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A study on the prevalence of cyathostomin and *Anoplocephala perfoliata* infections in Italian horses: diagnostic testing and analysis of factors affecting infection risk

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ABSTRACT

Background: Cyathostomins and *Anoplocephala perfoliata* infect equids worldwide and both are of concern due to their potential to cause clinical disease. Although coprological techniques are commonly used for assessing helminth egg shedding these do not provide information about an individual's total parasite burden. Antibody-based tests provide information on infection levels within individuals.
Aims/objectives: This study aimed to evaluate cyathostomin and *Anoplocephala perfoliata* occurrence in Italian horses using coprological and serum-based antibody detection methods and to analyse risk factors for infection at the individual level.
Methods: Samples from 173 horses were collected on 35 farms. Coprological examinations were performed using Mini-FLOTAC or a centrifugation/flotation technique. Parasite-specific antibody levels were assessed by ELISA using the Tapeworm Blood Test and the Small Redworm Blood Test.
Results: Intestinal strongyle and tapeworm eggs were detected in 140 (80.9 %) and five (2.9 %) faecal samples, respectively. Cyathostomin ELISA results revealed 39 horses (22.5 %) below the 1,000 total worm burden threshold, with 75 (43.0 %) below the 10,000. Tapeworm ELISA results indicated 136/173 horses (78.6 %) were below the test's treatment threshold. Small Redworm serum score result category was associated with sex, access to pasture, strongyle FEC, no recent anti-nematode treatments and tapeworm serum score category. Tapeworm ELISA results were associated with living area, age class, sex, access to pasture, quarantine procedures, dung removal, strongyle FEC, no recent anti-nematode treatments and SR serum score category.
Conclusion: Cyathostomin and *A. perfoliata* infections in Italian horses are influenced by horse signalment, specific management practices and, in the case of tapeworm, living area.

1. Introduction

Grazing horses are infected with several intestinal parasites, of which small strongyles (cyathostomins) are the most common; with other parasites such as the tapeworm, *Anoplocephala perfoliata,* of concern due to their potential clinical impact. Cyathostomins often represent over 95 % of the total worm population recorded in horses [1] and generally infection is asymptomatic [2]. However, where management is poor, susceptible horses can accumulate large numbers of encysted larvae (in some cases, 1,000,000′s-1,000,000′s cyathostomins) in the mucosa and submucosa of the caecum and large colon, particularly if <5 years-old [3]. These encysted larvae can emerge simultaneously from the intestinal wall causing a clinical syndrome known as larval cyathostominosis and, although it is a relatively uncommon disease, it is characterised by weight loss, diarrhoea, colic, subcutaneous oedema, pyrexia and severe colitis [4,5]. The degree of parasite exposure, age, season, concurrent

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disease and anthelmintic treatment history are factors associated with larval cyathostominosis which can have a poor prognosis, with case fatality reports as high as 50 % [5].

Three drug classes are available for treating nematode infections in horses: benzimidazoles, pyrimidines and macrocyclic lactones [6]. Among these, only two regimens have been demonstrated as effective against all stages of cyathostomin encysted larvae: a five day-course of fenbendazole and single administration of moxidectin. However, resistance to fenbendazole is widespread in cyathostomins, including reduced effectiveness of the five-day regimen [7]. Increasing reports of reduced effectiveness of moxidectin, usually reported as a reduced strongyle egg reappearance period observed after treatment, also indicate that this compound may not be as effective at killing all stages of cyathostomins compared to when the anthelmintic was first licensed in the 1990′s [8,9].

The three tapeworm species infecting horses worldwide are *A. perfoliata, Anoplocephala magna* and *Paranoplocephala (Anoplocephaloides) mamillana*, with infection occurring more frequently in young animals [10,11]. Among these, *A. perfoliata* is the most frequently reported [12] and appears to be the only species of clinical significance, causing severe mucosal and sub-mucosal tissue damage and, in some cases, resulting in medical (i.e., spasmodic) and surgical colic of the ileo–caecal-colic region (i.e. ileocaecal, caecal–caecal and caecal–colic intussusceptions, ileal impaction) [13,14]. A significant association between the presence of *Anoplocephala spp.* eggs in the faeces and the onset of colic signs has been reported in some European countries [15,16].

Coprological examination such as faecal egg count (FEC) flotation methods and centrifugal/flotation methods (as proposed by Proudman and Edwards [17]) historically represent the commonest techniques for the diagnosis of intestinal strongyles and cestodes, respectively. However, FEC tests do not provide accurate information about the total number of helminths present within individuals, as these tests do not account for immature stages, which can comprise the majority of an individual's burden [18], or, in the case of nematode infections, male parasites. Encysted larvae can remain in the mucosa/sub-mucosa for many months [19], and animals with high encysted larval burdens often show a low or negative FEC [18,20]. In managed groups, 20 % of horses generally shed 80 % of all eggs, illustrating the 20/80 rule [21]. Egg shedding appears to be influenced by different risk factors such as breed, age, season and management practice [22,23]. Moreover, strongyle egg shedding can be affected by acquired immunity that can limit egg production by female worms [24]. Although the Proudman and Edwards technique [17] is reported as the best-performing technique for detecting *Anoplocephala* spp. eggs [25], this method is associated with many false negative results, particularly in horses with a low tapeworm burden [17]. Coprological tests do not account for non-continuous proglottid shedding behaviours by adult parasites [26], nor the fact that many tapeworm infections can comprise large proportions of immature non-egg releasing worms [12,27].

Enzyme-linked immunosorbent assays (ELISA) have been developed to provide information on cyathostomin and *A. perfoliata* infections in horses [28]. The cyathostomin ELISA (Small Redworm Blood Test, Austin Davis Biologics), available since 2019, is based on serum IgG(T) responses to recombinant antigens expressed in encysted larval and luminal stages [29]. This test can discriminate the total cyathostomin burden (TCB) at specific thresholds (1,000, 5,000 and 10,000 cyathostomins) and is recommended for informing anthelmintic treatments in horses kept in low infection risk circumstances [3]. The sensitivity and specificity of the test depend on the threshold selected: TCB >1,000 (sensitivity 97.65 %; specificity 85.19 %); TCB >5,000 (sensitivity 96.10 %; specificity 75.43 %) and TCB >10,000 (sensitivity 91.55 %; specificity 75.61 %) [29]. Based on a veterinarian risk assessment, cyathostomin treatment can be applied at any of the three TCB thresholds.

A serological ELISA test (Tapeworm Blood Test, Austin Davis Biologics), that measures parasite-specific IgG(T), in this case to 12/13 kDa excretory/secretory antigens [15], is also available for diagnosis of *A. perfoliata* infection, with results reported as 'low' (<2.7), 'borderline' (2.7-6.3) and 'moderate/high' (>6.3). Cestocidal treatment is recommended for horses with results in the latter two categories [30]. At a cut-off value of 2.7, the serological ELISA test showed a sensitivity of 85.2 % and specificity of 77.6 %.

This study aimed to evaluate the occurrence of small strongyle and *A. perfoliata* infections in horses managed under different conditions in Italy. Coprological examinations and serum-based antibody assays were used for parasite detection, and information gathered through owner questionnaires was analysed to identify risk factors associated with infection by each parasite at the individual horse level.

2. Materials and methods

2.1. Study animals

The study was performed between November 2023 and February 2024 and included 173 horses based on 35 horse farms located in nine Italian regions classified in northern, central and southern Italy [31]. Under the framework of the national project, "*Horse endoparasite and anthelmintic resistance control plan*", veterinary practitioners were involved in collecting faecal and blood samples during routine clinical visits. Written informed consent was obtained from each owner.

On each farm, owners were interviewed using a purpose-designed questionnaire to obtain a complete history of the farm and the horses included in the study. The questionnaire consisted of 54 closed-ended questions (multiple choice and yes/no options), and was divided into three sections (general information, farm management and grazing practices, anthelmintic treatment and helminth control practice). Horses were divided into two groups: horses that did not have access to pasture (low risk - LR) and horses that had access to pasture (high risk - HR).

For each horse involved in the study, sex (intact male, female, gelding), age, body condition score (BCS - <3; ≥3), size of farm (≤10 horses; >10 horses), management conditions and deworming strategy were obtained from owners on the sampling day. The age of horses was determined matching owners' information with teeth examination [32]. Horses aged one year or older were included in the study and these were classified into two age groups: <5 years and ≥5 years. Body condition score was determined by veterinary practitioners involved in the study, using a five-point system with 0.5 increments (1 = poor, 2 = moderate, 3 = ideal, 4 = fat and 5 = obese) [33].

2.2. Parasitological examinations

Individual faecal samples were collected from all horses according to general recommendations proposed in Nielsen et al. [34]; faeces were taken directly from the rectum of each animal or picked from the ground from fresh deposits using plastic gloves. Faecal samples were stored in portable refrigerators (approximately 4 °C) and delivered to the Department of Veterinary Medicine and Animal Production of the University of Naples Federico II. Individual FECs were performed within 48 h of collection, using the Mini-FLOTAC technique [35] with a detection limit of 5 eggs per gram (EPG) and Sheather's sugar solution as flotation medium (specific gravity, 1.250) [36]. Furthermore, a qualitative centrifugation/flotation technique [17], using 40 g of faeces and a Sheather's sugar solution, was used for the detection of *Anoplocephala* spp. eggs.

Serum samples (5 mL) were collected from all horses by jugular venipuncture into tubes without anticoagulant, allowed to clot and centrifuged at 2000 × g, 10 min, and the extracted sera placed into 1.5 ml Eppendorf tubes and stored at −20 °C until analysis. Serum samples were analysed with the Small Redworm Blood Test [29] and the Tapeworm Blood Test [30], carried out at Austin Davis Biologics (Northamptonshire, UK), to generate small redworm and tapeworm serum scores, respectively. Small redworm serum scores were analysed across into four categories: 1 (serum score <14.37; TCB <1,000), 2 (serum

score 14.37-30.46; TCB 1,000-10,000), 3 (serum score 30.46-50; TCB between 10,000 threshold and off-scale value - 50) and 4 (serum score >50; TCB above off-scale value) [29]. Animals showing a serum score ≥ 14.37 (1,000 TCB) were considered positive for small strongyles. Serum tapeworm scores were calculated as 'low' (<2.7), 'borderline' (2.7-6.3) and 'moderate/high' (>6.3). The low threshold was considered negative, while the borderline and moderate/high thresholds were considered positive [37].

2.3. Data analysis

All data collected were entered and organised using a Microsoft Excel spreadsheet. The chi-square test of association was used to investigate relationships between categorical variables describing premise management practices and small redworm and tapeworm serum score categories using IBM SPSS Statistics for Windows, Version 29.0.2.0 (Armonk, NY: IBM Corp). P-values were calculated using the Fisher-Freeman-Halton exact test. Adjusted standardised residuals for each contingency table cell that were either >1.96 or <−1.96 were considered to indicate significantly higher or lower counts than expected, respectively. Individual p-values for each of these cells were calculated in Excel using the function =CHISQ.DIST.RT(adjusted residual^2,1). These values were evaluated against modified alpha values for the null hypothesis (i.e. no difference between observed and expected counts) following Bonferroni adjustment of the alpha values depending on the structure of the contingency table.

3. Results

3.1. Study animals and questionnaire data

This study involved 173 horses on 35 Italian horse farms (15 from northern Italy, 10 from central Italy, and 10 from southern Italy). Most of the horses were female (n. 109/173; 63.0 %), and the mean age ± standard deviation was 7.3 ± 3.1 years. The majority of horses (n. 161; 93.1 %) were involved in sport activity, whereas on one farm, 12 horses (6.9 %) were bred for the production of meat for human consumption. The horses were evenly distributed in the two risk classes: 87/173 (50.3 %) belonged to the HR group (access to pasture) and 86/173 (49.7 %) belonged to the LR group (no access to pasture). In the HR group, the pasture size ranged between 0.3 and 30 hectares. General data of the study horses are reported in Table 1.

Data from the questionnaire survey revealed that access to pasture was available on 27/35 farms (77.1 %; 95 % CI 63.2-91.1); specifically access to pasture was performed for 'some hours' on 11/27 farms (40.7 %; 95 % CI 22.2-59.3); 'half a day' on 3/27 farms (11.1 %; 95 % CI 0-23) and 'all day – 24 hours' on 13/27 farms (48.1 %; 95 % CI 29.3-67.0). Of the 35 farms, 11 (31.4 %) kept 10 or fewer horses, while 24 (68.6 %) had >10 horses (Table 2). Regarding anthelmintic treatments, 113/173 (65.3 %) horses based on 23/35 (65.7 %) farms were dewormed following an interval dosing programme, whereas selective treatment based on coprological examination was performed on 60/173 (34.7 %) horses based on 12/35 (34.3 %) farms. None of the farms had previously used antibody-based testing to inform anthelmintic treatments. Application of FEC reduction testing for assessing anthelmintic efficacy was performed on 11/35 (31.4 %) premises. Where interval treatments were administered, the frequency of anthelmintic administration was annually, every six months and every four months on 6/23 (26.1 %), 12/23 (52.2 %) and 5/23 (21.7 %) of farms, respectively. Ivermectin (IVM) and IVM + praziquantel (PZQ) combinations were the commonest anthelmintics administered; specifically, IVM was used on 28/35 farms (80.0 %), while IVM+PZQ on 25/35 farms (71.4 %), followed by fenbendazole (22 farms; 62.9 %), pyrantel pamoate (15 farms; 42.9 %), moxidectin (11 farms; 31.4 %) and moxidectin + praziquantel (eight farms, 22.9 %).

Table 1
Data on the 173 horses from 35 Italian horse farms included in the study.

Parameter	Value
Living Area	
Northern Italy	57 (32.9%)
Central Italy	46 (26.6%)
Southern Italy	70 (40.5%)
Number of horses on site (min – max)	2-78
≤10 horses	43 (24.9%)
>10 horses	130 (75.1%)
Type of farm	
Open herd	166 (96.0%)
Closed herd	7 (4.0%)
Quarantine practice (in case of open herd - – n. of horses 166)	
Yes	84 (50.6%)
No	82 (49.4%)
Total hectares of pasture on site (mean; min-max)	7.2 (0.3-30)
Access to pasture	
Yes (high risk – HR)	87 (50.3%)
No (low risk - LR)	86 (49.7%)
Dung removal from pasture (in case of access to pasture – n. of horses 87)	
Yes	36 (58.6%)
No	51 (41.4%)
Horses sampled per farm (mean; min – max)	4.9 (1-12)
Sex	
Intact males	18 (10.4%)
Females	109 (63.0%)
Geldings	46 (26.6%)
Mean age ± standard deviation (min – max)	7.3 ± 3.1 (1-19 years)
Age class	
<5 years	38 (22.0%)
≥5 years	135 (78.0%)
BCS	
<3	7 (4.0%)
≥3	166 (96.0%)
Time between last anthelmintic treatment and sampling (months)	0.5-24
Anthelmintic treatment scheme	
Strategic treatment	113/173 (65.3%)
Selective treatment (based on FEC test results)	60/173 (34.7%)
Last anthelmintic drug administered	
Pyrantel pamoate	6 (3.5%)
Fenbendazole	12 (6.9%)
Ivermectin	110 (63.6%)
Ivermectin + Praziquantel	29 (16.8%)
Moxidectin	3 (1.7%)
Moxidectin + Praziquantel	0 (0.0%)
No answer	13 (7.5%)
Anthelmintic treatment against small strongyles in the last six months	
Yes	70 (40.5%)
No	103 (59.5%)
Anthelmintic treatment against tapeworms in the last six months	
Yes	5 (2.9%)
No	168 (97.1%)

3.2. Coprological test results

Intestinal strongyle eggs were reported in the faeces of 140/173 horses (80.9 %; 95 % CI 75.1-86.8). Regarding egg shedding class, 33/173 (19.1 %; 95 % CI 13.2-24.9) horses had a FEC = 0; 65/173 (37.6 %; 95 % CI 30.4-44.8) had a positive FEC of under 200 EPG, 35/173 (20.2 %; 95 % CI 14.2-26.2) had a FEC between 200 and 500 EPG and 40/173 (23.1 %; 95 % CI 16.8-29.4) had a FEC >500 EPG. Intestinal strongyle eggs were found in samples from 34 of 35 farms, a yard-level prevalence

Table 2
Number of horses for each study farm.

ID Farm	Total number of horses	Number of study horses
1	70	4
2	20	2
3	25	11
4	24	5
5	7	3
6	18	10
7	34	11
8	50	12
9	25	2
10	6	5
11	6	2
12	8	3
13	15	3
14	21	8
15	26	10
16	6	5
17	23	1
18	21	2
19	2	2
20	26	2
21	78	5
22	32	1
23	19	5
24	27	3
25	13	4
26	16	7
27	10	7
28	6	5
29	9	5
30	17	4
31	30	5
32	6	4
33	18	6
34	15	7
35	8	2
TOTAL	**737**	**173**

of 97.1 % (95 % CI 91.6-102.7 %). Of 140 horses found positive for intestinal strongyles, 99 (70.7 %) were female, 29 (20.7 %) were geldings and 12 (8.6 %) were intact males. Many strongyle FEC-positive horses were aged ≥5 years-old (n. 105/140; 75.0 %). More FEC-positive horses (80/140; 57.1 %) had access to pasture (HR group), whereas 60/140 (42.9 %) were reported as stabled without access to pasture (LR group). The distribution of horses according to egg shedding categories and risk group is reported in Fig. 1.

The overall coprological prevalence of *Anoplocephala* spp. was 2.9 % (5/173 horses; 95 % CI 0.4-5.4). Of the positive samples, two out of five (40.0 %) were positive only in the Mini-FLOTAC test, and 1/5 (20.0 %) were positive only in the centrifugal floatation test, with two out of five

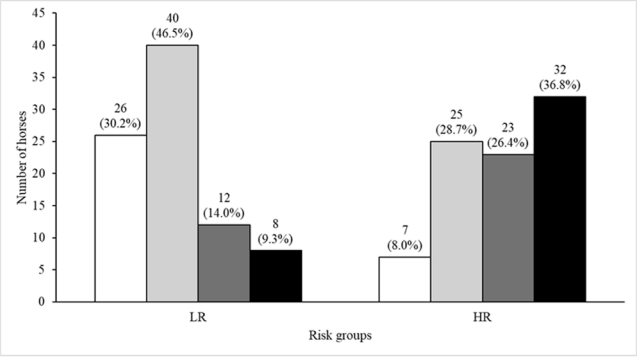

Fig. 1. Number and percentage of horses belonging to the following egg shedding categories: 0 eggs per gram (white), 5 - <200 EPG (light gray), ≥200 – 500 EPG (dark gray), >500 EPG (black). The data is reported according to risk groups: LR (n. = 86) and HR (n. = 87).

(40.0 %) positive in both tests. All positive horses were female; of which two out of five (40.0 %; 95 % CI 0-82.9 %) were aged <5 years, while three out of five (60.0 %; 95 % CI 17.1-102.9 %) were ≥5 years. Three out of five (60.0 %) horses belonged to the HR group, while two out of five (40.0 %) belonged to the LR group. *Anoplocephala* spp. eggs were found in the faeces of horses based at four of the 35 farms (one from northern Italy, one from Central Italy and two from southern Italy), with a farm prevalence of 11.4 % (95 % CI 0.9-22.0).

3.3. Serum ELISA test results

Of 173 serum samples examined using the cyathostomin ELISA, 134 (77.5 %; 95 % CI 71.2-83.7) were above the serum score threshold (14.37) for 1,000 cyathostomins and were based at 32/35 (91.4 %; 95 % CI 82.2-100) farms. Of 173 serum samples examined, 39/173 (22.5 %; 95 % CI 16.3-28.8) belonged to category 1 (TCB < 1,000 cyathostomins); 36/173 (20.8 %; 95 % CI 14.8-26.9) belonged to category 2 (TCB 1,000-10,000 cyathostomins); 33/173 (19.1 %; 95 % CI 13.2-24.9) belonged to category 3 (TCB >10,000 cyathostomins-off-scale value) and 65/173 (37.6 %; 95 % CI 30.4-44.8) belonged to category 4 (above the off-scale value).

Of 173 serum samples examined with the tapeworm ELISA, 37 (21.4 %; 95 % CI 15.3-27.5) tested positive and were from horses based at 13/35 (37.1 %; 95 % CI 21.1-53.2) farms. Of 173 serum samples examined, 136/173 (78.6 %; 95 % CI 72.5-84.7) were diagnosed as low (serum score <2.7); 17/173 (9.8 %; 95 % CI 5.4-14.3) as borderline (serum score 2.7-6.3) and 20/173 (11.6 %; 95 % CI 6.8-16.3) as moderate/high (>6.3).

3.4. Comparison of coprological and serum ELISA test data

To evaluate relationships between FEC and small redworm serum scores, two categories of FEC were considered (<200 EPG and ≥200 EPG) with respect to the proportion of horses above or below the serum score threshold of 14.37. The relationship between copromicroscopic examination and serum-based cyathostomin ELISA test is shown in Fig. 2a. Out of 98 horses with a FEC <200 EPG, 30 (30.6 %) were under this threshold serum score.

Three horses that were positive for *Anoplocephala* spp. eggs by coprological examination were in the low score category in the serum-based ELISA, while two horses positive by coprological examinations had a moderate/high serum score (7.29 and 12.11) in the ELISA. The relationship between copromicroscopic examination and serum-based tapeworm ELISA testing is shown in Fig. 2b.

3.5. Risk factor analysis

The descriptive relationship between each variable and the ELISA test results is summarised in Table 3. Similar proportions of horses had small redworm serum scores below the 14.37 threshold across living area; however, the highest proportion (47.8 %) of horses with serum scores >50 was observed in central Italy. A higher proportion of horses with serum scores in this category (>50) were based on larger farms (i.e., >10 horses) and those with open grazing systems (38.5 % and 38.6 %, respectively). Farms implementing quarantine measures had a lower percentage (34.5 %) of horses above a serum score of 50, whereas those allowing pasture access and not practising dung removal had higher proportions (57.5 % and 64.7 %, respectively) of horses with small redworm serum scores >50. Female horses were more likely to have serum scores >50 (48.6 %), as were those with FEC ≥200 EPG (58.7 %). Additionally, the time since the last anthelmintic treatment was associated with higher serum scores; 44.7 % horses that had not received treatment in the past six months fell into the highest serum score category.

The highest proportion (17.1 %) of horses with tapeworm serum scores >6.3 was found in southern Italy and on farms with >10 horses

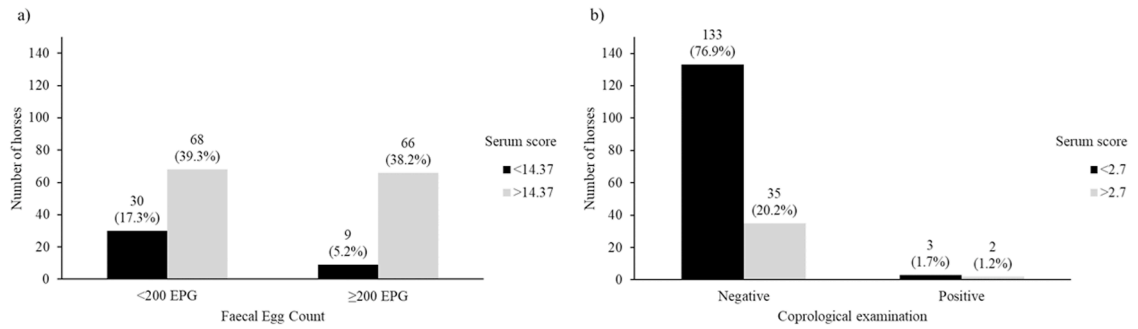

Fig. 2. a) The correlation between strongyle faecal egg count results and serum small redworm scores in the studied horses ($n = 173$). b) The correlation between copromicroscopic examination results for tapeworm eggs and serum-based tapeworm ELISA test results in the studied horses ($n = 173$).

(13.8 %). All cases of high tapeworm serum scores (>6.3) occurred on open farms (12.0 %), with no affected horses on closed farms. Farms without quarantine measures had a higher proportion (20.7 %) with higher tapeworm serum scores. Additionally, horses with pasture access and those on farms that did not remove dung from pastures showed a higher proportion of horses (19.5 % and 29.4 %, respectively) with tapeworm scores >6.3. Female horses had the highest prevalence (16.5 %) of higher tapeworm serum scores, followed by intact males (5.6 %) and geldings (2.2 %). High tapeworm serum scores were also associated with horses having FEC ≥200 EPG (17.3 %). Notably, all 20 horses (11.9 %) with high tapeworm serum scores had not received tapeworm treatment in the previous six months. Furthermore, 23.1 % of horses with a higher tapeworm serum score had a small redworm serum score >50.

The result of the chi-square tests for risk factors associated with serum score values for cyathostomins and *Anoplocephala* spp. are summarised in Tables 4 and 5. Regarding the cyathostomin ELISA results, sex, access to pasture, strongyle FEC, no anti-nematode treatment in the last 6 months and tapeworm serum score category were significantly associated with the small redworm serum score category. Regarding the tapeworm ELISA, living area, age class, sex, access to pasture, quarantine procedures, dung removal from pasture, strongyle FEC, no anti-nematode treatment in the last 6 months and small redworm serum score category were significantly associated with tapeworm serum score category.

4. Discussion

This is the first nationwide study in Italy comparing the copromicroscopic prevalence and antibody test results both for cyathostomins and tapeworms, providing new data on the distribution of these two parasites across the country. This study was conducted over a four-month winter period, which, characterised by higher cyathostomin larval burdens and lower strongyle egg excretion, is considered the optimal time for performing serological tests to guide larvicidal treatment [38].

The strongyle FEC results reported herein are similar to those of studies in other regions, confirming the high prevalence of these parasites globally [1]. The current study also found prevalence estimates closely aligned with those obtained via coprological analysis when the cyathostomin ELISA was applied at a threshold of 1,000 TCB.

According to the coprological examinations, in the present study, many horses (80.9 %) were positive for intestinal strongyle eggs, with a high farm prevalence (97.1 %); this is in line with prevalence data reported in a previous nationwide study (86.4 %), performed from January 2015 to December 2016 by Scala et al. [22], although the latter reported a lower horse-level prevalence (39.5 %). The copromicroscopic higher prevalence reported in the present study compared to Scala et al. [22] could be ascribed to: *(i)* the time since the last anthelmintic treatment (the majority of the study horses received anthelmintic treatment within the six months preceding the coprological analyses, with most of them treated using macrocyclic lactones); *(ii)* presence of encysted larvae in the intestinal mucosa and submucosa that progressively develop into adult stages resulting in a positive FEC and *(iii)* a more sensitive diagnostic method being in the current study (Mini-FLOTAC technique - detection limit 5 EPG) compared to the method used by Scala et al. [22] (McMaster - detection limit of 15 EPG).

The FEC test is a valuable diagnostic tool for identifying horses that require anthelmintic treatment; although it does not provide information on cyathostomin worm burden, including mucosal and luminal stages [28]. As the number of encysted larvae tends to increase at the end of the grazing season, an annual larvicidal anthelmintic treatment was previously recommended for all horses in autumn/early winter, regardless of FEC results [39,40]. This approach is likely to select for anthelmintic resistance; the cyathostomin ELISA can guide treatment decisions during this season, particularly in horses considered at low risk of helminth infection [28]. In this study, out of 86 low-risk horses, 27 (31.4 %) had a serum score below the lowest threshold (14.37; 1,000 TCB) and 52 (60.5 %) below the 10,000 TCB threshold, whereas among 87 high-risk horses, 23 (26.4 %) fell below the 10,000 TCB threshold. The choice of which serum score threshold to use depends on individual or group features. In high helminth transmission scenarios (i.e., high-density horse farms, presence of young horses, high stocking density, no dung removal), it may be advisable to apply a lower serum score threshold. Conversely, in low-risk settings (i.e., no pasture access, low-density stocking, no young horses, regular dung removal), a higher serum score threshold may be appropriate [3]; based on the results of this study, that approach would lead to a 60.5 % reduction in larvicidal treatments compared to an all-group treatment approach.

A statistically significant relationship was observed between strongyle FEC and small redworm serum scores. Horses exhibiting FECs ≥200 EPG demonstrated higher serum scores, whereas a greater proportion of horses with FECs <200 EPG were categorised in groups 1 and 2 (serum scores <14.37 and 14.37–30.46, respectively). Notably, 30 of the 98 horses with FECs <200 EPG had serum scores below 14.37. Among these 98 horses, for the 66 animals identified as low risk, applying a serum score cutoff of 14.37 would result in a 33.3 % reduction in treatments; furthermore, withholding treatment from the 44 horses within this group who registered serum scores under 30.46 would yield a 66.7 % decrease in anthelmintic administration relative to an all-group treatment approach.

Statistical analysis revealed a significant association between sex and small redworm serum score. In this study, geldings were less likely to exhibit high small redworm serum scores, whereas most females showed higher scores. Although there was an unequal distribution of sex in this study, this finding may be attributed to differences in management practices; notably, 62.4 % of females had access to pasture, compared to only 21.7 % of the geldings. In this study, no statistical differences in FEC and small redworm serum scores were observed between the two age groups (<5 and ≥5 years). Although intestinal strongyle infections

Table 3
Relationship between small redworm serum score and tapeworm serum scores according to each variable included in the study. The number (and proportion) of horses in each test result category is indicated for each variable for both tests.

	Small redworm serum score category				Tapeworm serum score category		
	<14.37	14.37-30.46	30.46-50	>50	<2.7	2.7-6.3	>6.3
Living Area							
Northern Italy	15 (26.3%)	13 (22.8%)	13 (22.8%)	16 (28.1%)	52 (91.2%)	3 (5.3%)	2 (3.5%)
Central Italy	11 (23.9%)	10 (21.7%)	3 (6.5%)	22 (47.8%)	32 (69.6%)	8 (17.4%)	6 (13.0%)
Southern Italy	13 (18.6%)	13 (18.6%)	17 (24.3%)	27 (38.6%)	52 (74.3%)	6 (8.6%)	12 (17.1%)
N. of horses on farm							
≤10 horses	12 (27.9%)	9 (20.9%)	7 (16.3%)	15 (34.9%)	38 (88.4%)	3 (7.0%)	2 (4.7%)
>10 horses	27 (20.8%)	27 (20.8%)	26 (20.0%)	50 (38.5%)	98 (75.4%)	14 (10.8%)	18 (13.8%)
Type of farm							
Closed farm	3 (42.9%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	7 (100%)	0 (0.0%)	0 (0.0%)
Open farm	36 (21.7%)	34 (20.5%)	32 (19.3%)	64 (38.6%)	129 (77.7%)	17 (10.3%)	20 (12.0%)
Quarantine practiced							
Yes	15 (17.9%)	18 (21.4%)	22 (26.2%)	29 (34.5%)	72 (85.7%)	9 (10.7%)	3 (3.6%)
No	21 (25.6%)	16 (19.5%)	10 (12.2%)	35 (42.7%)	57 (69.5%)	8 (9.8%)	17 (20.7%)
Not applicable*	3 (42.9%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	7 (100%)	0 (0.0%)	0 (0.0%)
Access to pasture							
Yes	12 (13.8%)	11 (12.6%)	14 (16.1%)	50 (57.5%)	56 (64.4%)	14 (16.1%)	17 (19.5%)
No	27 (31.4%)	25 (29.1%)	19 (22.1%)	15 (17.4%)	80 (93.0%)	3 (3.5%)	3 (3.5%)
Dung removal from pasture							
Yes	6 (16.7%)	6 (16.7%)	7 (19.4%)	17 (47.2%)	27 (75.5%)	7 (19.4%)	2 (5.6%)
No	6 (11.8%)	5 (9.8%)	7 (13.7%)	33 (64.7%)	29 (56.9%)	7 (13.7%)	15 (29.4%)
Not applicable	27 (31.4%)	25 (29.1%)	19 (22.1%)	15 (17.4%)	80 (93.0%)	3 (3.5%)	3 (3.5%)
Sex							
Intact male	6 (33.3%)	5 (27.8%)	3 (16.7%)	4 (22.2%)	17 (94.4%)	0 (0.0%)	1 (5.6%)
Female	17 (15.6%)	20 (18.3%)	19 (17.4%)	53 (48.6%)	76 (69.7%)	15 (13.8%)	18 (16.5%)
Gelding	16 (34.8%)	11 (23.9%)	11 (23.9%)	8 (17.4%)	43 (93.5%)	2 (4.3%)	1 (2.2%)
Age classes							
<5 years	7 (18.4%)	7 (18.4%)	10 (26.3%)	14 (36.8%)	24 (63.2%)	4 (10.5%)	10 (26.3%)
≥5 years	32 (23.7%)	29 (21.5%)	23 (17.0%)	51 (37.8%)	112 (83.0%)	13 (9.6%)	10 (7.4%)
Body Condition Score							
<3	0 (0.0%)	1 (14.3%)	2 (28.6%)	4 (57.1%)	5 (71.4%)	1 (14.3%)	1 (14.3%)
≥3	39 (23.5%)	35 (21.1%)	31 (18.7%)	61 (36.7%)	131 (78.9%)	16 (9.6%)	19 (11.4%)
Intestinal strongyles - FEC							
<200 EPG**	30 (30.6%)	30 (30.6%)	17 (17.3%)	21 (21.4%)	88 (89.8%)	3 (3.1%)	7 (7.1%)
≥200 EPG**	9 (12.0%)	6 (8.0%)	16 (21.3%)	44 (58.7%)	48 (64.0%)	14 (18.7%)	13 (17.3%)
Tapeworm – coprological examination							
Positive	0 (0.0%)	0 (0.0%)	3 (60.0%)	2 (40.0%)	3 (60.0%)	0 (0.0%)	2 (40.0%)
Negative	39 (23.2%)	36 (21.4%)	30 (17.9%)	63 (37.5%)	133 (79.2%)	17 (10.1%)	18 (10.7%)
Intestinal strongyle treatment in the last 6 months							
Yes	29 (41.4%)	12 (17.1%)	10 (14.3%)	19 (27.1%)	64 (91.4%)	4 (5.7%)	2 (2.9%)
No	10 (9.7%)	24 (23.3%)	23 (22.3%)	46 (44.7%)	72 (69.9%)	13 (12.6%)	18 (17.5%)
Tapeworm treatment in the last 6 months							
Yes	3 (60.0%)	0 (0.0%)	1 (20.0%)	1 (20.0%)	5 (100%)	0 (0.0%)	0 (0.0%)
No	36 (21.4%)	36 (21.4%)	32 (19.0%)	64 (38.1%)	131 (78.0%)	17 (10.1%)	20 (11.9%)
Last anthelmintic administered							
Fenbendazole	4 (33.3%)	2 (16.7%)	1 (8.3%)	5 (41.7%)	11	0 (0.0%)	1 (8.3%)
Ivermectin	25 (22.7%)	24 (21.8%)	19	42 (38.2%)	(91.7%)	10 (9.1%)	18 (16.4%)
Iveremctin +	3 (10.3%)	4 (13.8%)	(17.3%)	14 (48.3%)	82 (74.5%)	5 (17.2%)	1 (3.4%)
Praziquantel	1 (33.3%)	0 (0.0%)	8 (27.6%)	0 (0.0%)	23 (79.3%)	0 (0.0%)	0 (0.0%)
Moxidectin	4 (66.7%)	1 (16.7%)	2 (66.7%)	1 (16.7%)	3 (100%)	0 (0.0%)	0 (0.0%)
Pyrantel	2 (15.4%)	5 (38.5%)	0 (0.0%)	3	6 (100%)	2 (15.4%)	0 (0.0%)
No anthelmintic treatment			3 (23.1%)	(23.1%)	11 (84.6%)		
Small redworm serum score							
<14.37			-		38 (97.4%)	1 (2.6%)	0 (0.0%)
14.37-30.46					34 (94.4%)	0 (0.0%)	2 (5.6%)
30.46-50					28 (84.8%)	2 (6.1%)	3 (9.1%)
>50					36 (55.4%)	14 (21.5%)	15 (23.1%)
Tapeworm serum score							
<2.7	38 (27.9%)	34 (25.0%)	28 (20.6%)	36 (26.5%)			
2.7-6.3	1 (5.9%)	0 (0.0%)	2 (11.8%)	14 (82.4%)		-	
>6.3	0 (0.0%)	2 (10.0%)	3 (15.0%)	15 (75.0%)			

* Closed farms with no or little horse movement.
** EPG, Eggs per gram.

Table 4
Contingency tables listing frequency distributions for variables associated with serum-based small redworm ELISA score categories. Statistically significant p-values are highlighted in bold (chi-square test of association).

Variable	Category	Parameter	Small Redworm Test Serum Score (Category)				Total
			<14.37 (1)	14.37-30.46 (2)	30.46-50 (3)	>50 (4)	
Sex	Gelding	Number (%)	16 (34.8%)	11 (23.9%)	11 (23.9%)	8 (17.4%)	46 (100%)
		Adjusted Residual	2.3	0.6	1	-3.3	
		p value	0.021	0.549	0.317	**0.001**	
	Female	Number (%)	17 (15.6%)	20 (18.3%)	19 (17.4%)	53 (48.6%)	109 (100%)
		Adjusted Residual	-2.9	-1	-0.7	3.9	
		p value	**0.004**	0.317	0.484	**<0.001**	
	Intact male	Number (%)	6 (33.3%)	5 (27.8%)	3 (16.7%)	4 (22.2%)	18 (100%)
		Adjusted Residual	1.2	0.8	-0.3	-1.4	
		p value	0.230	0.424	0.764	0.162	
Access to pasture	Yes	Number (%)	12 (13.8%)	11 (12.6%)	14 (16.1%)	50 (57.5%)	87 (100%)
		Adjusted Residual	-2.8	-2.7	-1	5.4	
		p value	**0.005**	0.007	0.317	**<0.001**	
	No	Number (%)	27 (31.4%)	25 (29.1%)	19 (22.1%)	15 (17.4%)	86 (100%)
		Adjusted Residual	2.8	2.7	1	-5.4	
		p value	**0.005**	0.007	0.317	**<0.001**	
Intestinal strongyles FEC	>200 EPG	Number (%)	9 (12.0%)	6 (8.0%)	16 (21.3%)	44 (58.7%)	75 (100%)
		Adjusted Residual	-2.9	-3.6	0.7	5	
		p value	**0.004**	**<0.001**	0.484	**<0.001**	
	<200 EPG	Number (%)	30 (30.6%)	30 (30.6%)	17 (17.3%)	21 (21.4%)	98 (100%)
		Adjusted Residual	2.9	3.6	-0.7	-5	
		p value	**0.004**	**<0.001**	0.484	**<0.001**	
Anthelmintic treatment against small strongyles in the last six months	Yes	Number (%)	29 (41.4%)	12 (17.1%)	10 (14.3%)	19 (27.1%)	70 (100%)
		Adjusted Residual	4.9	-1	-1.3	-2.3	
		p value	**<0.001**	0.317	0.194	0.021	
	No	Number (%)	10 (9.7%)	24 (23.3%)	23 (22.3%)	46 (44.7%)	103 (100%)
		Adjusted Residual	-4.9	1	1.3	2.3	
		p value	**<0.001**	0.317	0.194	0.021	
Serum Tapeworm Score Category	Low	Number (%)	38 (27.9%)	34 (25.0%)	28 (20.6%)	36 (26.5%)	136 (100%)
		Adjusted Residual	3.3	2.6	1	-5.8	
		p value	**0.001**	**0.009**	0.317	**<0.001**	
	Borderline	Number (%)	1 (5.9%)	0 (0%)	2 (11.8%)	14 (82.4%)	18 (100%)
		Adjusted Residual	-1.7	-2.2	-0.8	4	
		p value	0.089	0.028	0.424	**<0.001**	
	Moderate/ High	Number (%)	0 (0%)	2 (10.0%)	3 (15.0%)	15 (75%)	20 (100%)
		Adjusted Residual	-2.6	-1.3	-0.5	3.7	
		p value	0.009	0.194	0.617	**<0.001**	

are more common in young animals, primarily due to their immune susceptibility [41], the low number of young animals included here is likely to have influenced this outcome.

In horses with no access to pasture, 60 out of 86 (69.8 %) had a positive FEC. These results were supported by those of the cyathostomin ELISA, which indicated that 68.6 % (n. 59/86) of these horses had a serum score >14.37. FEC and ELISA test positivity in horses reported as having no access to pasture could be ascribed to different reasons: for example, intestinal strongyles could be transmitted in a box stall in the case of non-optimal hygiene practices [22], or individual horses had access to contaminated grass when travelling. Moreover, out of the 86 horses that had no access to pasture, 81 (94.2 %) lived in an open herd, and parasite transmission could be associated with frequent movements [42].

Horses with access to pasture were far more likely to belong to small redworm serum group 4 (>50), whereas horses with no access to pasture

Table 5
Contingency tables listing frequency distributions for variables associated with serum-based tapeworm ELISA score categories. Statistically significant p-values are highlighted in bold (chi-square test of association).

Variable	Category	Parameter	Serum-based tapeworm ELISA score (category)			Total
			<2.7 (Low)	2.7-6.3 (Borderline)	>6.3 (Moderate/ High)	
Living area	Northern Italy	Number (%)	52 (91.2%)	3 (5.3%)	2 (3.5%)	57 (100%)
		Adjusted Residual	2.8	-1.4	-2.3	
		p value	**0.005**	0.162	0.021	
	Central Italy	Number (%)	32 (69.6%)	8 (17.4%)	6 (13.0%)	46 (100%)
		Adjusted Residual	-1.7	2	0.4	
		p value	0.089	0.046	0.689	
	Southern Italy	Number (%)	52 (74.3%)	6 (8.6%)	12 (17.1%)	70 (100%)
		Adjusted Residual	-1.1	-0.5	1.9	
		p value	0.271	0.617	0.057	
Age	≥5 years	Number (%)	112 (83.0%)	13 (9.6%)	10 (7.4%)	135 (100%)
		Adjusted Residual	2.6	-0.2	-3.2	
		p value	0.009	0.841	**0.001**	
	<5 years	Number (%)	24 (63.2%)	4 (10.5%)	10 (26.3%)	38 (100%)
		Adjusted Residual	-2.6	0.2	3.2	
		p value	0.009	0.841	**0.001**	
Sex	Gelding	Number (%)	43 (93.5%)	2 (4.3%)	1 (2.2%)	46 (100%)
		Adjusted Residual	2.9	-1.5	-2.3	
		p value	**0.004**	0.134	0.021	
	Female	Number (%)	76 (69.7%)	15 (13.8%)	18 (16.5%)	109 (100%)
		Adjusted Residual	-3.7	2.3	2.7	
		p value	**<0.001**	0.021	0.007	
	Intact male	Number (%)	17 (94.4%)	0 (0%)	1 (5.6%)	18 (100%)
		Adjusted Residual	1.7	-1.5	-0.8	
		p value	0.089	0.134	0.424	
Access to pasture	Yes	Number (%)	56 (64.4%)	14 (16.1%)	17 (19.5%)	87 (100.0%)
		Adjusted Residual	-4.6	2.8	3.3	
		p value	**0.005**	**<0.001**	**0.001**	
	No	Number (%)	80 (93.0%)	3 (3.5%)	3 (3.5%)	86 (100.0%)
		Adjusted Residual	4.6	-2.8	-3.3	
		p value	**0.005**	**<0.001**	**0.001**	
Dung removal	Yes	Number (%)	27 (75.0%)	7 (19.4%)	2 (5.6%)	36 (100%)
		Adjusted Residual	1.7	0.7	-2.8	
		p value	0.089	0.484	**0.005**	
	No	Number (%)	29 (56.9%)	7 (13.7%)	15 (29.4%)	51 (100%)
		Adjusted Residual	-1.7	-0.7	2.8	
		p value	0.089	0.484	**0.005**	
Quarantine Practice	Yes	Number (%)	72 (85.7%)	9 (10.7%)	3 (3.6%)	84 (100%)
		Adjusted Residual	2.5	0.2	-3.4	
		p value	0.012	0.841	**0.001**	
	No	Number (%)	57 (69.5%)	8 (9.8%)	17 (20.7%)	82
		Adjusted Residual	-2.5	-0.2	3.4	
		p value	0.012	0.841	**0.001**	
Intestinal strongyles FEC	≥200 EPG	Number (%)	48 (64.0%)	14 (18.7%)	13 (17.3%)	75 (100%)
		Adjusted Residual	-4.1	3.4	2.1	
		p value	**0.001**	**<0.001**	0.036	
	<200 EPG	Number (%)	88 (89.8%)	3 (3.10%)	7 (7.1%)	98 (100%)
		Adjusted Residual	4.1	-3.4	-2.1	
		p value	**0.001**	**<0.001**	0.036	

(*continued on next page*)

Table 5 (*continued*)

Variable	Category	Parameter	Serum-based tapeworm ELISA score (category)			Total
			<2.7 (Low)	2.7-6.3 (Borderline)	>6.3 (Moderate/ High)	
Anthelmintic treatment against small strongyles in the last six months	Yes	Number (%)	64 (91.4%)	4 (5.7%)	2 (2.9%)	70 (100%)
		Adjusted Residual	3.4	-1.5	-3	
		p value	**0.001**	0.134	**0.003**	
	No	Number (%)	72 (69.9%)	13 (12.6%)	18 (17.5%)	103 (100%)
		Adjusted Residual	-3.4	1.5	3	
		p value	**0.001**	0.134	**0.003**	
Small Redworm Serum Score Category	1	Number (%)	38 (97.4%)	1 (2.6%)	0 (0%)	39 (100%)
		Adjusted Residual	3.3	-1.7	-2.6	
		p value	**0.001**	0.089	0.009	
	2	Number (%)	34 (94.4%)	0 (0%)	2 (5.6%)	36 (100%)
		Adjusted Residual	2.6	-2.2	-1.3	
		p value	0.009	0.028	0.194	
	3	Number (%)	28 (84.4%)	2 (6.1%)	3 (9.1%)	33 (100%)
		Adjusted Residual	1	-0.8	-0.5	
		p value	0.317	0.424	0.617	
	4	Number (%)	36 (55.4%)	14 (21.5%)	15 (23.1%)	65 (100%)
		Adjusted Residual	-5.8	4	3.7	
		p value	**<0.001**	**<0.001**	**<0.001**	

were more frequently observed in group 1 (score <14.37) and 2 (score 14.37-30.46). In this study, 6 out of 7 (85.7 %) horses with a negative FEC (FEC = 0) and access to pasture reported a serum score >14.37, indicating that despite 0 EPG, these horses had been exposed to cyathostomins, confirming pasture access as a significant risk factor for acquiring infection [43]. Such horses may have larval or luminal burdens not detectable by FEC analysis. Effective pasture management is essential to minimise contamination and, in turn, reduce the risk of infection. Removing faeces from pastures is a key method for reducing parasite infection levels, especially during summer when larval development is rapid and the associated risk of infection is high [44]. Twice weekly removal of faeces from paddocks has been shown to be effective in significantly reducing the number of infective larvae on pasture [44] or the level of strongyle egg-shedding [45,46]. However, regular pasture hygiene is not performed in several countries [47,48], including Italy [49]. According to this study, pasture hygiene was performed once a week or at longer intervals, on the majority of farms. This likely resulted in a non-statistical difference between farms that followed these hygiene practices and those that did not. Although the infectious larval stage of strongyles can survive through winter, their numbers are significantly reduced by the following summer. This suggests that resting pastures for a year in a cold, temperate climate can lead to a decrease in infectivity levels [44]. Therefore, on farms where horses are grazed, improved management practices, including appropriate resting periods, would help to reduce contamination with infective stages [50].

Newly arrived horses should be dewormed or tested on arrival, quarantined in a separate paddock, and undergo a follow-up efficacy test before being introduced to communal pastures [51]. In this study, quarantine practices were stated to be performed by 51.5 % of respondents; however, on only eight farms were new horses dewormed upon arrival, and no significant relationship was identified between the application of quarantine practices and small redworm serum scores.

The serum half-life of equine Ig(T) ranges from 21 to 35 days [52,53]; to reduce the number of false positives, the serum test should not be performed within four months of the last anthelmintic treatment. Furthermore, to provide additional insight into serological outcomes following recent deworming 25/173 (14.5 %) horses that received anthelmintic treatment in the previous four months were included. Among these, eight horses were treated with fenbendazole, of which four were SRW test-positive; 12 horses were treated with ivermectin, of which six were SRW test-positive and five horses were treated with pyrantel, of which one was SRW test-positive. Overall, 11/25 (44.0 %) of the horses showed a positive small redworm serum score (>14.37). Moreover, horses treated in the previous six months before testing showed a significantly lower mean FEC and mean small redworm serum score than those treated more than six months previously, confirming that these treatments may have been effective in reducing strongyle transmission.

This study found a significant association between horses with high small redworm serum scores (categories 3 and 4) and those with moderate to high serum tapeworm scores. Additionally, a higher strongyle FEC was linked to a greater percentage of horses testing positive for antibodies against *Anoplocephala*. The life cycles of both intestinal strongyles and tapeworms include pasture stages, with both transmitted during grazing. Therefore, access to pasture and poor hygiene conditions may increase the risk of acquiring both infections. These findings align with those reported in Germany, where strongyle FEC were compared to tapeworm test results [54].

The low copromicroscopic prevalence of *Anoplocephala* spp. reported herein is in line with a nationwide study recently performed in horses in Italy (3.7 %) [55] and in previous European investigations, such as in Germany (0.8 %) [56] and Slovakia (1.99 %) [37]. However, the positive seroprevalence reported here (21.4 %) suggests that *Anoplocephala* spp. infections are underestimated by coprological analysis, as previously reported [37,56], due to the low sensitivity of the latter [17,28]. The tapeworm ELISA test effectively addresses the low sensitivity of coprological techniques in detecting tapeworm eggs. In this study, tapeworm ELISA seroprevalence with moderate/high score (11.6 %) was similar to farm prevalence according to coprological analysis (11.4 %). Thus, in addition to informing treatment decisions, the ELISA test can be a useful tool to indicate farm-level exposure, which, if the prevalence of positivity is high, can be addressed with management improvements in addition to appropriate anti-tapeworm treatments. However, antibody-based assays may yield false positives due to persistent parasite-specific antibodies after treatment, with an IgG(T) half-life of about 35 days [57]. It is therefore advised to avoid blood testing for tapeworms until four months after anthelmintic treatment [28]. In this study, only five horses had received anti-cestode treatment

within the last 4.5 months, and all showed low serum tapeworm antibody levels (four had a score of 0 and one had 0.43). Three horses that tested positive by coprological examination had low tapeworm serum scores. Using a one-tapeworm cut-off, the serum-based test demonstrated a sensitivity of 85 % and specificity of 78 % [30]. At this threshold, the test correctly identified 100 % of horses harbouring >20 tapeworms; however, around 15 % of horses with 1–20 tapeworms fell into the low serum score category, explaining why some horses with low serum scores could still test positive for cestode eggs. A low serological result, therefore, indicates the presence of no tapeworms or a total burden of fewer than 20 tapeworms, which is generally not considered pathogenic. Furthermore, given the low genus-level specificity of coprological techniques, it cannot be excluded that all eggs detected belonged to *A. perfoliata* [58].

Statistical analysis showed a significant difference in the distribution of tapeworm serum scores according to living area. Most horses living in northern Italy reported a low serum score, whereas a higher mean tapeworm serum score was reported in horses in central Italy. *Anoplocephala* spp. infection is linked to the ingestion of its oribatid mite intermediate host. As for the previous national survey [55], a higher copromicroscopic prevalence was found in horses of central regions, likely due to the greater abundance of oribatid mites [59]. Moreover, the higher prevalence reported herein could be associated with the higher number of horses with access to pasture in central Italy than in northern Italy.

Young horses (<5 years) showed a higher probability of belonging to the moderate/high category tapeworm serum score category than older horses (≥5 years). A recent Italian study also demonstrated the highest rate of copromicroscopic positivity in young horses [55]. Similarly, higher copromicroscopic prevalence in young animals has also been reported in Denmark [10] and in France [11]. These observations align with recent reports from Slovakia, where older horses exhibited a significantly lower risk of positive tapeworm ELISA test results [37]. Females were more likely to have a moderate/high tapeworm serum score than geldings and males. This finding is in contrast to those reported in Slovakia, as no differences in management practices were observed between sexes [37]; the current study indicated that a majority of females (62.4 %) had access to pasture, compared to 50 % of intact males and only 21.7 % of geldings. In the present study, access to pasture influenced the distribution of horses across the different tapeworm serum score categories: animals that had access to pasture were more likely to have a borderline or moderate/high tapeworm serum score, as reported in Germany [54] and in Slovakia [37]. Accordingly, horses that have access to pasture have an increased probability of being infected by oribatid mites and consequently are at increased risk of tapeworm infection.

In the current study, the implementation of quarantine practices was associated with a significantly higher percentage of horses showing a serum tapeworm score of <2.7. This finding underscores the critical importance of carefully managing the introduction of new horses into a population. Effective quarantine measures are essential for reducing the risk of infection. By monitoring and treating new arrivals as necessary, managers can minimise the transmission of new, and potentially anthelmintic-resistant, helminth infections into a herd.

Horses with high strongyle FEC may not exhibit any clinical signs, and there is generally no correlation between FEC and BCS [60]. Horses with low strongyle FEC can carry significant burdens of encysted cyathostomin larvae, making them more susceptible to disease [61]. Additionally, the generally low sensitivity of coprological testing for *A. perfoliata* can lead to false-negative results in horses at risk of tapeworm-associated colic. From a clinical standpoint, using ELISA tests for these parasites can be advantageous in assessing the need for anthelmintic treatment in susceptible horses to help prevent the development of clinical signs related to a pathogenic burden, particularly when a considerable number of immature stages are present. The widespread occurrence of anthelmintic resistance in cyathostomin populations [8] and recent reports of resistance in *Anoplocephala* spp. [62,63] highlight the importance of strategically using testing to reduce anthelmintic use. Combining antibody tests with FEC tests (conducted at the appropriate time to reduce strongyle egg shedding), can aid in making informed treatment decisions. This approach can help reduce the amount of anthelmintics used throughout the year, thereby decreasing the selection pressure for resistance.

5. Conclusion

Copromicroscopic examinations are the most commonly used diagnostic methods for detecting helminth parasite infections in horses. However, these methods can produce false negatives and do not provide information about the total worm burden. For this reason, as demonstrated in this study, combining serological methods with coprological examination will provide a more reliable diagnosis of an individual's infection status with cyathostomins or *A. perfoliata*. This can lead to more informed decisions regarding the administration of anthelmintics. In this study, access to pasture, age, time since last anthelmintic treatment and overall farm management practice were identified as significant risk factors for acquiring strongyle and/or tapeworm infections. Therefore, enhancing farmers' and horse owners' understanding of effective pasture management - such as removing faeces two times/week, and, ideally, more frequently in high transmission settings and seasons, as well as implementing appropriate quarantine procedures for newly introduced or returning horses - will help reduce the overall parasite burden in equine populations.

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Ethics in publishing statement

- We confirm that this manuscript has not been published elsewhere and is not under consideration by any other journal.
- All authors have read and approved the final version of the manuscript.
- If accepted the manuscript will not be published elsewhere in the same form.

CRediT authorship contribution statement

F. Buono: Writing – original draft, Investigation, Data curation, Conceptualization. **E. Castaldo:** Writing – review & editing, Data curation. **S. Scarcelli:** Investigation. **D. Piantedosi:** Writing – review & editing. **G. Oliveto:** Investigation, Funding acquisition. **G. Sgroi:** Writing – review & editing. **K.L. Lightbody:** Writing – review & editing, Data curation. **N. Peczak:** Investigation. **S. Engeham:** Investigation, Data curation. **P.A. Lambert:** Formal analysis. **J.B. Matthews:** Writing – review & editing, Writing – original draft, Data curation. **V. Veneziano:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

JBM, NP and SE are employees of Austin Davis Biologics, the company that markets the Small Redworm Blood Test, the Tapeworm Blood Test and the EquiSal® Saliva Test and so have a financial interest in the results of this study. The remaining authors declare no conflicts of interest.

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