Parasitological investigation in an organic dairy donkey farm

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SUMMARY

A parasitological survey was carried out in an organic dairy donkey farm. Individual fecal, skin and fur samples were collected from 29 donkeys (14 jennies, 2 jacks, 9 fillies and 4 foals) and examined for the search of ecto- and endoparasites. Blood samples were also collected from jennies and fillies and examined for the presence of IgG antibodies to *Toxoplasma gondii*. All donkeys were found infected by intestinal strongyles (100%), with a fecal egg count ranging from 50 to 2400 eggs per gram of feces. Moreover, the following endoparasite species were also identified: *Oxyuris equi* 37.9%, *Parascaris equorum* 31%, *Dicrocoelium dendriticum* 14.3%, *Fasciola hepatica* 14.3%, Anoplocefalidae cestodes (*Anoplocephala perfoliata*, *Anoplocephala magna* and *Anoplocephaloides mamillana*) 10.3%, and *Strongyloides westeri* 6.8%. A single jenny (4.3%) was found positive to *T. gondii* at serological analysis. The louse species *Haematopinus asini* was detected in 27.6% animals. Multiple parasite infections were found in the 76% of examined donkeys. This is the first parasitological investigation in an organic dairy donkey farm worldwide. Results showed that pathogenic helminths and *H. asini* are prevalent in the examined donkeys, suggesting the need for effective parasite control measures.

KEY WORDS

Dairy donkeys, ectoparasites, endoparasites, central Italy, prevalence, organic farm.

INTRODUCTION

Recently, the interest in donkey farming has increased in several European countries, mainly due to the recent popularity gained by donkey milk use for the cosmetic industry or for human consumption, and the use of donkeys for social and recreational activities[1,2]. Consequently, the interest in the diseases of donkeys has also increased[3,4]. Amongst donkey pathogens, parasite infections are frequently observed and may negatively affect the health, the productive and reproductive performances of infected animals[3,4,5]. Prevalent donkey endoparasites include the intestinal roundworm species *Parascaris equorum* and intestinal strongyles, that in infected donkeys may be the cause of weight loss and reduced growth and productions, decline in general conditions, intestinal obstruction, colic and diarrhea[5]. Among donkey endoparasites, the tickborne apicomplexan protozoa *Babesia caballi* and *Theileria equi* are prevalent in donkeys in Italy and are considered a possible cause of poor work performance in asymptomatic donkeys[6,7]. Some endoparasites, especially *Toxoplasma gondii*, are zoonotic species and *T. gondii* infected donkeys are considered a potential source for human infections through the consumption of donkey milk and meat products[8,9].

Among ectoparasites, lice and other arthropods are frequently observed in donkeys and are often responsible for anemia, pruritus, skin lesions and debilitation, mainly in younger animals[10].

The diffusion, intensity and species composition of parasite infections may be highly variable in donkeys, depending on several factors, such as farming system, management practices and geographical area[5,11]. Although parasitic infections are considered extremely important in donkeys, few recent studies concern with parasite infections in donkeys in Europe[3,4,8,10,12,13]. Moreover, data on parasitic infections in organic donkey farms are completely absent.

In this study, a parasitological investigation was carried out in an organic dairy donkey farm aimed at evaluating the prevalence and species composition of ecto- and endoparasite infections.

MATERIALS AND METHODS

Animals

Twenty-nine donkeys of different breed (Romagnola, Amiatina and cross-bred animals), gender and age, reared outdoor in a semi-extensive system in an organic dairy donkey farm according to the EU regulations 834/2007 and 889/2008, and located in a natural reserve of central Italy (Arezzo, Italy; 43° 39' 3.3" N, 12° 10' 10.51" E), were examined. Except for five foals, all animals reared in the farm were included in the study. The reserve is mainly a vast wooded area (1540 hectares) covered with forests for about 86% of its surface,

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rich in waterways and placed at an altitude ranging from about 520 to 1453 meters above sea level. Several wild animal species live in this natural reserve, including deer.
The farm covers about 280 hectares and includes stables and grassy or bushy pastures and mowing grassland delimited by wooden and electric fences. The donkeys had free access to pastures during the experimental period. The main farm production is pasteurized donkey milk for human consumption according to E.U. regulation 853/2004, and for the cosmetic industry. Moreover, donkeys are also used for educational and recreational activities. In the farm, donkeys are fed ad libitum with an in farm-produced mixed-grass hay. About 2.5-5 kg of concentrate/day/donkey are also administered, depending on the age and the productive and reproductive needs of animals.
Examined animals included 14 jennies (8-15 years old), 2 jacks (5 years old), 9 fillies (3-4 years old) and 4 foals (10-12 months old). No antiparasitic treatments had been performed in the farm in the 12 months prior the study.
Approval for this study was obtained from the Ethical Committee on Animal Experimentation of the University of Pisa. In addition, authors declare that the work was carried out in compliance with relevant European guidelines regarding ethical use of animals and in adherence to a high standard of veterinary care.

Sampling

Individual fecal samples were collected from the rectal ampoule of all examined animals and examined for the detection of endoparasites. Moreover, the perianal region of each animal was dabbed with strips of transparent adhesive tape for the search of oxyurid eggs, that were adhered on a microscope slide (scotch test). Blood samples were also collected from jennies and fillies, via venipuncture of a jugular into tubes without anticoagulant. Sera were separated by centrifugation for 10 min at 1000 × g and stored at -20°C until examination. Furthermore, individual hair and skin samples were collected both by skin scrapings and by using the scotch-tape test from animals showing skin lesions (alopecia, erythema, scabs), to detect ectoparasite arthropods. All samples were individually labeled, immediately refrigerated at 4°C after the collection, and processed within 12-24 hours. When visible to the naked eye, ectoparasites were directly collected with a needle from the infested animals and placed in test tubes containing 80% ethyl alcohol. Except for blood samples that were collected once, sampling was performed twice, in summer (July) and autumn (November).

Parasitological analysis

For the search of endoparasites, faecal samples were qualitatively examined with a sedimentation/flotation technique[14] using a saturated ZnCl solution (specific gravity 1.56). In addition, a McMaster technique with a sensitivity of 50 eggs per gram of faeces (EPG) was also performed for counting nematode and cestode eggs, by using saturated NaCl as flotation solution (specific gravity 1.2). The Baermann technique was used for the detection of *Dictyocaulus arnfieldi* larvae in faecal samples. Moreover, a commercial rapid immunoassay (RIDA QUICK®) was used to detect *Giardia duodenalis* and *Cryptosporidium* spp. faecal antigens. For the identification of intestinal strongyles such as Strongylinae or Cyathostominae, coprocultures were also made with pooled faecal samples positive for these parasites, placed in an incubator at 27°C for seven days and larvae were recovered using the Baermann technique[15]. Larvae (about 100 larvae) were microscopically examined and identified according to morphological keys as previously reported[16]. Anti-*Toxoplasma gondii* IgG antibodies were detected from sera of all adult females and fillies using a modified agglutination test (MAT), as previously described[17].
Skin and hair samples collected by scotch-tape and skin scrapings were microscopically evaluated for the presence of ectoparasite arthropods as fresh samples or after exposure to 10% NaOH at 30°C for about 30 min, to dissolve hairs and epidermal scales. Moreover, collected ectoparasites were mounted in Hoyer medium, observed under an optical microscope and identified at species level using morphological keys and descriptions given by Ferris[18].

Statistical analysis

Data were analyzed using R software (R Core Team 2015). The prevalence of identified parasites was estimated as the number of positive animals/total number of examined animals. Prevalence of each identified parasite in the two different seasons (summer and autumn), were compared by using the Student's T test. The significance level was set at $P< 0.05$.

RESULTS

All examined animals were found infected by at least a single parasite species (Table 1, Figure 1). Multiple parasite infections were found in about 76% (22/29) of examined donkeys (Tables 1 and 2).
Among faecal parasites, all animals (29/29, 100%) were found infected by intestinal strongyles. Coprocultures revealed a high prevalence of cyathostomins (>90%) in the examined farm.
At quantitative analysis, the EPG number of these parasites was found highly variable, ranging from 50 to 2400 EPG. Considering both seasons, 23 out of 29 animals showed a fecal count ≥200 EPG, and in 7 donkeys the fecal egg count was higher than 1000 EPG. In adult donkeys, the average EPG number of intestinal strongyles was similar in the two seasons, i.e. 493 ± 530 EPG in summer and 482 ± 458 EPG in autumn (Table 1).
The ascarid species *P. equorum* showed an overall prevalence of 31% (9/29), but its prevalence was highly variable in the different age-groups of animals. In fact, the prevalence of *P. equorum* was 31.25% (5/16) in adult donkeys, 22.2% (2/9) in fillies and 75% (3/4) in foals (Table 2).
The pinworm *Oxyuris equi* showed a prevalence of 37.9% (11/29). However, this nematode was identified only in adult animals, including the adult males (2/2, 100%) and 9/14 jennies (64.3%).
Strongiloydes westeri showed an overall prevalence of 6.8% (2/29) and was identified only in 2 fillies.
Anopocephafalidae cestodes showed an overall prevalence of 10.3% (3/29) and were identified only in jennies and fillies. *Anoplocephala perfoliata* was the most frequent species (3/29, 10.3%), while *Anoplocephaloides* (*Paranoplocephala*) *mamillana* (2/29, 6.8%) and *Anoplocephala magna* (1/29, 3.4%) were less frequent. In summer, these parasites showed a lo-

wer prevalence (6.9%) and a lower mean EPG number (50 EPG) than in autumn (prevalence 10.3%, 200 EPG in average), but these differences were not statistically significant.

Among trematodes, *Fasciola hepatica* and *Dicrocoelium dentriticum* were identified and both these parasites showed an overall prevalence of 13.8% (4/29). However, *F. hepatica* was

Table 1 - Ecto- and endoparasites identified in animals of an organic dairy donkey farm from central Italy.

Donkey	Summer	Autumn
JENNIES		
1	50 EPG Gastrointestinal Strongyles	Deceased
2	200 EPG Gastrointestinal Strongyles	100 EPG Gastrointestinal Strongyles
3	250 EPG Gastrointestinal Strongyles	200 EPG Gastrointestinal Strongyles *Haematopinus asini*
4	300 EPG Gastrointestinal Strongyles	200 EPG Gastrointestinal Strongyles
5	50 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	300 EPG Gastrointestinal Strongyles
6	1050 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	200 EPG Gastrointestinal Strongyles
7	2400 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	100 EPG Gastrointestinal Strongyles
8	350 EPG Gastrointestinal Strongyles *Oxyuris equi** *Parascaris equorum** *Fasciola hepatica**	100 EPG Gastrointestinal Strongyles
9	500 EPG Gastrointestinal Strongyles	300 EPG Gastrointestinal Strongyles
10	300 EPG Gastrointestinal Strongyles 50 EPG *Anoplocephaloides mamillana* *Anoplocephala perfoliata**	500 EPG Gastrointestinal Strongyles *Anoplocephala perfoliata**
11**	250 EPG Gastrointestinal Strongyles	900 EPG Gastrointestinal Strongyles 200 EPG *Parascaris equorum* *Haematopinus asini*
12	400 EPG Gastrointestinal Strongyles	200 EPG Gastrointestinal Strongyles *Oxyuris equi** *Haematopinus asini*
13	600 EPG Gastrointestinal Strongyles	600 EPG Gastrointestinal Strongyles *Oxyuris equi** *Toxoplasma gondii* *Haematopinus asini*
14	1750 EPG Gastrointestinal Strongyles *Oxyuris equi** *Dicrocoelium dentriticum** *Haematopinus asini*	100 EPG Gastrointestinal Strongyles 100 EPG *Parascaris equorum* *Oxyuris equi** *Haematopinus asini*

Donkey	Summer	Autumn
JACKS		
1	600 EPG Gastrointestinal Strongyles	1100 EPG Gastrointestinal Strongyles 50 EPG *Parascaris equorum* *Oxyuris equi**
2	750 EPG Gastrointestinal Strongyles	2100 EPG Gastrointestinal Strongyles *Oxyuris equi**
FILLIES		
1	700 EPG Gastrointestinal Strongyles *Strongyloides westeri** *Dicrocoelium dentriticum**	100 EPG Gastrointestinal Strongyles *Haematopinus asini*
2	100 EPG Gastrointestinal Strongyles *Dicrocoelium dentriticum**	900 EPG Gastrointestinal Strongyles
3	150 EPG Gastrointestinal Strongyles *Dicrocoelium dentriticum**	100 EPG Gastrointestinal Strongyles
4	1100 EPG Gastrointestinal Strongyles 50 EPG *Parascaris equorum* *Haematopinus asini*	200 EPG Gastrointestinal Strongyles *Haematopinus asini*
5	200 EPG Gastrointestinal Strongyles 50 EPG *Strongyloides westeri*	200 EPG Gastrointestinal Strongyles
6	250 EPG Gastrointestinal Strongyles	800 EPG Gastrointestinal Strongyles
7	50 EPG Gastrointestinal Strongyles 50 EPG *Parascaris equorum* 50 EPG *Anoplocephala perfoliata*	300 EPG Gastrointestinal Strongyles 100 EPG *Parascaris equorum* 150 EPG / 50 EPG *Anoplocephala perfoliata*
8	300 EPG Gastrointestinal Strongyles	800 EPG Gastrointestinal Strongyles
9	250 EPG Gastrointestinal Strongyles	300 EPG Gastrointestinal Strongyles 100 EPG *Anoplocephaloides mamillana* 100 EPG *Anoplocephala magna* 100 EPG *Anoplocephala perfoliata*
FOALS		
1	100 EPG Gastrointestinal Strongyles 550 EPG *Parascaris equorum*	200 EPG Gastrointestinal Strongyles 450 EPG *Parascaris equorum* *Haematopinus asini*
2	850 EPG Gastrointestinal Strongyles	1100 EPG Gastrointestinal Strongyles 100 EPG *Parascaris equorum*
3	200 EPG Gastrointestinal Strongyles 300 EPG *Parascaris equorum*	700 EPG Gastrointestinal Strongyles
4	250 EPG Gastrointestinal Strongyles	800 EPG Gastrointestinal Strongyles

* = Positivity detected only at the sedimentation/flotation technique performed on faecal samples. ** = Jeanny tested positive to *T. gondii* antibodies at serological analysis.

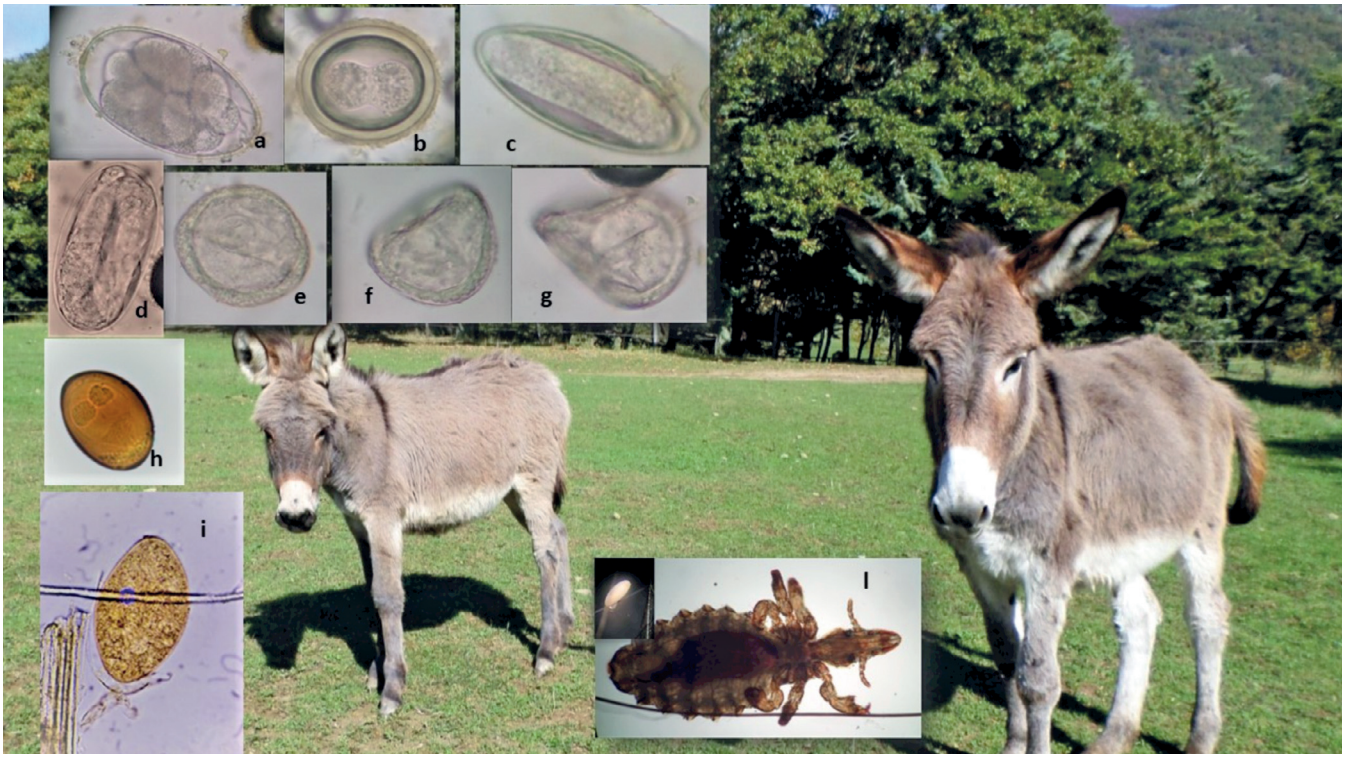

Figure 1 - Endo- and ectoparasites identified in an organic farm from central Italy: **a.** intestinal strongyle egg (200x); **b.** *Parascaris equorum* egg (200x); **c.** *Oxyuris equi* egg (200x); **d.** *Strongyloides westeri* egg (400x); **e.** *Anoplocephala magna* egg (200x); **f.** *Anoplocephala perfoliata* egg (200x); **g.** *Anoplocephaloides mamillana* egg (200x); **h.** *Dicrocoelium dendriticum* egg (400x); **i.** *Fasciola hepatica* egg (100x); **l.** *Haematopinus asini* adult and egg (40x).

identified only among jennies (28.6%, 4/14), while a single jenny (1/14, 7.1%) and 3/9 fillies (33.3%) were found positive for *D. dentriticum*. Moreover, these two trematode species were found significantly more prevalent in autumn than in summer ($p< 0.05$).

All examined donkeys scored negative for intestinal protozoa, i.e. *Cryptosporidium*, *Giardia duodenalis* and *Eimeria leuckarti*. Similarly, no animal was found positive for the respiratory nematode *Dictyocaulus arnfieldi*.

At serological analysis, a single jenny tested positive to *T. gondii* antibodies (1/23, 4.3%) with a low titre (1:40).

Regarding ectoparasites (Table 1), adults and eggs of the blood suckling louse species *Haematopinus asini* were identified at microscopical examination in 8/29 animals (27.6%), including jennies, fillies and foals. Most of the infested animals showed skin lesions, mainly characterized by crusty areas. Moreover, *H. asini* showed a significant lower prevalence ($p< 0.05$) in summer than in autumn.

Table 2 - Prevalence (%) of faecal parasites identified in an organic dairy donkey herd in central Italy according to the different age and gender. Examined animals included 14 mares (of 8-15 years in age), 2 adult males (of about 5 years in age), 9 fillies (of 3-4 years in age) and 4 foals (of 10-12 months in age).

	Jennies	Jacks	Fillies	Foals
Gastrointestinal strongyles	100%	100%	100%	100%
Parascaris equorum	35.7%	50%	22.2%	75%
Oxyuris equi	42.8%	100%	–	–
Strongiloydes westeri	–	–	11%	–
Dicrocoelium dentriticum	7.1%	–	33.3%	–
Fasciola hepatica	28.5%	–	–	–
Anoplocephaloides mamillana	7.1%	–	11.1%	–
Anoplocephala magna	–	–	11.1%	–
Anoplocephala perfoliata	7.1%	–	22.2%	–

DISCUSSION

Several parasite species are included among the main pathogens of donkeys[19]. Nevertheless, in Europe few data are available about the distribution and variability of donkey intestinal parasite infections[3,12]. This is especially true in the case of organic donkeys reared to produce milk for human consumption. Nevertheless, in organic farms the knowledge of prevalence, intensity, species composition and distribution of parasites among different age-groups of animals, is considered essential to perform effective and sustainable control measures to combat and manage parasite infections[20].

Among endoparasites, intestinal strongyles were identified in all donkeys examined in this study. These finding confirm the high prevalence of intestinal strongyles previously observed in donkeys worldwide[5], Italy included[12]. The high prevalence of intestinal strongyles in sampled animals may depend mostly on the lack of anthelmintic treatments and possible high pasture contamination. Intestinal strongyles are included among the most important donkey parasites and may damage animals both in larval and adult stage[5]. It is a group of nematodes with a cosmopolitan distribution that in adult stage localize in the large intestine of equids. Intestinal strongyle species infecting donkeys belong mostly to the subfamilies Strongylinae and Cyathostominae, also known as large and small strongyles, respectively[5]. As observed in this study, a higher prevalence of cyathostomins are generally ob-

served in European donkey farms[5,21]. Cyathostomin infections tend to be higher in young donkeys, but adult animals are often infected and may contribute to pasture contamination[5]. The faecal egg count of intestinal strongyles observed in donkeys is generally higher than in horses[22]. Nevertheless, most of donkeys found positive in this study showed a fecal count of intestinal strongyle eggs ranging from 500 to 1000 EPG or higher, at least in one of the two examined seasons.

The ascarid species *P. equorum* was a further species found prevalent (31%) in this study and both jennies and younger animals were found infected. These results confirm some previous observations on *P. equorum* infections in adult donkeys and the important role they may play in pasture contamination[5]. Nevertheless, in foals the prevalence of this nematode species was higher (75%) than in jennies and fillies.

The pinworm *O. equi* is a worldwide-diffused nematode whose localization site is the large intestine of equids and it is considered as a nuisance or irritant, low pathogenic parasite[23]. In this study, this nematode showed an overall prevalence of 37.9%, but it was identified only in adult animals, with a prevalence of 42.8% in jennies and 100% in males. These prevalence rates are higher than what reported in previous studies (about 1-4%)[24,25], probably because in the present survey both flotation test and scotch test of the perineal skin were used in parallel for the detection of this nematode.

About *S. westeri*, the prevalence observed in this study for this nematode species was low (6.8%). In equids, this parasite is considered widespread mostly among very young animals[26], while in the present study it was identified only among fillies.

In previous studies, *D. arnfieldi* has been reported as a nematode species occurring in donkeys with a prevalence ranging from about 3.6% to about 19%[5,12]. However, in the present survey none of the examined faecal samples was found positive for this respiratory nematode.

Among trematodes, *F. hepatica* showed an overall prevalence of 13.8%, but it was identified only in jennies (28.5%). *F. hepatica* is a common liver parasite of ruminants, but it can infect also other animals, including equids and humans[22,27]. In European donkeys, this trematode has been mainly found at necropsy examination of deceased animals[3]. It is generally accepted that clinical fascioliasis in equids is rare, but in heavily contaminated areas animals grazing with ruminants may suffer from sub-acute or chronic diseases[25].

D. dentriticum is a further liver fluke species that typically infects ruminants. In this study *D. dentriticum* showed a higher prevalence (overall 13.7%, 7.1% in jennies and 33.3% in fillies) when compared to that previously reported in donkeys (0.9-12.8%)[28]. As for *F. hepatica* infection, the contamination of pastures by infected wild ruminants may represent the main reason for the high prevalence of *D. dentriticum* in examined donkeys.

In this study, anopocephalid cestodes were detected with a higher prevalence (10.3%) compared to that reported in previous studies[24,29]. *A. perfoliata* was the most prevalent species (10.3%), as evidenced in previous studies[5], while *A. mamillana* and *A. magna* were less frequent. Although these cestodes are a recognised cause of colic in horses, their clinical effects in donkeys are not yet completely known[5].

The protozoans *G. duodenalis, Cryptosporidium* spp. and *Eimeria leuckarti* have been reported with variable prevalence in donkeys worldwide[30-32]. However, in the present study no positivity to these faecal protozoans was recorded.

T. gondii showed 4.3% seroprevalence in this study. This value is in line with what has been observed in a study carried out on donkeys in Italy[13]. Even though only one jenny showed a positive result to *T. gondii* antibodies, this finding may indicate a potential risk of human infection. Indeed, seropositive lactating jennies, also those with a low serological titre (1:20, 1:40), can be positive to *T. gondii* DNA in milk and blood as demonstrated in a previous study[8]. Thus, the consumption of infected raw milk and other raw donkey products can be a further source of infection to humans, mainly to babies and children where donkey milk is often administered in case of cow milk allergy due to its similarity in composition to woman's milk[2].

Among ectoparasites, the blood suckling louse *H. asini*, previously reported in donkeys also in Italy[10], was the only species identified in this study. In infested donkeys, *H. asini* is considered often responsible for anemia, pruritus and skin lesions, reduction of body conditions and weakness[10]. Subclinical infestations caused by a low number of parasites are also frequently observed and may contribute to the diffusion of *H. asini* among donkeys[10].

CONCLUSIONS

Our results showed that intestinal helminths and the louse species *H. asini* are prevalent in the donkey organic farm here examined. Among faecal parasites, mainly infections caused by intestinal strongyles, but also those caused by ascarids, pinworms and anoplocephalid cestodes, were found prevalent. The identification of zoonotic fluke species and of serological positivity to *T. gondii* is also noteworthy.

The high frequency of multiple infections and the pathogenicity and intensity of some identified parasite species may be responsible for important reductions in productive and reproductive performances and, potentially, also for overt disease in examined donkeys[5].

In this farm, it is therefore advisable the use of effective control measures. However, in organic dairy donkeys reared to produce milk for human consumption, the control of parasites should mainly be based on alternative methods to the use of drugs. Various alternative approaches for parasite control have been extensively studied in equine husbandry, although mainly in horses. Among these different management-based nematode control methods are included, such as the removal of faeces from pastures, rotational grazing and the administration of food supplements[33-35]. The use of nematophagous fungi[35] and of plant-derived compounds[33,36], has been also recommended for the control of nematodes and lice. Therefore, some or all these control methods could be used in the organic dairy farm examined in the present study. Effective methods to avoid the access of wild animals in donkey grazing areas may be also helpful for the control of liver fluke infections.

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