donkeys co-graze with horses.

Introduction

Donkeys (*Equus asinus*) are traditionally used as working animals for transportation and farm activities in developing countries (Ali *et al.*, 2014). In Europe, donkey populations have declined from the 1960s and nowadays, according to the Food and Agriculture Organization, the population is estimated to be about 395,910 animals (FAO, 2020). In Italy, the donkey population has gradually decreased due to industrialization and urbanization from 500,000 in the 1960s to about 23,000 in the early 2000s (FAO, 2020).

In recent years, donkeys have found increasing use in leisure activities, onotherapy (animal-assisted therapy) and milk production (Veneziano *et al.*, 2011). This has caused an increase of the Italian donkey population to currently 92,965 donkeys (AIA, 2020). In Italy, there is a long-established tradition in donkey breeding as confirmed by the presence of several autochthonous breeds such as Amiantino, Asinara, Martina Franca, Ragusano, Romagnolo, Pantesco, Sardo, Grigio Siciliano and Viterbese (Bigi & Zanon, 2008). However, despite the economic and sanitary relevance of parasitosis in farm animals, there are studies on ectoparasites and protozoa, but limited epidemiological data regarding helminths affecting this species available in the scientific literature (Matthews & Burden, 2013).

In general, all common helminth parasites of horses also infect donkeys (Matthews & Burden, 2013), and equine intestinal strongyles are considered ubiquitous in grazing equids worldwide, so co-grazing animals can act as a source of infection for both species (Matthews & Burden, 2013). Similar to horses, the most important parasitic nematodes of

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donkeys are Strongylidae (large strongyles – *Strongylus vulgaris*, *Strongylus edentatus*, *Strongylus equinus*, *Strongylus asini* – and small strongyles – Cyathostominae). Other relevant helminths are ascarids (*Parascaris* spp.), pinworms (*Oxyuris equi*), threadworms (*Strongyloides westeri*), tapeworms (*Anoplocephala* spp.) and lungworms (*Dictyocaulus arnfieldi*) (Matthews & Burden, 2013).

Donkeys represent the main competent host and reservoir for *D. arnfieldi* and the major source of pasture contamination for horses (Beelitz *et al.*, 1996), although infection from horse to horse is reported (Matthews, 2002). Clinical signs in donkeys are quite rare, but in horses, tachypnea and persistent cough could be present (Matthews, 2002).

Moreover, horses and donkeys can be parasitized by the liver fluke, *Fasciola hepatica*, in wetter areas (Matthews & Burden, 2013). Although massive parasitic infections are often subclinical in donkeys, the impact of endoparasites on their health, welfare and production is still unclear (Buono *et al.*, 2018). Generally, donkeys with a high parasite load of small strongyles appear healthy, and mainly overworked animals or those with poor physical status show clinical signs, as reported in developing countries where substandard environmental conditions exist (Burden *et al.*, 2010; Matthews & Burden, 2013; Tavassoli *et al.*, 2015). Regarding large strongyles, in an eight-year-old donkey, *S. vulgaris* caused obstruction of the cranial mesenteric artery, which led to death of the animal (Borji *et al.*, 2014).

Anthelmintic therapy represents a main strategy to control endoparasitosis in horses (Nielsen *et al.*, 2018), as well as in donkeys (Buono *et al.*, 2018); however, only few anthelmintic drugs are registered for use in donkeys, so extra-label use of products registered for horses and ruminants is the norm (Gokbulut & McKellar, 2018).

Previously in Italy, only three reports on a small number of donkeys (approximately 100 animals) were available about helminth infections (Giannetto *et al.*, 2008; Trentini *et al.*, 2010; Garippa *et al.*, 2016). In Europe, robust parasitological studies have been carried out by The Donkey Sanctuary (UK) but did not aim to conduct an epidemiological study on a national level (Matthews & Burden, 2013).

The aims of the present survey were to: (1) determine the prevalence and distribution of main helminth infections in Italian donkeys; (2) investigate the risk factors associated with infection; and (3) describe current parasite control practices adopted for donkeys.

Materials and methods

Study animals

The study was performed during 2014–2016 and included 1775 donkeys raised on 77 farms located in 13 Italian regions (nine farms from northern Italy; 28 from central Italy; 26 from southern Italy; 14 from islands). The sample size was calculated using the formula proposed by Thrusfield (2007), including the following information: study population at the time of the start of the study (58,647 donkeys; data supplied by Italian Breeders Association – AIA, 2013); expected prevalence (80%) based on the results of a regional study on intestinal strongyles in donkeys conducted in Sicily, southern Italy (Giannetto *et al.*, 2008); desired absolute precision (3%); and confidence interval (CI) (99%).

The age of donkeys was determined matching owners' information with examination of teeth, according to the guide proposed by The Donkey Sanctuary (2016). The animals were classified into four age groups: ≤1 year; 1–4 years; 4–10 years; and >10 years. Furthermore, body condition scores (BCS) were determined using the chart developed by The Donkey Sanctuary (2008). Briefly, a scale of 1–5 was applied (1 = poor, 2 = moderate, 3 = ideal, 4 = fat and 5 = obese).

All procedures on the donkeys were performed with the owner's consent and according to the European Communities Council Directive (86/609/EEC). The investigation was approved by the Ethical Animal Care and Use Committee of the University of Naples 'Federico II' number 889/04.

Questionnaires and data collection

On each farm, owners were interviewed using a purpose-designed questionnaire to obtain a complete history of the farms and examined animals. The questionnaire consisted of 36 closed-ended questions (multiple choice and yes/no options) and it was divided into three sections (general information, grazing practices and farm management, helminth control procedures). The questionnaire can be found in the supplementary material.

The first section included general information about the farms and donkeys: living area (northern, central, southern Italy and island) as previously defined (Otranto & Dantas-Torres, 2010), herd size (number of donkeys; small: ≤25; medium: 25–50; large: >50), breed (pure, such as Amiatino, Martina Franca, Ragusano, Romagnolo, or mixed; hybrids like mules and hinnies were not included in this study), sex (male, female, gelding), age (≤1 year; 1–4 years; 4–10 years; >10 years), BCS, primary use (milk production, meat production, onotherapy, recreational activities, milk production for cosmetic industry and pet animal).

The second section included information about farm management: availability of box stalls and access to grazing (yes or no); months of grazing per year; and co-grazing with horses (yes or no) or ruminants (yes or no).

The third section included information about anthelmintic treatments and deworming strategy: the number of treatments per donkey per year (0; 1–2; >2) for each anthelmintic drug type considered (ivermectin (IVM), moxidectin (MOX), benzimidazoles (BZ), pyrantel (PYR), IVM/praziquantel (PZQ), MOX/PZQ, other products).

Coprological analysis

Individual fecal samples were collected from all donkeys included in the study (1775) and, according to general recommendations proposed by Nielsen *et al.* (2010a), faeces were taken directly from the rectum of each animal, or alternatively picked up off the ground from fresh deposits (especially from nervous animals and foals) using plastic gloves. Fecal samples were stored in portable refrigerators (about 4°C) and individual fecal egg counts (FECs) were performed within 48 h, using a special modification of the McMaster method with a detection limit of ten eggs per gram (EPG) (Zajac & Conboy, 2011). The floatation medium used was the Sheather's sugar solution with a specific gravity of 1.250 (Lester & Matthews, 2014). Based on visual morphological identification (Zajac & Conboy, 2011), each egg was classified as belonging to intestinal strongyles, *Parascaris* spp., *S. westeri*, *O. equi*, *Anoplocephala* spp. Furthermore, a qualitative centrifugation/flotation technique (Proudman & Edwards, 1992) using

Sheather's sugar solution and a qualitative sedimentation technique (Ambrosi, 1991) were used for the detection of *Anoplocephala* spp. and *F. hepatica* eggs, respectively.

Fecal cultures and speciation of intestinal strongyle

Pooled fecal cultures (from a maximum of five donkeys) were set up for each farm. Briefly, 20 g of faeces from samples with FECs greater than the suggested 300 EPG selective therapy cut-off (Matthews & Burden, 2013) were used and then incubated at 27–30°C for 7–10 days for larval development. At the end of the incubation period, third-stage larvae (L3s) were recovered by the Baermann technique (MAFF, 1986) and identified according to their specific genera and/or species, based on the shape and number of gut cells, using the keys proposed by the Atlas of Diagnosis of Equine Strongylidosis (Cernea *et al.*, 2008). Where possible, up to 100 larvae (L3s) were identified.

Baermann technique and diagnosis of *D. arnfieldi*

For each donkey, individual Baermann technique was also used to ascertain the occurrence of lung worm infection on 30 g of faeces, in a double layer of gauze, closed with an elastic band and placed in a funnel (Zajac & Conboy, 2011). The funnel was filled with tap water until the faeces were immersed. After 24 h, the content of each Baermann was collected in 15 ml tubes and centrifuged at 400 g for 5 min. The supernatant was discarded, and 1 ml of each pellet was transferred to nematode slides and examined under microscope.

Statistical analysis

Animal-level prevalence was computed with the associated 95% CIs for all the considered parasites (intestinal strongyles, *Parascaris* spp. and *D. arnfieldi*) with the exception of *S. vulgaris*, whose prevalence was estimated at farm level. All the answers from the questionnaires were included as independent variables in a multivariate logistic regression analysis developed by a step-wise regression process to explore factors that could be considered predictors for positive status (>0 EPG) for intestinal strongyles, *Parascaris* spp. and *D. arnfieldi* (>0 larvae per gram) at the animal level (sex, breed, presence of pasture, co-pasture with ruminants, co-grazing with horses, BCS, age, living area, herd size and number of treatments/year) and *S. vulgaris* at the farm level (presence of pasture, co-pasture with ruminants, co-pasture with horses, presence of young stock (donkeys <1 year) living area, herd size and number of treatments/year). Results were presented as adjusted odds ratios. All the statistical analyses were performed using commercial software (SPSS, Version 22.0, Chicago, IL, USA).

Results

Study animals and questionnaire survey

Of 1775 donkeys, 396 (22.3%) were intact males, 1354 (76.3%) were females and 25 (1.4%) were geldings. Most of the donkeys (937, 52.8%) were mixed-breed, while 838 (47.2%) were pure-breed. The majority of donkeys (1133–63.8%) showed an ideal BCS (BCS = 3), 198 (11.2%) had a BCS < 3 and 206 (11.6%) > 3. For 238 (13.4%), BCS was not available. The mean age of animals

± standard deviation was 7.7 ± 5.8 years (range: three months–33 years).

Regarding location of farms, 28/77 (36.4%) were located in central Italy for a total of 693 donkeys (39.0%), 26/77 (33.8%) in southern Italy for a total of 379 donkeys (21.4%), 14/77 (18.2%) in Sicily for a total of 266 donkeys (15.0%) and 9/77 (11.7%) in northern Italy for a total of 437 donkeys (24.6%).

In most of the farms (66/77, 85.7%), donkeys lived at pasture, while in 11/77 (14.3%) donkeys had no access to pasture; 1612 donkeys (90.8%) had access to pasture, while 163 (9.2%) lived in box stalls with access to a small, annexed paddock. Of 1612 donkeys belonging to the 66 farms that had access to pasture, grazing was permanent for 1265 donkeys (78.5%), while seasonal pasture access was provided to 347 donkeys (21.5%). Grazing time ranged from three to 12 months. Mixed/alternate grazing with cattle was reported for 336/1612 donkeys (20.8%), while 797/1612 donkeys (49.4%) co-grazed with horses.

Owners reported that donkeys were involved mainly in milk production for human nutrition (816, 45.9%) and as pet animals (723, 40.7%); other uses included onotherapy (332, 18.7%), recreational outdoor activities (274, 15.4%), meat (153, 8.6%) and milk production for the cosmetic industry (97, 5.4%). Several donkeys were employed in more than one activity and not all owners raised donkeys for reproductive purposes.

Sixty out of 77 (78.0%) farm owners declared to perform anthelmintic treatments, and the overall number of treated donkeys was 1557 (87.7%), while 17 owners (22.0%) did not treat their animals with anthelmintics (218–12.3%). Regarding the treatment scheme, the highest percentage of owners (53.3%) reported deworming their donkeys once a year, followed by 43.3% and 3.3% treating twice and four times/year, respectively; none of the owners treated three times/year. The mean number of anthelmintic treatment/year was 1.4 (range 1–4). Owners declared using different anthelmintic drugs during the year following arbitrary and empirical rotational criteria. Macrocytic lactones (MLs) (IVM and MOX) were the drugs most frequently administered (84.8%) (IVM 71.8% and MOX 13.0%); sometimes, IVM was administered in association with PZQ (5.5%), whereas no donkey was treated with MOX + PZQ. Other drugs used were BZ (22.4%), PYR (21.3%), PZQ (0.2%) and other active principles such as phytotherapeutic dewormers (3.1%) (fig. 1).

Parasitological examinations

In total, 1536/1775 donkeys (86.5%) (95% CI 84.9–88.1) were helminth positive in single or mixed infections; 1271 (82.7%), 251 (16.3%), 10 (0.7%) and 4 (0.3%) were parasitized by one, two, three and four helminth categories, respectively (table 1).

Intestinal strongyle eggs were found in all tested farms (77/77–100%) with a prevalence at animal level of 84.9% (1507/1775) (95% CI 83.2–86.6), with a single strongyle infection in 1248. In 502 positive donkeys (28.3%), strongyle FEC was lower than the recommended donkey cut-off for selective anthelmintic therapy (300 EPG). The average EPG was 706.4 ± 747.8 (min 10–max 9200). Regarding the egg shedding level, 43.4% of donkeys had a FEC < 300 EPG (low contaminators), 19.1% had a FEC ranging between 300 and 600 EPG (moderate contaminators) and 37.5% had a FEC > 600 EPG (high contaminators) (fig. 2). The infection rate for intact males, females and geldings was 85.1%, 85.1% and 72.0%, respectively. No significant differences related to sex were

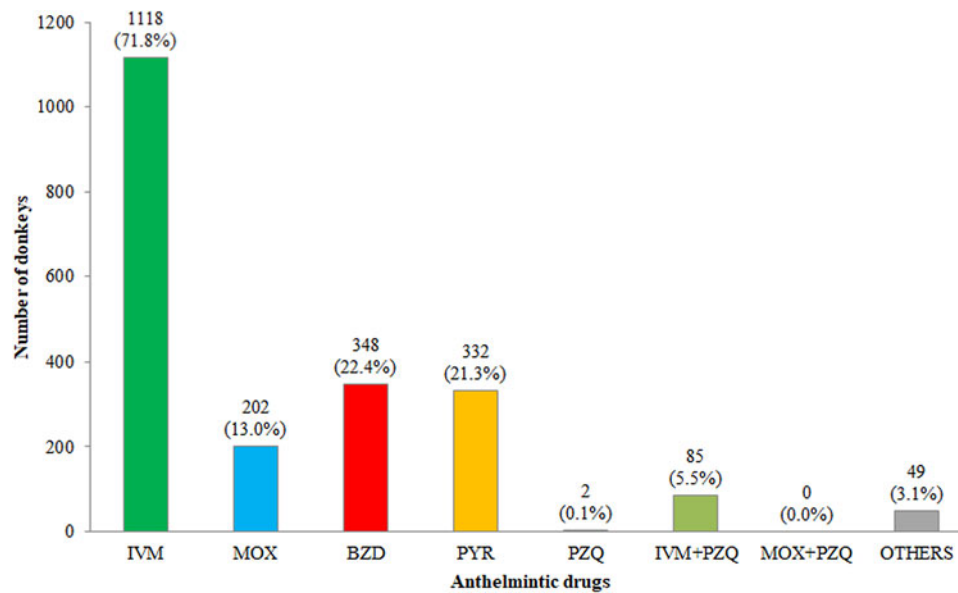


Fig. 1. Anthelmintics administered by donkey owners/breeders. Respondents could use more than one drug. Abbreviations: IVM, ivermectin; MOX, moxidectin; BZD, benzimidazoles; PYR, pyrantel pamoate; PZQ, praziquantel.

Table 1. Single and mixed helminth infections in study donkeys ($N = 1775$).

Categories of parasites	No. of donkeys
Intestinal strongyles	1248
<i>Parascaris</i> spp.	7
<i>Oxyuris equi</i>	7
<i>Dictyocaulus arnfieldi</i>	7
<i>Anoplocephala</i> spp.	2
Intestinal strongyles + <i>Parascaris</i> spp.	47
Intestinal strongyles + <i>S. westeri</i>	2
Intestinal strongyles + <i>O. equi</i>	80
Intestinal strongyles + <i>D. arnfieldi</i>	102
Intestinal strongyles + <i>Anoplocephala</i> spp.	14
<i>Parascaris</i> spp. + <i>O. equi</i>	2
<i>Parascaris</i> spp. + <i>D. arnfieldi</i>	2
<i>O. equi</i> + <i>D. arnfieldi</i>	1
<i>O. equi</i> + <i>Anoplocephala</i> spp.	1
Intestinal strongyles + <i>Parascaris</i> spp. + <i>O. equi</i>	3
Intestinal strongyles + <i>Parascaris</i> spp. + <i>D. arnfieldi</i>	2
Intestinal strongyles + <i>O. equi</i> + <i>D. arnfieldi</i>	4
Intestinal strongyles + <i>O. equi</i> + <i>Anoplocephala</i> spp.	1
Intestinal strongyles + <i>S. westeri</i> + <i>O. equi</i> + <i>D. arnfieldi</i>	3
Intestinal strongyles + <i>Parascaris</i> spp. + <i>O. equi</i> + <i>D. arnfieldi</i>	1
No infection	239

found. The infection rate of *Parascaris* spp. was 3.6% (64/1775 donkeys) (95% CI 2.8–4.5) and the average EPG was 391.6 ± 525.7 (min 10–max 2600) with a farm prevalence of 30.0%.

Faecal cultures revealed the presence of larvae (L3s) of *Cyathostomum sensu lato* (100%) followed by *S. vulgaris* (31.0%), *Poteriostomum* spp. (25.0%), *Triodontophorus* spp. (9.0%), *S. edentatus* (7.0%) and *S. equinus* (5.0%).

Dictyocaulus arnfieldi larvae were reported in 122 donkeys (6.9%; CI 5.7–8.1) with a farm prevalence of 24.7%. The mean number of larvae was 57.5 (min 1–max 285).

Oxyuris equi, *Anoplocephala* spp. and *S. westeri* eggs were reported in 103 (5.8%; CI 4.7–6.9%), 18 (1.0%; CI 0.6–1.5%) and in five (0.3%; CI 0.1–0.5%) donkeys, with a farm prevalence of 24.7%, 6.5% and 3.9%, respectively. None of the examined donkeys were positive for liver fluke *F. hepatica*.

Risk factor analyses

Results of logistic regression analysis for risk factors associated with patent infections of intestinal strongyles, *Parascaris* spp. and *D. arnfieldi* are summarized in tables 2–4. The results of the analysis at farm level of the presence of *S. vulgaris* are summarized in table 5. In the logistic regression analysis of intestinal strongyles, breed, co-pasture with horses, living area, herd size and number of treatments were significantly associated with the outcome variables (table 2).

In the logistic regression analysis of *Parascaris* spp., sex, age, living area and herd size were significantly associated with the outcome variables (table 3).

In the logistic regression analysis of *D. arnfieldi*, sex, grass, co-pasture with horses, living area and herd size were significantly associated with the outcome variables (table 4).

In the logistic regression analysis of *S. vulgaris*, living area and herd size were significantly associated with the outcome variable (table 5).

Discussion

The present study is the first European countrywide survey providing data on parasite dynamics in donkeys and provides

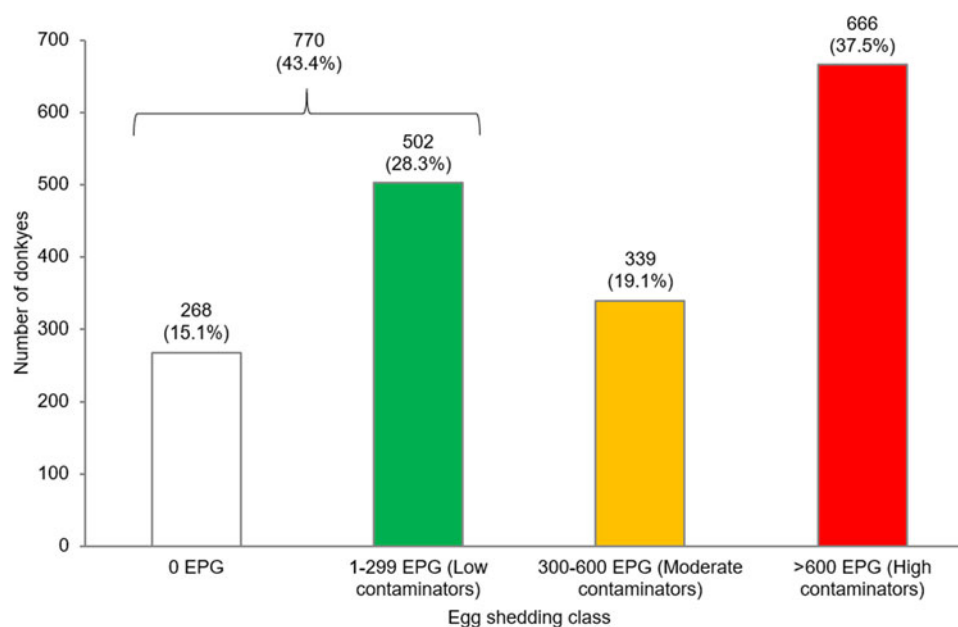


Fig. 2. Number and percentage of donkeys belonging to strongyle egg shedding categories. EPG, eggs per gram.

Table 2. Distribution of frequencies and risk factors associated with intestinal strongyle infection in studied donkeys according to the multivariable generalized linear model.

Variable	Category	Positive/examined (%; CI 95%)	Wald's Chi-square	df	P-value	$\beta \pm SE$	OR (95% CI)
Breed	Mixed-breed	825/937 (88.0, 86–90.1)	18.863	1	<0.001	0.926 \pm 0.213	2.525 (1.662–3.835)
Co-pasture horse	No	802/978 (82.0, 78.5–84.4)	11.453	1	0.001	0.764 \pm 0.226	2.148 (1.380–3.344)
Living areas	Island	228/266 (85.7, 81.5–89.9)	30.937	3	<0.001	–	–
	Northern	398/437 (91.1, 88.4–93.7)	5.415	1	0.020	–0.967 \pm 0.416	0.380 (0.168–0.859)
	Central	546/693 (78.8, 75.7–81.8)	21.105	1	<0.001	–1.671 \pm 0.364	0.188 (0.092–0.384)
	Southern	335/379 (88.4, 85.2–91.6)	4.011	1	0.045	–0.751 \pm 0.375	0.472 (0.226–0.984)
Herd size	Large (>50)	762/874 (87.2, 85–89.4)	22.340	2	<0.001	–	–
	Small (\leq 25)	360/418 (86.1, 82.8–89.4)	0.023	1	0.879	0.033 \pm 0.220	1.034 (0.672–1.590)
	Medium (25–50)	385/483 (79.7, 76.1–83.3)	19.537	1	<0.001	–0.885 \pm 0.200	0.413 (0.279–0.611)
Number of treatments	>2	10/11 (90.9, 73.9–100)	12.535	2	0.002	–	–
	0	183/218 (83.9, 79.1–88.8)	3.781	1	0.052	–2.222 \pm 1.143	0.108 (0.012–1.018)
	1–2	1314/1546 (85.0, 83.2–86.8)	0.841	1	0.359	–0.997 \pm 1.087	0.369 (0.044–3.107)

$\beta \pm SE$, coefficient \pm standard error; OR, odds ratio; CI, confidence interval.

valuable new information for the construction of meaningful parasite control programs.

The frequency of anthelmintic treatment reported in this study is quite different to what has been reported in horses in Italy (Veneziano *et al.*, 2015), where deworming was carried out by almost all owners (94.0%) and the frequency was mainly two or three times/year (68.6%) and comparable with other equine surveys (Schneider *et al.*, 2014; Nielsen *et al.*, 2018; Tzelos *et al.*, 2019). Moreover, MLs (mainly IVM) were the most common drugs used to control endoparasites in Italian

donkey farms, similarly to those used in horses (Veneziano *et al.*, 2015; Becher *et al.*, 2018; Nielsen *et al.*, 2018; Wilkes *et al.*, 2020). This supports the theory that deworming in donkeys is commonly performed with the same drugs and dosages used in horses (Gokbulut & McKellar, 2018). In Italy, given that a sizeable proportion of donkeys are raised mainly for milk production for human consumption, the use of MLs (IVM and MOX) should be avoided in lactating animals given the lack of established withdrawal time for milk in equids (Gokbulut & McKellar, 2018).

Table 3. Distribution of frequencies and risk factors associated with *Parascaris* spp. infection in studied donkeys according to the multivariable generalized linear model.

Variable	Category	Positive/examined (%, CI 95%)	Wald's Chi-square	df	P-value	$\beta \pm SE$	OR (95% CI)
Sex	Male	28/396 (7.1, 4.5–9.6)	12.005	2	0.002	–	–
	Gelding	0/25 (0)	0.000	1	0.998	–1.005±	0.000
	Female	36/1354 (2.7, 1.8–3.5)	12.005	1	0.001	–1.829±	0.282 (0.138–0.577)
Age (years)	>10	5/430 (1.2, 0.1–2.2)	48.378	3	<0.001	–	–
	≤1	36/147 (24.5, 17.5–31.4)	19.772	1	<0.001	2.467 ± 0.555	11.792 (3.974–34.986)
	1<x≤4	19/440 (4.3, 2.4–6.2)	1.271	1	0.260	0.640 ± 0.568	1.897 (0.623–5.774)
	4<x≤10	4/758 (0.5, 0–1)	1.931	1	0.165	–0.959 ± 0.690	0.383 (0.099–1.482)
Living areas	Island	15/266 (5.6, 2.9–8.4)	19.852	3	<0.001	–	–
	Northern	2/437 (0.5, 0–1.1)	8.245	1	0.004	–2.409 ± 0.839	0.090 (0.017–0.465)
	Central	21/693 (3.0, 1.8–4.3)	0.332	1	0.564	–0.307 ± 0.533	0.735 (0.259–2.090)
	Southern	26/379 (6.9, 4.3–9.4)	3.745	1	0.053	0.806 ± 0.417	2.240 (0.990–5.070)
Herd size	Large (>50)	31/874 (3.5, 2.3–4.8)	14.058	2	0.001	–	–
	Small (≤25)	14/418 (3.3, 1.6–5.1)	6.141	1	0.013	–1.005 ± 0.405	0.366 (0.165–0.810)
	Medium (25–50)	19/483 (3.9, 2.2–5.7)	11.789	1	0.001	–1.829 ± 0.533	0.161 (0.057–0.456)

$\beta \pm SE$, coefficient \pm standard error; OR, odds ratio; CI, confidence interval.

Table 4. Distribution of frequencies and risk factors associated with *Dictyocaulus arnfieldi* infection in studied donkeys according to the multivariable generalized linear model.

Variable	Category	Positive/examined (%, CI 95%)	Wald's Chi-square	df	P-value	$\beta \pm SE$	OR (95% CI)
Sex	Male	21/396 (5.3, 3.1–7.5)	11.531	2	0.003	–	–
	Gelding	0/25 (0)	0.000	1	0.998	–16.837 ± 8010.365	0.000
	Female	101/1354 (7.5, 6.1–8.9)	11.531	1	0.001	1.548 ± 0.456	4.703 (1.924–11.493)
Grass	Yes	122/1612 (7.6, 6.5–8.7)	0.000	1	0.995	18.787 ± 3005.153	1442.143
Co-pasture horse	No	78/978 (8.0, 6.4–9.8)	13.846	1	0.000	1.093 ± 0.294	2.984 (1.678–5.307)
Living areas	Island	55/266 (20.7, 15.8–25.5)	84.685	3	<0.001	–	–
	Northern	12/437 (2.7, 1.2–4.3)	31.309	1	<0.001	–2.077 ± 0.371	0.125 (0.061–0.259)
	Central	45/693 (6.5, 4.7–8.3)	66.223	1	<0.001	–2.601 ± 0.320	0.074 (0.040–0.139)
	Southern	10/379 (2.6, 1.4–3.8)	15.965	1	<0.001	–1.638 ± 0.410	0.194 (0.087–0.434)
Herd size	Large (>50)	54/874 (6.2, 4.6–7.8)	47.551	2	<0.001	–	–
	Small (≤25)	35/418 (8.4, 5.7–11)	8.920	1	0.003	0.787 ± 0.263	2.197 (1.311–3.682)
	Medium (25–50)	33/483 (6.8, 4.6–9.1)	31.454	1	<0.001	–2.812 ± 0.501	0.060 (0.022–0.161)

$\beta \pm SE$, coefficient \pm standard error; OR, odds ratio; CI, confidence interval.

It is important to keep in mind that donkeys are not small horses with long ears, as they are characterized by a greater level of activity of some P450 isoenzymes than horses (Lizzaraga *et al.*, 2004). Thus, for certain drugs, the recommended dose and posology for horses may not be appropriate for donkeys (Grosenbaugh *et al.*, 2011). Regarding anthelmintic drugs, some studies confirm the use of horse dosage in donkeys, despite the apparent differences in intestinal absorption and plasma disposition (Gokbulut &

McKellar, 2018). PYR pamoate in oral paste and granule formulations administered to donkeys at the horse dose rates, showed different bioavailability and persistence compared to horses, but were effective in controlling intestinal strongyle infections (Gokbulut *et al.*, 2014). In donkeys, mebendazole was reported to be highly effective against small strongyles both at the horse dose rate and at double the horse dosage, and egg reappearance period was five weeks for both dosages (Gokbulut *et al.*, 2016). In donkeys, a recent

Table 5. Distribution of frequencies and risk factors associated with *Strongylus vulgaris* infection in studied farms according to the multivariable generalized linear model.

Variable	Category	Positive/examined (%; CI 95%)	Wald's Chi-square	df	P-value	$\beta \pm SE$	OR (95% CI)
Living areas	Island	1/14 (7.1, 0–20.6)	11.271	3	0.010	–	–
	Northern	2/9 (22.2, 0–49.4)	2.277	1	0.131	2.153 \pm 1.427	8.609 (0.525–141.061)
	Central	8/28 (28.6, 11.8–45.3)	2.559	1	0.110	1.886 \pm 1.179	6.591 (0.654–66.420)
	Southern	13/26 (50.0, 30.8–69.2)	8.320	1	0.004	3.509 \pm 1.217	33.418 (3.079–362.674)
Herd size	Large (>50)	7/14 (50.0, 23.8–76.2)	6.413	2	0.041	–	–
	Small (\leq 25)	14/48 (25.0, 12.8–37.3)	6.247	1	0.012	–1.974 \pm 0.790	0.139 (0.30–0.653)
	Medium (25–50)	5/15 (33.3, 9.5–57.2)	1.424	1	0.233	–1.096 \pm 0.918	0.334 (0.055–2.022)

P-value < 0.05. $\beta \pm SE$, coefficient \pm standard error; OR, odds ratio; CI, confidence interval.

trial showed that in well-managed farms, anthelmintic drugs administered at the horse dose rate were generally effective in the control of small strongyle infections (Buono *et al.*, 2018). Based on the results of these studies, the horse dosage for some anthelmintics may also be effective for the donkey dosage. In the present survey, owners and veterinarians generally treated donkeys like horses.

Intestinal strongyle infections are common in grazing horses and donkeys; however, in this survey, access to pasture was not associated with a higher prevalence of positive strongyle FECs. A similar finding was made in a previously published equine study (von Samson-Himmelstjerna *et al.*, 2009). Donkeys raised in box stalls with an annexed paddock showed high intestinal strongyle prevalence, and it is possible that parasites could be transmitted in stalls in cases of suboptimal hygiene (Nielsen *et al.*, 2010b). Moreover, it should keep in mind that encysted and arrested larvae will gradually develop into adult parasites, causing positive FECs over time, and several anthelmintic treatments could be needed to achieve a negative FEC.

In the examined farms, donkeys generally grazed with a low number of ruminants, and the ratio between these two species was very low. This would explain why a 'dilution effect' was not observed and why there were no significant differences in strongyle egg excretion between donkeys that co-grazed and those that did not co-graze with ruminants.

Donkeys raised in farms in northern Italy had a significantly higher prevalence of strongyle infection. This is probably due to the climatic difference between northern and central-southern Italy. Pasture contamination with small strongyles is widely influenced by climatic conditions (Leathwick *et al.*, 2015); in northern Italy, rainfalls are more abundant than central-southern Italy and this would allow a greater migration of the L3 on the pastures.

Small (\leq 25 animals) and large farms (\geq 50 animals) were more associated with strongyle infection than medium-sized farms (25–50 donkeys). Similar findings have been reported in horses (Kornaś *et al.*, 2010). In small farms, donkeys are usually housed with horses and are often neglected and just sheltered into the farms without being involved in management and anthelmintic control practices, whereas in larger farms a high stocking density could be correlated to higher infection pressure.

The distribution of strongyle egg count levels reported herein are different from those reported in managed horses, where an overwhelming majority (50–75%) is in the low contaminator category (Relf *et al.*, 2013; Lester *et al.*, 2018). Consequently, the 80:20 distribution rule that is often cited for horses (Becher *et al.*, 2010; Nielsen *et al.*, 2018), according to which 20% of

animals shed about 80% of intestinal strongyle eggs, does not appear directly applicable for this donkey population. Here, about 40% of adult donkeys were shedding approximately 80% of the eggs, suggesting an 80:40 distribution (fig. 3). In this study, 770 donkeys (43.4%) had <300 EPG intestinal strongyles, and, thus, these animals may not require deworming.

Faecal cultures documented a wide biodiversity with a high prevalence of large strongyles, most notably *S. vulgaris*. Given the distinct pathogenicity of this parasite (Pihl *et al.*, 2018), more attention should be given to controlling it in donkey operations. It was reported that *S. vulgaris* caused obstruction of the cranial mesenteric artery, which led to death of the animal in an eight-year-old donkey (Borji *et al.*, 2014). Moreover, in a two-year-old intact male donkey, aberrant migrations of *S. vulgaris* through the spinal cord resulted in progressive paraparesis and tetraplegia (Mayhew *et al.*, 1984). The farm-level analysis showed a significant association between *S. vulgaris* and location, suggesting that donkeys raised on farms located on the island of Sicily are less exposed to *S. vulgaris* infection. The reason for this difference is not clear, but climatic influence and management practices may play a role.

The low anthelmintic treatment intensity reported in this study could be a risk factor for *S. vulgaris* infection in donkeys as also demonstrated in horses (Nielsen *et al.*, 2012; Hedberg-Alm *et al.*, 2020). Considering the high prevalence of *S. vulgaris* in the donkey population and that many donkeys co-graze with horses, it is necessary to assess the presence of large strongyles in donkey species to avoid the risk of infection in horses. Thus, if coprocultures show the presence of *S. vulgaris*, it is important to also treat the low contaminators (FEC <300 EPG) to limit its spread on the pasture. It is also important to highlight that IVM treatment may not be completely effective against *S. vulgaris* migrating fifth-stage larvae (Nielsen *et al.*, 2015). Although horses demonstrate different degrees of immunity to *Parascaris* spp. in relation to age and exposure with patent infections (Clayton & Duncan, 1979), adult donkeys can harbour ascarids without clinical signs, and could be playing an important role in pasture contamination (Matthews & Burden, 2013). The reason for female donkeys having a lower ascarid prevalence than males is unclear, but could be due to differences in management strategies. A large proportion of females were kept for milk production purposes, whereas males were used for a variety of purposes, including meat production. Consequently, males and females could be managed substantially differently, which could affect the risk of acquiring patent ascarid infection.

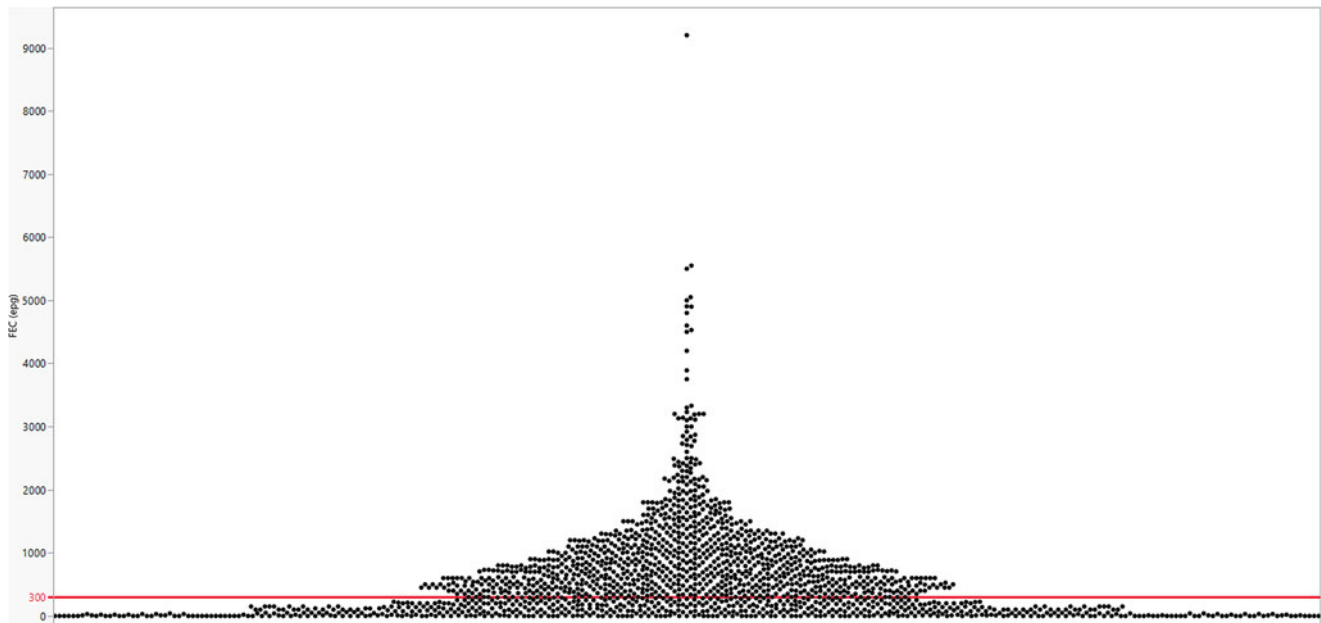


Fig. 3. This graph presents the overdispersion in the intestinal strongyles faecal egg count (FEC) in donkeys (80:40 rule). In the study donkeys (1775), 1005 animals (56.6%) were above the red threshold line, with FEC > 300 eggs per gram (EPG), while 770 (43.4%) were below the red threshold line, with FEC < 300 EPG.

Although ascarid eggs were found in faeces of all age groups, younger donkeys (≤ 1 year) had a significantly higher prevalence than other age groups. This contrasts findings among working donkeys in Ethiopia, where a high prevalence (>50%) regardless of the animal age was reported (Getachew *et al.*, 2008). This discrepancy could be explained by the inability of the animals to develop immunity against ascarids due to poor farm management and poor nutrition for working equids in developing countries (Getachew *et al.*, 2010). Donkeys on farms located in southern Italy had significantly higher *Parascaris* spp. prevalence, and this can be explained considering that in these farms donkeys were kept mainly for milk production and, therefore, characterized by a greater presence of animals aged less than one year.

Donkeys and mules are natural hosts for *D. arnfieldi*, whereas horses are primarily at risk of getting infected while co-grazing with these animals. Although the prevalence was relatively low in this study, it could be underestimated considering that the first-stage larvae (L1) recovery of *D. arnfieldi* by Baermann technique is strongly influenced by the method of storage of faecal samples. Considering that faecal samples were stored in portable refrigerators, temperature fluctuations during the first 48 h could adversely affect the parasitological examination (Rode & Jorgensen, 1989). Female donkeys were at higher risk of infection than male and gelding, probably due to the fact that they spent more time grazing. Donkeys that had access to pasture were at major risk of infection by *D. arnfieldi*, suggesting that animals acquire infections when grazing on a contaminated pasture (Matthews, 2002). Donkeys co-grazing with horses had a lower prevalence of *D. arnfieldi*, which is probably due to the dilution principle, considering that horses rarely harbour adult stages and considering that horses are not permissive hosts of the full life cycle, they generally do not excrete larvae in the faeces (Matthews & Burden, 2013). The highest prevalence was reported in animals aged less than one year and in animals with BCS < 3. This makes sense as young age and poor BCS represent potential risk factors, probably related to impaired immunity to infections (Tihitna *et al.*, 2007).

It is advisable to perform diagnosis for lungworm infection in all donkeys co-grazing with horses, and the animals should be treated using MLs to reduce pasture contamination by L1 of *D. arnfieldi* (Matthews, 2002). In our study, IVM was the compound mostly used for deworming and this could explain the low prevalence of lungworm infections. Moreover, the frequency of anthelmintic treatments was associated with a lower infection rate, suggesting that the routine deworming scheme in the donkey farms could be correlated to a reduction of lungworm parasitic load.

The prevalence of *Anoplocephala* spp. was low (1.0%) but in agreement with data reported in donkeys in few studies on the presence of tapeworms in Europe (Matthews & Burden, 2013). In horses, *Anoplocephala perfoliata* was detected with higher prevalence (Tzelos *et al.*, 2019; Hedberg-Alm *et al.*, 2020). This low prevalence could be correlated with the irregular excretion of tapeworm eggs in the faeces (Gasser *et al.*, 2005). Furthermore, considering that the Proudman test can result in false negatives if the parasitic load is less than 20 worms (Proudman & Edwards, 1992), it is possible that tapeworm infection was not diagnosed in those animals with a low parasitic load.

In Europe, *F. hepatica* is widespread in ruminants, but with a clustered spatial distribution, and regional variation in epidemiology and prevalence (4% in southern Italy) (Beesley *et al.*, 2017). In Italy, the low prevalence of this liver fluke could justify its absence in the studied donkey population.

In conclusion, this study represents the first large-scale epidemiological survey in Europe on the prevalence of helminth infections in donkeys, adding useful data to the scarce literature available about the parasite dynamics in this neglected animal. Our findings revealed the presence of several helminth species, representing the most important pathogenic parasites of equids and donkeys worldwide. Considerations for parasite control in donkeys could be different to those suggested in horses, because large strongyles in donkeys are more common than in horses. Furthermore, in donkeys the 80:20 strongyle egg shedding

distribution rule is not applicable, because about 40% of donkeys were shedding around 80% of the eggs. Furthermore, this study highlighted the prominent occurrence of *S. vulgaris* in managed donkeys and a farm prevalence exceeding that of *D. arnfieldi*. Thus, parasite control programs in donkeys require specific consideration of strongyle egg shedding patterns as well as occurrence of individual parasite species with pathogenic potential for both the donkeys and possible co-grazing horses.

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