Gastrointestinal parasites, liver flukes and lungworms in domestic ruminants from central Italy

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SUMMARY

Introduction - In ruminants, gastrointestinal, liver and lung parasites may represent a limiting factor for farm production.
Aim - The aim of this study was to evaluate the occurrence of gastrointestinal, liver and lung parasites in adult ruminants living in two different areas of Tuscany, central Italy.
Materials and methods - Between April 2012 and December 2014, 178 adult ruminants (76 cattle, 61 sheep and 41 goats) from 16 extensive farms located in two different areas (A1 and A2) of Tuscany, were examined to assess the occurrence of gastrointestinal parasites, liver flukes and lungworms. A1 included 111 animals from farms located in flat areas subject to water stagnation in rainy seasons, while A2 included 67 animals from farms located in hilly and drier areas. Individual faecal samples collected from all animals were analysed using qualitative and quantitative parasitological techniques. A total of 94 animals were examined for *Fasciola hepatica* also by using two commercial Elisa kits for the detection of faecal antigens and antibodies in serum, respectively. Data were statistically analysed.
Results and discussion - An overall prevalence of 83.7% was found in the examined animals. Higher prevalence values ($p<0.001$) were found in small ruminants than in cattle and in Area 2 compared to Area 1. With regard to isolated parasites, gastrointestinal strongyles and coccidia were prevalent in all ruminant species and in both areas, while the prevalence of *F. hepatica* was higher in small ruminants and in Area 1 than in cattle and Area 2, respectively.
Conclusion - Results indicated that in both areas and in all ruminant species, gastrointestinal parasites and liver flukes require more effective control measures.

KEY WORDS

Domestic Ruminants, Gastrointestinal Parasites, Liver Flukes, Lungworms, Central Italy.

INTRODUCTION

In ruminants, gastrointestinal, liver and lung parasites are very common and may represent a limiting factor for animal production, as they may cause reductions in weight gain, milk production and fertility[1;2]. Although most of these parasites rarely cause death, they may be responsible for severe clinical signs or even promote the susceptibility of infected animals to other pathogens, such as bacteria or viruses[3;4;5].
Among gastrointestinal parasites, strongyles and coccidia are common in domestic ruminants and are some of the main causes of farm production losses[6;7]. In Europe, prevalence of up to 85% in cattle[8] and up to 88% in small ruminants[2] has been recorded for gastrointestinal strongyles, while the prevalence of coccidian infections can reach 83% in cattle[9] and 99% in small ruminants[10]. Young animals (< 1 year of age) are more susceptible to these parasites compared to adult animals (> 1 years of age), however adults may greatly contribute to contaminate pastures[6]. Liver flukes include zoonotic species that raise public health concerns, such as *Fasciola hepatica*, which joined the list of important worm diseases with a great impact on human development[1]. In many European areas, an increase in the prevalence of *F. hepatica* has been observed over the last decade[11] and this observation has been associated mainly with climate changes[12;13]. In Europe, *F. hepatica* has shown a prevalence ranging from 0% to 27% in cattle[14;15;16] and from 4% to about 62% in small ruminants[2;11]. This liver fluke may lead to a reduction of up to 9% in weight gain and up to 15% in milk production[12].
Lungworms are parasites closely related to pasture and are very common in Europe, particularly in temperate climates, with a reported prevalence of up to 70% in grazing ruminants[17].
In Italy, few recent studies have dealt with the prevalence of gastrointestinal parasites, liver flukes and lungworms in ruminants[11;18;19;20;21].
The aim of this study was to obtain data on the occurrence and species composition of these parasites in adult ruminants living in two different areas of Tuscany, central Italy.

MATERIAL AND METHODS

Animals and Farms

A total of 178 adult ruminants (76 beef cattle of 3-9 years old, 61 dairy sheep and 41 dairy goats of 3-5 years old) were enrolled in a two years-prospective study (2012 - 2014).
Animals were from 16 extensive farms in Tuscany located in two areas, Area 1 and Area 2, with different environmental conditions. Area 1 (n= 10 farms) is a flat area subjected to

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Table 1 - Adult ruminants and farms from two areas (A1 and A2) of central Italy examined for gastrointestinal parasites, lungworms and liver flukes. A1: flat areas subject to water stagnation during rainy periods; A2: hilly and drier areas.

Area	Farms	Cattle	Sheep	Goats	Total
Area 1	10	52	42	17	111
Area 2	6	24	19	24	67
Total	16	76	61	41	178

water stagnation after rainy periods, while Area 2 (n= 6 farms) is a hilly and drier area. The mean animal consistency was about of 15-20 animals/farm.

Of the animals examined, 111/178 and 67/178 came from Area 1 and from Area 2, respectively (Table 1). Based on the will of farmers, anthelmintic treatments were performed only in Area 1 with netobimin or albendazole twice per year (summer and winter) in 6 out of 10 farms.

All the animals were examined to evaluate the occurrence of faecal parasites, while in 94 out of 178 examined animals *F. hepatica* faecal antigens and anti- *F. hepatica* antibodies were also evaluated. Of these latter animals, 50/94 (25 cattle, 15 sheep and 10 goats) were from 8/10 farms located in Area 1 and 44/94 (18 cattle, 16 sheep and 10 goats) were from 4/6 farms located in Area 2.

Sampling

Individual faecal samples were collected from all the animals enrolled in the study and used for copromicroscopical analysis. In the study period, most of the samples were collected from October until the end of June.

An aliquot of faecal samples taken from 94 animals was stored at -20°C to be used later for the detection of *F. hepatica* faecal antigens. From these same 94 animals, blood samples were also collected and stored at -20°C until examined for the detection of anti- *F. hepatica* antibodies.

Parasitological analysis

All collected fresh faecal samples were promptly analysed by a sedimentation-flotation technique using a saturated zinc chloride solution with 1.560 specific gravity on 4 gr of faeces[22], to detect the presence of worms eggs and/or protozoal (oo)cysts. In order to have an estimation of infection intensity and of pasture contamination, gastrointestinal strongyle and *Eimeria* faecal egg and oocyst count was performed, respectively, by using a McMaster technique with a sensitivity of 50 eggs per gram of faeces (EPG)/ oocysts per gram of faeces (OPG)[23]. For the isolation of lungworms, the Baermann technique was used and isolated first-stage larvae (L1) were identified at the species level[23]. In order to identify isolated coccidian species, oocysts from positive faecal samples were allowed to sporulate by suspending them in 2.5% potassium dichromate ($K_2Cr_2O_7$) in Petri dishes at 20°C ± 1°C, until the sporulation of the oocysts that were identified at the species level[23].

Immune-diagnosis of *Fasciola hepatica*

In 94 out of 178 animals and by using two commercial ELISA kits, the occurrence of *F. hepatica* infections was also evaluated by the detection of *F. hepatica* antigens in faecal samples (Bio-X Diagnostics *Fasciola hepatica* antigen ELISA kit, Jewelle, Belgium), and of anti- *F. hepatica* antibodies in serum from blood samples (Bio-X Diagnostics antibody anti-*Fasciola hepatica* ELISA kit, Jewelle, Belgium). Tests were performed according to the manufacturer's instructions and the results were expressed as *S/P* ratios where *S* is the optical density (OD) reading of the test sample and *P* is the OD reading of a positive control run on each plate. Test results were multiplied by 100 and expressed as percentages. The ODs were read with the Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Shanghai, China).

Data analysis

The prevalence of isolated parasites was estimated as the number of positive animals/total number of examined animals. Differences among prevalence values were statistically evaluated using a χ^2 test with the Yates correction, considering two groups of animals (cattle and small ruminants) and two different areas (A1 and A2). The statistical set was $P<0.05$.

Statistical analysis was performed using GraphPad Prism® program and Microsoft Excel: MAC® 2011 program.

RESULTS

Overall, all farms and 83.7% of the examined ruminants were found positive for at least one parasitic species. With the copromicroscopic techniques, 80.3% of the examined animals were found positive, with significant differences ($p<0.001$) between cattle (57.9%) and small ruminants (95.1% in sheep and 87.8% in goats) (Table 2).

Animals from A2 showed a significant higher prevalence (94.2%) of parasites than A1 animals (69.7%) ($p<0.001$) (Table 2).

Of the identified parasites, gastrointestinal strongyles (71.3%), *Strongyloides papillosus* (10.7%) and *Eimeria* spp. (56.7%) were isolated from all the species of ruminants. Cattle were also infected by *Capillaria* spp. (2.5%) and *Buxtonella sulcata* (8.9%), while small ruminants by *Trichuris* spp. (12.4%), lungworms (10.8%), *Dicrocelium dendriticum* (14.05%) and *Moniezia benedeni* (3.4%) (Table 3).

Gastrointestinal strongyles and coccidia (*Eimeria* spp.) were the prevalent parasites in all species of ruminants, with higher prevalence ($p<0.001$) in small ruminants than in cattle. Prevalence values of 85.2% and 85.4% for gastrointestinal strongyles and of 75.4% and 85.4% for coccidia were found in sheep and goats, respectively, while cattle showed prevalence values of 52.6% for gastrointestinal strongyle and of 26.3% for *Eimeria* spp. infections. Consi-

Table 2 - Prevalence of gastrointestinal parasites, lungworms and liver flukes observed by copromicroscopical analysis in cattle, sheep and goats from flat areas subject to water stagnation during rainy periods (A1) and from hilly and drier areas (A2).

Animals	Overall	A1	A2
Cattle	57.9% (44/76)	42.3% (22/52)	91.6% (22/24)
Sheep	95.1% (58/61)	92.8% (39/42)	100% (19/19)
Goats	87.8% (36/41)	82.3% (14/17)	91.6% (22/24)
Total	80.3% (143/178)	68.4% (76/111)	97.01% (65/67)

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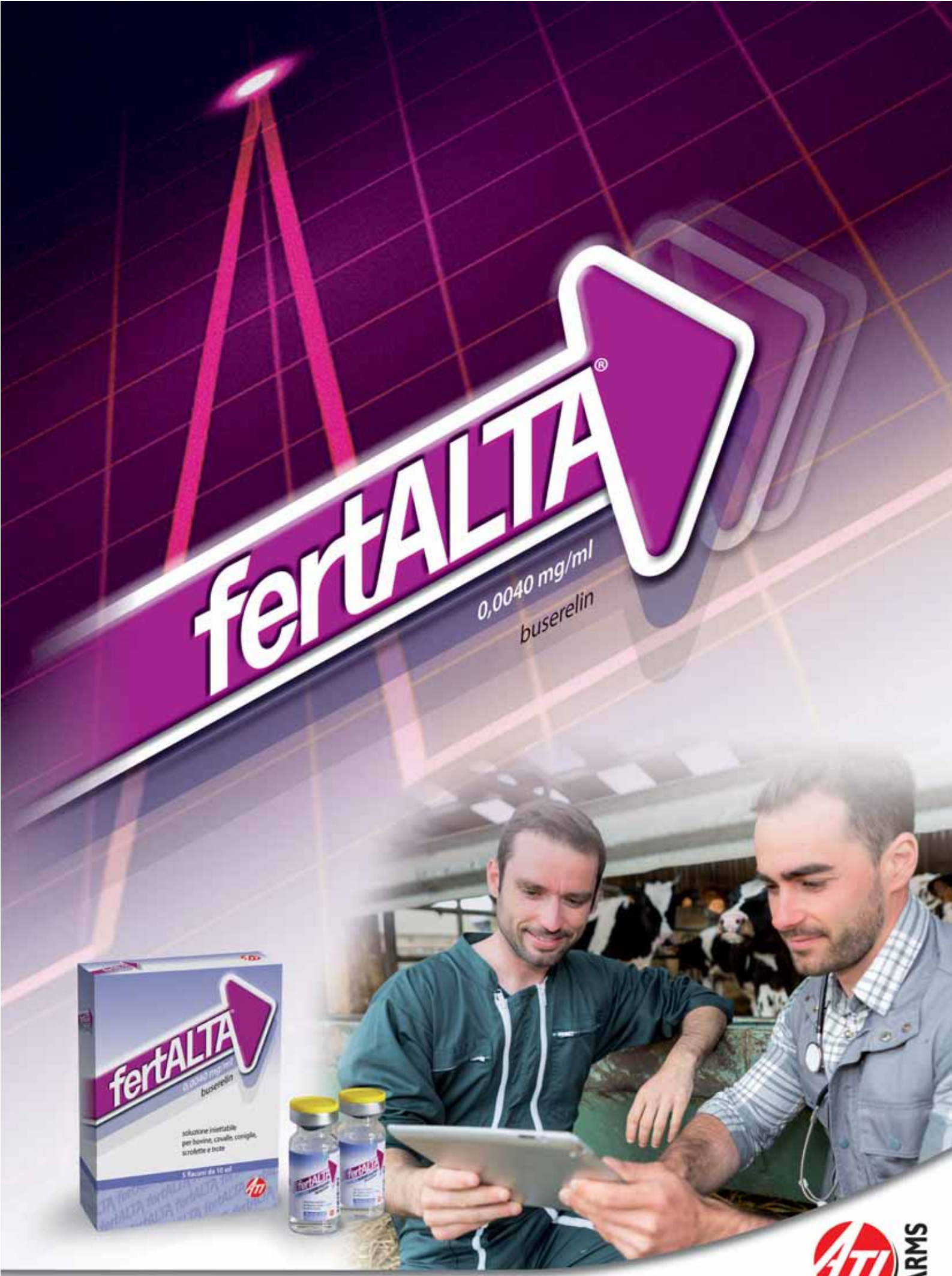

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Table 3 - Prevalence of isolated gastrointestinal, lung and liver parasitic species observed by faecal analysis in cattle, sheep and goats from flat areas subject to water stagnation during rainy periods (A1) and from hilly and drier areas (A2).

Parasites	Overall	A1	A2	Cattle	Sheep	Goats
Gastronitestinal strongyles	71.3% (127/178)	63.4% (71/111)	83.6% (56/67)	52.6% (40/76)	85.2% (52/61)	85.4% (35/41)
Strongyloides papillosus	10.7% (19/178)	5.4% (6/111)	19.4% (13/67)	5.3% (4/76)	18.1% (11/61)	9.8% (4/41)
Trichuris spp.	12.4% (22/178)	8.1% (9/111)	19.4% (13/67)	0% (0/76)	19.7% (12/61)	24.4% (10/41)
Capillaria spp.	2.2% (4/178)	0.9% (1/111)	4.5% (3/67)	5.3% (4/76)	0% (0/61)	0% (0/41)
Lungworms	10.1% (18/178)	3.6% (4/111)	20.9% (14/67)	0% (0/76)	18% (11/61)	17.1% (7/41)
Fasciola hepatica	0% (0/178)	0% (0/111)	0% (0/67)	0% (0/76)	0% (0/61)	0% (0/41)
Dicrocelium dendriticum	14% (25/178)	9.1% (10/111)	22.4% (15/67)	0% (0/76)	32.8% (20/61)	12.2% (5/41)
Eimeria spp.	56.7% (101/178)	43.2% (48/111)	79.1% (53/67)	26.3% (20/76)	75.4% (46/61)	85.4% (35/41)
Buxtonella sulcata	8.9% (16/178)	1.8% (2/111)	20.9% (14/67)	21% (16/76)	0% (0/61)	0% (0/41)
Moniezia benedeni	3.4% (6/178)	2.7% (3/111)	4.5% (3/67)	0% (0/76)	6.5% (4/61)	4.9% (2/41)

Table 4 - Mean ± standard deviation of gastrointestinal strongyle eggs per gram of faeces (EPG) and coccidian oocysts per gram of faeces (OPG) counted in cattle, sheep and goats from flat areas subject to water stagnation during rainy periods (A1) and from hilly and drier areas (A2).

Animal	Gastrointestinal strongyles EPG			*Eimeria* OPG		
	Overall	A1	A2	Overall	A1	A2
Cattle	401.2±187.2	195.4±99.8	652.7±134	505±288.8	170±54	616.6±285
Sheep	547.7±107.7	542.6±139.2	563.6±69	491.2±176.2	428.3±201.2	680±169.9
Goats	475.7±206.7	473.3±188.6	477.5±127	587.5±212.3	436.6±37	640.6±213.7

dering the areas, gastrointestinal strongyles ($p<0.001$) and coccidia ($p<0.002$) were significantly more prevalent in A2 than in A1 (Table 3).

Eimeria bovis (65%), *E. ovinoidalis* (74%) and *E. caprina* (82.9%) were the most prevalent coccidian species identified in cattle, sheep and goats, respectively. The other identified *Eimeria* species were *E. zuernii* (50%), *E. canadensis* (45%), *E. cilindrica* (35%) and *E. subspherica* (30%) in cattle, *E. ahsata* (54.3%), *E. granulosa* (43.9%), *E. parva* (15.2%) and *E. weybridgensis* (8.7%) in sheep and *E. caprovina* (54.3%), *E. christenseni* (31.4%), *E. ninakohlyakimovae* (22.8%) and *E. arloingi* (17.1%) in goats.

Among lungworms, *Muellerius capillaris* and *Protostrongylus rufescens* were isolated both in sheep and goats, although *M. capillaris* was the most prevalent species in sheep (53.8%), while *P. rufescens* showed a higher prevalence in goats (80%).

With regard to the quantitative analysis, mean EPG and OPG numbers were higher in cattle from A2, while in small ruminants very similar values were found in both areas (Table 4). Data from quantitative analysis are summarised in Table 4.

With the immune-diagnostic tests for *F. hepatica*, the 18.1% of the examined animals had positive scores at the faecal antigens ELISA kit, with higher prevalence in small ruminants, while 38.3% animals had positive scores with the antibody anti-*F. hepatica* ELISA kit (Table 5). Higher *F. hepatica* prevalence values were found in A1 than in A2 (Table 5).

On average, the 19.1% of examined ruminants were infected by only one parasitic species (27.7% of cattle, 14.7% of sheep and 9.7% of goats), 25.3% by two different parasites (11.8% of cattle, 29.5% of sheep and 43.9% of goats) and 33.7% were positive for more than two parasites (18.4% of cattle, 32.8% of sheep and 39% of goats).

Table 5 - Prevalence of *Fasciola hepatica* observed in cattle, sheep and goats from flat areas subject to water stagnation during rainy periods (A1) and from hilly and drier areas (A2) with two commercial (Bio-X Diagnostics *Fasciola hepatica* antigen ELISA kit, Jewelle, Belgium and Bio-X Diagnostics antibody anti-*Fasciola hepatica* ELISA kit, Jewelle, Belgium) ELISA kits.

Animal	Antigen ELISA			Antibody ELISA		
	Overall	A1	A2	Overall	A1	A2
Cattle	6.9% (3/43)	8% (2/25)	5.5% (1/18)	25.6% (11/43)	40% (10/25)	5.5% (1/18)
Sheep	35.5% (11/31)	40% (6/15)	31.2% (5/16)	61.3% (19/31)	73.3% (11/15)	50% (8/16)
Goats	15% (3/20)	20% (2/10)	10% (1/10)	30% (6/20)	50% (5/10)	10% (1/10)
Total	18.1% (17/94)	20% (10/50)	18.2% (7/44)	38.3% (36/94)	52% (26/50)	22.7% (10/44)

DISCUSSION

In adult ruminants, gastrointestinal parasites, lungworms and liver flukes are very common and can represent a limiting factor for productions[1;2]. In addition, the contamination of pastures by adult ruminants may be responsible for the infections of young animals, which are more susceptible to pathogenic effects of most of these parasites[1;25;26].

The main aim of the present study was to assess the occurrence of gastrointestinal parasites, liver flukes and lungworms in adult ruminants in central Italy.

Overall, 83.7% of the examined animals were found positive, but a significantly higher number of positive animals was found in small ruminants than in cattle.

Gastrointestinal strongyles and coccidia were found to be the most prevalent parasites in all the species examined, in accordance with data reported in previous European studies performed in cattle[26;27], sheep[2] and goats[2;10].

In cattle, the prevalence of gastrointestinal strongyles found in this study was higher[27], similar[26] and lower[28] than previous European data. The prevalence found in sheep was similar to that found in previous European studies[2;29], but higher than that previously observed in southern Italy[20]. In goats, the prevalence was higher than that reported in other studies performed both in Italy[21] and in other European countries[2;10;30].

The prevalence of coccidian infections in cattle (26.3%) was lower than in other areas in Europe[9] while in small ruminants it was higher than previous European data both for sheep[25] and goats[31].

Regarding the most prevalent coccidian species, obtained results confirmed previous data reported in cattle[9], sheep[24] and goats[32], according to which *E. bovis* and *E. zuernii* are prevalent in cattle, *E. ovinoidalis* in sheep and *E. caprina* in goats. In domestic ruminants, these coccidian species are considered to be the most pathogenic ones[24;25;33].

The high prevalence of gastrointestinal strongyle and coccidian infections observed in adult ruminants here examined and the high pathogenicity of the prevalent coccidian species highlight the need to implement effective control procedures, which can limit the spread of these parasites and the contamination of pastures by adult animals[2;9;21]. Although in all species of the examined ruminants mean values of gastrointestinal strongyle EPG and of *Eimeria* OPG observed in this study are indicative of low intensities of gastrointestinal strongyle and coccidian infections, often associated with subclinical forms of diseases[3;6;31], it is known that in domestic ruminants even low infection intensities of these parasitic species may lead to significant production losses[4;9].

In the present study, lungworms, *D. dendriticum, Trichuris* and *M. benedeni* were found only in small ruminants. Among lungworms, *M. capillaris* was the most prevalent species isolated in sheep, as already observed in Spain[17]. The fact that no lungworm infections were found in cattle confirms previous observations in the same area regarding the unfavorable environmental conditions of central Italy for the life-cycle of *Dictyocaulus viviparus*[19].

D. dendriticum showed a higher prevalence in sheep than in goats. This could be associated with the higher susceptibility of sheep to this fluke species[34]. Finally, the prevalence of *Trichuris* and *M. benedeni* was lower if compared to that observed in small ruminants in previous studies[2;21].

In cattle, *Capillaria* showed a low prevalence (2.5%) as reported by other authors[26;27] while the prevalence of *B. sulcata* was lower than findings reported in England[35].

While no *F. hepatica* eggs were found at microscopy, the positivity detected with the ELISAs confirmed the occurrence of *F. hepatica* infections in ruminants in Italy[11;13;18;19]. In particular, the 18.1% of the ruminants here examined were found to be positive to the *F. hepatica* coproantigen ELISA, with higher values in small ruminants than in cattle. These results may be due to the high susceptibility of small ruminants to this fluke species[36]. The commercial kit used in this study enables the presence of *F. hepatica* antigens to be evaluated in faeces with a sensitivity of 98% and a specificity close to 100%[37;38]. This test is able to detect prepatent infections from four weeks post-infection onwards and animals infected with as low as 1-2 flukes[37]. This test is therefore able to diagnose *F. hepatica* infections earlier than coprological analysis, which is not able to detect *F. hepatica* until 8-10 weeks post infection[12]. Considering also that the sedimentation-flotation technique used in the present study for the microscopic diagnosis of *F. hepatica* was performed on an amount of faeces able to detect only high-rates of infection[22], it can be assumed that in positive animals *F. hepatica* eggs were absent or in low numbers. The antibody ELISA kit used in this study is able to detect anti-*F. hepatica* antibodies with a sensitivity and a specificity of 98% and 96%, respectively[39]. In ruminants, circulating antibodies are present from 2-4 weeks post infection[40;41;42] to 12 weeks after recovery[43]. Thus, this test may allow an earlier diagnosis of *F. hepatica* than the coproantigen ELISA kit. However, the positivity may not always be correlated with active infections, but it may only show exposure to the parasite. Indeed, the positivity to the antibody ELISA kit (38.3%) was higher than that to the antigen ELISA kit (18.1%). Except for *F. hepatica*, a higher prevalence of parasitic infections was found in A2 than A1. This finding could be related to the treatments and environmental conditions. In fact, the two anthelmintic treatments/year performed in 6 out of 10 farms sited in A1, may have significantly affected the prevalence of helminthic infections in this area. On the other hand, the hilly areas with dry pastures and abundant vegetation of A2, may favor the spread of some parasites, as *D. dendriticum*, whose intermediate hosts are very common in this kind of environment[18;44].

On the other hand, *F. hepatica* was the only isolated parasite that showed a higher prevalence in flat areas subjected to water stagnation (A1). This confirms the importance of these environmental conditions for the spread of this fluke species[1] and the need in the relative farms to perform effective management practices, such as pasture drainage, and effective treatments[4;45] for the control of this parasitic infection.

Finally, the high percentage (33.7%) of animals here examined found to be positive for more than two parasites may be a further cause of reduced productions, as the negative effects of different parasites may be additive, thus increasing production losses[4]. Economic benefits could thus be obtained by also performing a control of concomitant parasitic infections[3;4;46].

CONCLUSION

In conclusion, results from the present study showed that gastrointestinal parasites, especially gastrointestinal strongyles and coccidia, and liver flukes are very common in ruminants from central Italy and require further control measures.

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