

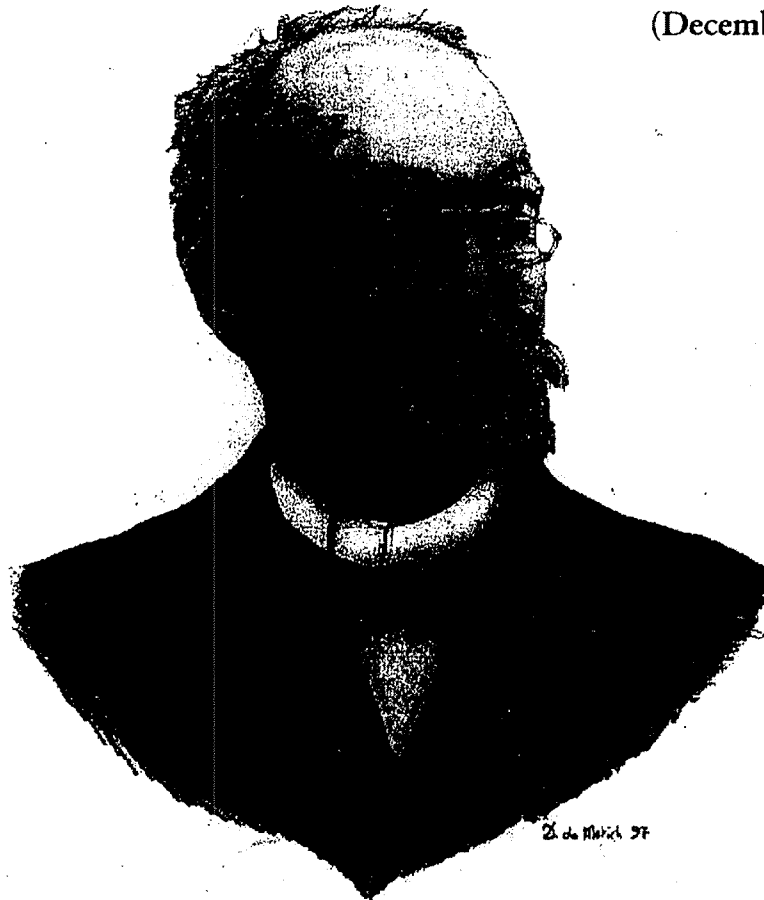
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Economic efficacy of anthelmintic treatments in dairy sheep naturally infected by gastrointestinal strongyles

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Abstract. The aim of the present paper was to assess benefit of strategic anthelmintic treatments on milk production in six commercial dairy sheep farms, located in southern Italy, whose animals were naturally infected with gastrointestinal strongyles. On each farm, two similar groups were formed, one untreated control group and one treated group. In all the treated groups, the strategic anthelmintic schemes were based on: (i) only one treatment with moxidectin in the periparturient period (February, Farm No. 6), or; (ii) two treatments, i.e. the first with moxidectin performed in the periparturient period (February, Farms Nos. 1, 2, 3 and 4) or in the postparturient period (April, Farm No. 5), and the second with netobimin at the mid/end of lactation (June, Farms Nos. 1, 2, 3, 4 and 5). Faecal egg count reduction (FECR) tests were performed on each farm in order to assess the anthelmintic efficacy of the drugs used. In addition, milk yield measurements for each animal fortnightly in each farm for the lactation period were performed. In terms of FECR, both moxidectin and netobimin were effective in all the 6 studied farms. Regarding milk production, overall in the 6 study farms the mean daily milk productions of the treated groups were higher than those of the control group. However, there were important differences between the 6 farms, i.e. the increase of milk production in the treated groups *versus* the control groups was as follows: +18.9% (Farm 1), +30.4% (Farm 2), +4.0% (Farm 3), +37.0% (Farm 4), +5.5% (Farm 5) and +40.8% (Farm 6). The results of the study showed that the economic efficacy of an anthelmintic treatment is not a cause-effect issue, but is a multifactorial issue which depends upon the qualitative-quantitative parasitological status of the animals, the pathogenesis of the species of parasites, the virulence of the strains of parasites, the local epidemiology, the timing of treatment, the breed of animal in terms of genetics and production types, nutrient supply.

Key words: sheep, gastrointestinal strongyles, anthelmintic treatment, milk production, economic efficacy.

Gastrointestinal (GI) strongyle infection (caused by different genera of nematodes, e.g. *Teladorsagia*, *Haemonchus*, *Bunostomum*, *Cooperia*, *Nematodirus*, *Trichostrongylus*, *Chabertia* and *Oesophagostomum*) is one of the main constraints to sheep production both in temperate and tropical countries. In many cases, GI strongyle parasitism can be assimilated to a nutritional disease, because the presence of worms usually induces a decrease in appetite, a decreased digestibility of the food and a diversion of nutrients from production sites towards the repair of tissue-damage caused by the parasites. Therefore, economic loss due to GI strongyles is related to decreased production, costs for treatments and even animal death. GI strongyles are the most commonly found helminths in pasturing sheep bred in central and southern Italy (Cringoli *et al.*, 2000, 2007; Rinaldi *et al.*, 2007).

In Italy, the control of these parasitic infections in small ruminants relies almost exclusively on multiple and regular dosing with anthelmintics. Besides

the parasitological efficacy of an anthelmintic treatment in livestock, it is very important to consider its strategic and economic efficacy.

The beneficial impact of anthelmintic treatment on milk production has been extensively documented in dairy cows (Nansen, 1987; Fox *et al.*, 1989; Ploeger *et al.*, 1990; Corwin, 1997; Huckle *et al.*, 2001; Nodtvedt *et al.*, 2002; Reist *et al.*, 2002; Forbes *et al.*, 2004); however, a very few studies have been conducted on this topic in dairy goats (Veneziano *et al.*, 2004) and sheep (Jordan and Perez, 1991; Fthenakis *et al.*, 2005; Cringoli *et al.*, submitted).

The aim of the study was to assess the benefit of strategic anthelmintic treatments on milk production in six commercial dairy sheep farms, located in central-southern Italy, whose animals were naturally infected with GI strongyles. The anthelmintic schemes were based on: (i) only one treatment with moxidectin in the periparturient period (February, Farm No. 6), or; (ii) two treatments, the first with moxidectin performed in the periparturient period (February, Farms Nos. 1, 2, 3 and 4) or in the postparturient period (April, Farm No. 5), and the second with netobimin at the mid/end of lactation (June, Farms Nos. 1, 2, 3, 4 and 5).

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Materials and methods

Study farms

The controlled field trials were conducted on six commercial sheep farms (2 in 2006 and 4 in 2007) located in central-southern Italy (provinces of Campobasso, Avellino and Salerno).

The breeds of sheep were different between the study farms, characterized by different production types (dairy/meat) and alimentation; they were exclusively dairy sheep in Farm No. 3 (Sarda breed), basically dairy sheep in Farms Nos. 1 (Pinzirita × Comisana breed) and 2 (Pinzirita × Crossbreed), and basically meat sheep in Farms Nos. 4 (Crossbreed), 5 (Gentile di Puglia breed) and 6 (Île-de-France × Gentile di Puglia breed).

In all the farms, most of the parturitions took place from 10th February to 15th March and the lactation period from parturitions to June/August.

Flock parasitological status

The sheep of the six farms had naturally acquired mixed parasite infections as determined by coprological examinations (see below). The species of GI strongyles in the animals were identified 3 days before the beginning of the trial, by slaughter and necropsy of three sheep randomly selected from animals coprologically positive for GI strongyles. The viscera were processed for sample collection, further worm counts and identification of parasites present in the abomasum and small and large intestines, following the procedures described in the WAAVP guidelines for evaluating the efficacy of anthelmintics in ruminants (Wood *et al.*, 1995).

Study animals and treatments

On each farm, two similar groups were formed for breed, age, weight, number of pasturing seasons, and positive GI strongyle eggs per gram of faeces (EPG) and randomly assigned to two groups, one untreated control group (C-group) and one treated group (T-group). The allocation into groups was performed using a computer random number generation.

Table 1 reports the details regarding treatments (months and drugs) and groups (Nos. of control and treated animals) in each study farm.

The strategic anthelmintic schemes were based on:

- (i) only one treatment with moxidectin in the periparturient period (February, Farm No. 6);
- (ii) two treatments, the first with moxidectin performed in the periparturient period (February, Farms Nos. 1, 2, 3 and 4) or in the postparturient period (April, Farm No. 5), and the second with netobimin at the mid/end of lactation (June, Farms Nos. 1, 2, 3, 4 and 5).

During the treatments, moxidectin (Cydectin™ 0.1%, Fort Dodge Animal Health) was given *per os* at the dosage of 0.2 mg/kg body weight, and netobimin (Hapadex™ 5%, Schering-Plough) was given *per os* at the dosage of 20 mg/kg body weight.

Both drugs are licensed for the treatment of various helminth infections of sheep (Bishop, 1998) and are commonly used for sheep in Italy.

At each treatment date, for each treated sheep, individually weighed, the dosage of the drug was calculated on the basis of its body weight. Sheep of the C-group did not receive any treatment but were subjected to the same handling procedures as the sheep of the treated groups, receiving *per os aqua fontis*.

On each farm, after the treatment the study animals of the two groups were maintained together under the same conditions. Veterinary care was available to the animals throughout the study.

Coprological examinations

On each study farm, faecal samples were collected from each study animal by inserting a gloved finger into the rectum. Samples were placed in bags labelled with protocol number, date of sampling, and animal identification number.

Faecal egg counts were performed on each study animal before the start of the trial (day -3), at days 0 and 14 after treatment and then monthly until the end of the study (July).

Individual faecal egg counts were carried out by the FLOTAC® technique (Cringoli, 2006), with a sensitivity of two EPG, using a sucrose-based flotation medium (specific gravity = 1.250) (Cringoli *et al.*, 2004).

On each sampling day, composite pooled faecal cultures were made. Third stage larvae were identified using the morphological keys proposed by MAFF (1986). When a coproculture had 100 or less third

Table 1. Treatments (months and drugs) and groups (No. of control and treated sheep) in each study farm.

Farms Nos.	I treatment		II treatment		No. of sheep	
	Month	Drug	Month	Drug	Control	Treated
Farm 1	February	MOX	June	NET	20	20
Farm 2	February	MOX	June	NET	20	20
Farm 3	February	MOX	June	NET	20	20
Farm 4	February	MOX	June	NET	20	20
Farm 5	April	MOX	June	NET	20	20
Farm 6	February	MOX	-	-	30	30

MOX = moxidectin 0.2 mg/kg body weight; NET = netobimin 20 mg/kg body weight.

stage larvae, all were identified; when a coproculture had more than 100 larvae, only 100 were identified.

Faecal Egg Count Reductions (parasitological efficacy)

EPG values were transformed to natural logarithms [$\ln(x+1)$] to calculate their geometric means (GM). In each farm, for each treatment group, percent efficacy (%) was calculated in terms of Faecal Egg Count Reduction (FECR) at the day 14 after treatment using the formula used by Dorchies *et al.* (2001):

$$\text{FECR} = \frac{\text{GM EPG from controls} - \text{GM EPG from treated}}{\text{GM EPG from controls}} \times 100$$

Milk production (economic efficacy)

Depending on the farm management practices, or the morning and evening (Farms Nos. 1, 2, 3 and 5), or only the morning (Farm No. 4) or only the evening (Farm No. 6), milk production (ml) was recorded for each study animal fortnightly for whole lactation period (April-August).

It should be noted that the first milk records were performed during or soon after the suckling period (April).

Milk samples were collected into plastic containers. In order to avoid misreading by foam formation, milk samples were then slowly poured into a volumetric glass cylinder graduated to 5 ml and allowed to stand 5-10 minutes before reading.

Data analysis

All the statistical analyses were performed using SPSS software (Version 13).

On each farm, at each faecal sampling time, the Mann-Whitney *U*-test was used to compare the mean GI strongyle EPG values from treated and

control groups. $P < 0.05$ was considered as statistically significant.

On each farm, at each date of milk sampling, a Generalized Linear Model (GLM) repeated measure analysis of the effect of the group (treated *versus* control) on milk production was performed. In particular, milk production values (ml) were introduced into the model as dependent variables, group as categorical fixed factor, and differences (days) between milk sampling date and parturition date as covariates. $P < 0.05$ was considered as statistically significant.

Results

Necropsies

The necropsies performed 3 days before the beginning of the trials allowed us to identify the species of GI strongyle in the animals of the study farms.

The adult nematodes recovered and identified in sheep were: *Teladorsagia circumcincta* (Farms Nos. 4, 5 and 6) and *Haemonchus contortus* (Farms Nos. 1, 2, 3, 4 and 6) in the abomasums, *Trichostrongylus vitrinus* (Farms Nos. 1, 2, 4 and 6) and *Trichostrongylus colubriformis* (Farms Nos. 1, 2, 3, 4 and 5) in the small intestine, *Oesophagostomum venulosum* (Farms Nos. 1, 3, 4, 5 and 6) in the large intestine.

Flock parasitological status

Faecal examinations performed on sheep from the six farms before the beginning of the study showed naturally acquired mixed parasite infections; Table 2 reports the flock parasitological status of the 6 studied farms, i.e. the prevalence and the mean eggs/larvae/oocysts per gram of faeces (EPG/LPG/OPG) for each group/genus of parasites.

Table 2. Flock parasitological status of the 6 studied farms (prevalence and intensities).

Parasites (groups, genera or species)	Percentages (%) and intensities (EPG/OPG/LPG) of parasites											
	Farm 1		Farm 2		Farm 3		Farm 4		Farm 5		Farm 6	
	%	EPG	%	EPG	%	EPG	%	EPG	%	EPG	%	EPG
GI strongyles	100	724	100	600	100	244	100	700	100	540	100	272
<i>Teladorsagia</i> spp.	0	ND	0	ND	0	ND	11	ND	1	ND	40	ND
<i>Haemonchus</i> spp.	4	ND	59	ND	32	ND	9	ND	0	ND	20	ND
<i>Trichostrongylus</i> spp.	93	ND	41	ND	65	ND	59	ND	79	ND	31	ND
<i>Oesophagostomum</i> spp.	3	ND	0	ND	3	ND	21	ND	20	ND	9	ND
<i>Nematodirus</i> spp.	12	8	0	0	0	0	48	4	56	4	38	4
<i>Trichuris</i> spp.	17	6	12	12	16	2	16	2	3	2	11	2
Lungworms	20	28*	47	16*	3	2*	58	8*	52	4	78	44
<i>Dicrocoelium dendriticum</i>	100	90	100	76	0	0	80	16	96	100	100	120
<i>Moniezia</i> spp.	25	ND	17	ND	0	ND	0	ND	0	ND	0	ND
<i>Eimeria</i> spp.	100	580**	100	280**	100	110**	100	150**	100	280**	100	80**

ND = not determined; * LPG; **OPG.

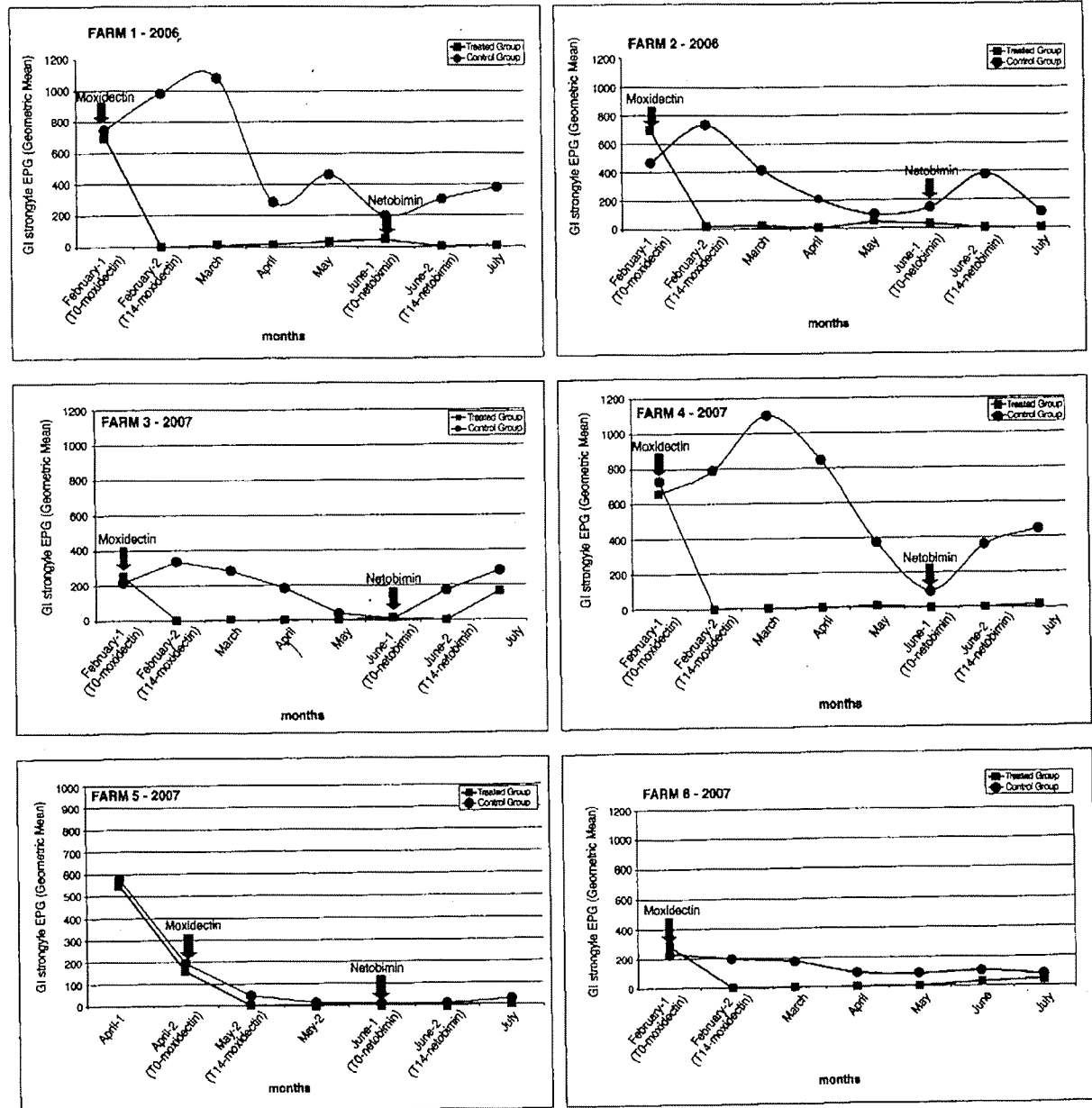


Fig. 1. GI strongyle faecal egg counts (geometric mean EPG) in the groups treated with the strategic anthelmintic scheme compared with the control untreated groups in the six studied farms.

Faecal Egg Count Reductions (parasitological efficacy)

The percentage reductions in GI strongyle faecal egg counts (FEGR) in the T-groups, compared to the untreated C-groups on day 14 were 100% (Farms Nos. 1, 3, 4, 5 and 6) and 97.3% (Farm No. 2) for moxidectin, and 100% (Farms Nos. 1, 2, 3, 4 and 5) for netobimin.

In all the six farms, the Mann-Whitney U-test showed that at day -3 and day 0 no differences between the mean GI strongyles egg emission of T-groups and C-groups were found, whereas control

animals showed significantly ($P < 0.05$) higher EPG values than treated animals during trial in all the farms except Farm No. 5 (Fig. 1).

Milk production (economic efficacy)

Estimated marginal means of milk yield production for the control and treated groups in the six farms on each date are shown in Table 3. At each milk sampling date, the treated groups of each farm had milk yields that were higher than their control groups and on some occasions these differences

Table 3. Estimated marginal means by GLM of milk production (ml) in the six studied farms.

Dates	Milk Production (ml) - Estimated marginal means by GLM																	
	Groups - Farm 1 ^a			Groups - Farm 2 ^a			Groups - Farm 3 ^a			Groups - Farm 4 ^b			Groups - Farm 5 ^a			Groups - Farm 6 ^c		
	C	T	P-value	C	T	P-value	C	T	P-value	C	T	P-value	C	T	P-value	C	T	P-value
Apr-1	1081.1	1299.5	*	298.1	482.0	*	1424.0	1536.2	NS	218.0	336.0	NS	373.1	378.0	NS	411.2	556.3	*
Apr-2	1113.3	1284.7	NS	296.4	400.8	*	1325.4	1393.3	NS	209.7	324.5	NS	470.2	463.2	NS	405.7	537.5	*
May-1	796.6	998.4	*	291.3	340.6	NS	1272.2	1297.7	NS	240.4	291.3	NS	373.8	367.0	NS	293.3	445.5	*
May-2	758.1	893.6	*	282.9	305.4	NS	1168.3	1178.6	NS	208.1	239.9	NS	388.1	417.7	NS	315.0	459.8	*
Jun-1	612.8	699.4	NS	237.2	285.9	NS	1039.2	1060.3	NS	109.8	157.2	NS	343.6	340.1	NS	239.1	358.7	*
Jun-2	536.3	670.1	*	197.5	275.5	NS	883.9	937.4	NS	125.2	175.8	NS	290.1	333.2	NS	164.1	279.5	*
Jul-1	532.7	649.3	*	-	-	-	799.4	844.3	NS	60.7	81.4	NS	219.2	234.0	NS	160.0	203.8	NS
Jul-2	500.3	559.2	NS	-	-	-	706.4	745.4	NS	-	-	-	170.3	235.1	NS	147.4	186.1	NS
Aug-1	-	-	-	-	-	-	599.2	596.2	NS	-	-	-	117.6	149.2	NS	98.3	133.7	NS
Aug-2	-	-	-	-	-	-	-	-	-	-	-	-	142.1	129.6	NS	117.5	150.0	NS
Mean	741.4	881.8	*	267.2	348.4	*	1024.2	1065.5	NS	167.4	229.4	*	288.8	304.7	NS	235.2	331.1	*
%	+18.9			+30.4			+4.0			+37.0			+5.5			+40.8		

a = morning and evening milk production; b = only morning milk production; c = only evening milk production; * = $P < 0.05$; NS = not significant.

were significant. Overall, in four farms the mean daily milk production of the treated groups were significantly ($P < 0.05$) higher than those of the control groups as follows: 881.8 ml versus 741.4 ml (+18.9%) in Farm No. 1; 348.4 ml versus 267.2 ml (+30.4%) in Farm No. 2; 229.4 ml versus 167.4 ml (+37.0%) in Farm No. 4; and 331.1 versus 235.2 ml (+40.8%) in Farm No. 6.

However, in two farms the difference in milk production between treated and control groups was not significant ($P > 0.05$) as follows: 1065.5 versus 1024.2 ml (+4.0%) in Farm no. 3, and 304.7 versus 288.8 ml (+5.5%) in Farm No. 5.

Discussion

Effective chemical anthelmintics remain irreplaceable for worm control and their elimination is not practical on animal welfare and economic grounds. Any anthelmintic scheme has three main goals, i.e. the parasitological efficacy, the strategic efficacy and the economic efficacy.

In fact, for producers, the three primary aims of anthelmintic treatment strategies are, in the face of ongoing parasite challenge, firstly to maintain or improve animal performance, secondly to reduce EPG in the animals, and third to reduce pasture contamination (Cringoli *et al.*, submitted).

The two drugs used in the present trials, i.e. moxidectin and netobimin, were effective and safe from a parasitological point of view, thus having a full parasitological efficacy. In fact, following the standards for rating anthelmintic efficacy as put forward in the WAAVP guidelines (Wood *et al.*, 1995), in terms of FECR, at days 14 moxidectin was highly effective in 5 farms (100%) and effective in one

farm (97.3%), whereas netobimin was highly effective (100%) in the 5 study farms.

In addition, the GI strongyle EPG of the treated groups were significantly lower than those of the control groups during the study in all the farms (except Farm No. 5), thus resulting in a lower pasture contamination and in a clear strategic efficacy.

Regarding milk production, overall in the 6 study farms the mean daily milk productions of the treated groups were higher than those of the control groups. However, there were important differences between the 6 farms, i.e. the increase of milk production in the treated groups vs the control groups was as follows: +18.9% (Farm No. 1), +30.4% (Farm No. 2), +4.0% (Farm No. 3), +37.0% (Farm No. 4), +5.5% (Farm No. 5) and 40.8% (Farm No. 6). Thus, the economic efficacy of the treatment was fully achieved in four of the six study farms.

These results could be explained by a series of issues depending upon the timing of treatment, the farm management, the characteristics of animals, and the parasitological and epidemiological scenario of the study area. The first issue which is extremely important for the economic and strategic efficacy of an anthelmintic treatment is the timing of treatment as already showed in dairy goats (Veneziano *et al.*, 2004). In fact, in the present study the treatment with moxidectin during the periparturient period (February) caused a consistent increase in milk yield across four farms (Farms Nos. 1, 2, 3 and 6) and all sampling times that resulted in an overall 19% to 41% greater milk yield. These results are in general agreement with studies in dairy sheep (Fthenakis *et al.*, 2005) that have used lamb liveweights to provide an indirect measure of milk production, and have reported milk yield to be increased by 15% following

anthelmintic treatment with moxidectin towards the end of the gestation.

The importance of the timing of treatment is testified by the fact that in Farm No. 5 the treatment performed with moxidectin in April (postparturient period) instead of February produced a very low difference in milk production between treated and control groups (+5.5%).

However, the results obtained in Farm No. 3 are not in line with the other ones; in fact, although the periparturient treatment with moxidectin and the mid/end lactation treatment with netobimin (as also done in Farms Nos. 1, 2 and 4), milk production increased only 4.0% in the treated group compared to the control group. This latter result could be explained by the absence of highly pathogenic species (e.g. *Teladorsagia circumcincta*) or strains of GI strongyles or by the fact that the sheep were very well supplied with nutrients. Further studies are needed to clarify these latter aspects.

Most of the sheep used in the present trials were also infected by lungworms (Metastrongylidae) and *Dicrocoelium dendriticum*, besides GI strongyles; it should be noted that moxidectin is also highly effective against lungworms (Papadopoulos *et al.*, 2004) and netobimin against *D. dendriticum* (Veneziano *et al.*, 2006). In the present trials, the treatment with moxidectin reduced the lungworm infection and the treatment with netobimin had an important effect on the decreasing of *D. dendriticum* infection in the treated sheep (data not shown), and thus on the detrimental effects of these helminths on milk production. Previous studies in small ruminant dairy animals have shown that GI strongyle, lungworm and *D. dendriticum* infections act to reduce the availability of nutrients that are likely to be partitioned for milk production (Jordan and Perez, 1991; Hoste and Chartier, 1993; Veneziano *et al.*, 2004).

Very few data are available in scientific literature regarding the impact of anthelmintic treatments against GI strongyles on milk production in sheep. The results of the present study showed that the economic efficacy of an anthelmintic treatment is not a cause-effect issue, but is a multi-factorial issue which depends upon the qualitative parasitological status of the animals, the pathogenesis of the species of parasites, the virulence of the strains of parasites, the local epidemiology, the timing of treatment, the breed of animal in terms of genetics and production types, nutrient supply, etc. All these factors must be taken into consideration before making a final evaluation and must be widely studied in order to deeply understand this very complex phenomenon.

Acknowledgements

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