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Secnidazol Treatment to Improve Milk Yield in Sheep with Giardiasis

Deniz ALIC URAL[1]✉, Adnan AYAN[2], Nuran AYSUL[2], Canberk BALIKÇI[3], Kerem URAL[3]

1. Adnan Menderes University, Faculty of Veterinary, Faculty Farm, Isikli, Aydin, TURKEY.
2. Adnan Menderes University, Faculty of Veterinary, Department of Parasitology, Isikli, Aydin, TURKEY.
3. Adnan Menderes University, Faculty of Veterinary, Department of Internal Medicine, Isikli, Aydin, TURKEY.

Abstract: The purpose of this multidiciplinary (agricultural and veterinary fields) study was to assess the effect of single secnidazol treatment on milk production in dairy ewes naturally infected with Giardia duodenalis. Thirty dairy ewes with Giardiasis, were enrolled into 3 equal groups and Groups I and II were treated with secnidazole at a single dose rate of 10 mg/kg or 30 mg/kg, respectively, perorally and G III ewes were controls. Throughout the study ewes in G III remained positive for Giardiasis, with some of them showed an increase without statistical significance in cyst counts on day 10 (ranged 1300-241650) compared to the initial values (ranged between 5600-274600). The least square means and standard error of means of cyst excretion on D0 and D10 revealed that there was a significant reduction (P<0.001) in cyst reduction in GI and GII animals. Both group GI and GII ewes produced significantly more milk than group G III ones (P<0.001). The change of mean milk yield over time was statistically significant (P<0.001) among group GI and GII ewes; besides a statistically significant (P<0.001) reduction in the mean milk yield of group GIII ewes was observed. Given the efficacy of secnidazol treatment for cyst reduction and increased milk yield, it may be safely suggested that Giardiasis adversely affects the production of the infested animals.

Key words: Giardiasis, Milk yield, Secnidazol, Sheep.

Giardiasis'li Koyunlarda Süt Verimini Arttırmaya Yönelik Seknidazol Sağaltımı

Özet: Giardia duodenalis ile doğal enfekte sütçü koyunlar üzerine yapılan bu multidisipliner çalışmada tek dozda seknidazol sağaltım etkinliğinin belirlenmesi amaçlanmıştır. Giardiasisli 30 sütçü koyun, 3 eşit gruba ayrılmıştır. Grup I ve Grup II'de tek doz seknidazol (sırasıyla 10 mg/kg, 30 mg/kg p.o.) sağaltımı uygulanmış ve Grup III kontrol grubu olarak belirlenmiştir. Çalışma süresince kontrol grubu olan grup III'deki sütçü koyunlar Giardia yönünden pozitivitelerini sürdürmüşlerdir ve başlangıç değerleri (5600-274600) ile 10. gündeki (1300-241650) kist sayıları karşılaştırıldığında gruptaki bazı hayvanlarda istatistiksel önemi bulunmayan artış gözlenlenmiştir. Sıfırıncı ve 10. günlerindeki kist saçılımının en küçük kare ve standart hata değerlerine bakıldığında farklı doz seknidazol ile sağaltımı yapılan GI ve GII'deki hayvanların kist atılımında önemli derecede azalma (P<0.001) olduğu görülmüştür. Gerek GI, gerekse GII'deki koyunlar, GIII'dekilere oranla istatistiksel olarak belirgin şekilde daha fazla süt üretmişlerdir (P<0.001). Süt verimi ortalamalarında GI ve GII'de istatistiksel olarak anlamlı (P<0.001) değişiklikler saptanırken, GIII'deki koyunlarda süt veriminde azalma (P<0.001) gözlemlendi. Seknidazol sağaltımının kist atılımında azalma ve süt veriminde artış gibi etkileri nedeniyle; Giardiasis'in, enfekte koyunların süt verimini olumsuz derecede etkilediği rahatlıkla söylenebilinir.

Anahtar kelimeler: Giardiasis, Koyun, Seknidazol, Süt verimi.

✉ Deniz ALIC URAL
Adnan Menderes University, Faculty of Veterinary, Faculty Farm, Isikli, Aydin, TURKEY.
e-mail: alicdeniz@gmail.com

INTRODUCTION

Giardia duodenalis (namely *G. lamblia* and *G. intestinalis*) is the vast majority and freuqently diagnosed protozoon parasite of livestock species worldwide. The prevalence of *G. duodenalis* infection in ruminants may possess rates from 9 to 38% in adult sheep (Olson et al., 1995; Ryan et al., 2005; Geurden et al; 2009). Prevalence rates in animals are often underestimated through intermittent cyst excretion and relatively low sensitivity of some parasite detection methods. In Turkey, the real prevalence of giardiasis in sheep remains unclear with relatively few studies indicating 36.6% (Ozmen et al., 2006) and 48.48% (Ozdal et al., 2009).

Giardia has been associated with reduced animal production in ruminants (Olson et al., 1995; Degerli et al., 2005) it appears to occur in animals of all ages (Meloni et al., 1995) Giardia infections have been reported for sheep in many parts of the world (Diaz et al., 1996; Olson et al., 1997). Giardiasis in domestic ruminants is an economically important disease, thus necessitating control or elimination of the infection.

As aforementioned above, a few therapy choices, specifically nitroimidazole derivatives are commercially available (Rossignol, 2010). On a large scale, 5-nitroimidazole compounds, (metronidazole, ornidazole and secnidazole), are first line anti-Giardial treatment choice and are still been widely using (Rossignol, 2010; Busatti et al., 2009). According to the authors experience there is clearly a need for evaluating novel treatment options both in human-being and for small and large animal veterinary fields.

The second generation Nitroimidazole derivative, such as secnidazole, might have efficacy even in a single dose, and has proven to be efficacous and inexpensive (Rossignol, 2010). The latter drugs present an activity against anaerobic micro-organisms and are effective for the treatment of giardiasis. (Gillis and Wiseman, 1996). Secnidazole is rapidly and completely absorbed after oral administration and has a longer terminal elimination half-life (approximately 17 to 29 hours) than commonly used drugs in this class (Gillis and Wiseman, 1996; Videau et al., 1978). It is commercialized for the treatment of giardiasis in humans. It has the advantage to be administered in a single dose with promising curative effects. Anti-Giardial therapy within secnidazole has been the subject of various research articles in humanbeing (Di Prisco et al., 2000; Escobedo et al., 2003; Almirall et al., 2011). Besides in line with the purpose of this study, the efficacy of secnidazol against Giardiasis in lambs (Ural et al., 2014) and Balantidiasis in cattle (Tarrar et al., 2008; Bilal et al., 2009) have been reported elsewhere. Both of the studies comprised livestock animals tretaed with secnidazole, with advantages of convenience and ease of administration associated with single-dose therapy, combined with a good tolerability profile, making it an appropriate option. Given above mentioned 3 different studies, and besides the present study there is strong evidence that secnidazol might be safely used in livestock, without side effects.

The objectives of this study were: (i) the investigation of the efficacy of secnidazol against Giardiasis and (ii) the evaluation of the benefits of the secnidazol treatment on the milk yield of ewes.

MATERIALS and METHODS

Animals, Housing and Husbandry Conditions

A sample size of a total of 30 Sakiz ewes at the age of 2 to 4 years of age belonging to 3 different commercial flock, where they were housed in Soke province, Aydin, Turkey. During the allocation period (10 days) all ewes were screened twice with a 10 days interval to confirm the presence/absence of *G. duodenalis* cysts and *Cryptosporidium parvum* oocysts in the faeces. All animals were previously treated with toltrazuril (Cevazuril® Ceva-Vet, 20

mg/kg bodyweight) to prevent possible coccidiosis. Disinfection by use of a product containing quaternary ammonium was performed prior to trial, for elimination of probable existing environmental contamination.

In the present study, 30 dairy ewes, 65–80 days into their first or second lactation and naturally infected with Giardiasis (fecal flotation and microscopic examination of fecal samples, as detailed below), were allocated into three equal groups, GI, GII or GIII (n= 10, for each). The animals in groups GI and GII were treated with secnidazole (Flagentyl® 500 mg tblt., Eczacıbasi) at a single dose rate of 10 mg/kg or 30 mg/kg perorally, GIII ewes were controls and were adminestered oral water at the same dose rate of secnidazol. Fig. 1 showed one of the ewes involved in the present study.

Fig. 1. Group I ewe with Giardiasis with an individual milk yield of 243,61 ml.
Şekil 1. Grup I'de yer alan ve bireysel süt verimi 243,61 ml olan Giardiasis'li bir koyun.

Fecal Flotation and Microscopic Examination of Fecal Samples

Prior to applications day 0 (D0) was designated as as the initial treatment of the trial. Sample collections from each ewes were obtained on 2 occasions, furthermore were designated etiher D0 or day 10 (D10) (following treatment by all researchers). Ten gr. fecal samples were withdrawn from the rectum of all animals manually which were then submitted immediately to Department of Parasitology for fecal flotation. Obtained material was thorughly mixed with 15 ml of 33% (w/v) zinc sulphate solution, then were strained onto centrifuge tubes, followed by spunning in centrifuge at 880 x g for 5 minutes, similar to what have been reported previously (Wilson and Hankenson, 2009; Ural et al., 2014). After centrifugation, a relatively few sample of the fecal mixture solution was collected, then were treated on a microscope slide including Lugol iodine, which was covered by a slip. The slide was microscpically examined under 40x power for possible viewing of Giardia cysts. The latter application was repeated for 2 times from different samples for each sample collected on day 0. The major criteria for enrollment in the present study was that mono infection with *G. doudenalis* proven only by microscopic examination, similar to what have been described elsewhere by Escobedo et al (Escobedo et al., 2003)

Milk Yield Measurements

Milk yield measurements were carried out by one of the authors on the evening of each test day, as described by Fthenakis and Jones (1990). Ewes were hand-milked out. The final yields of both mammary glands of each ewe were added.

Treatment Efficacy

Secnidazol treatment efficacy in the present study was assessed by microscopic examination of fecal samples collected on D0 and D10 (after treatment completion), in order to avoid the bias that would be introduced by reinfection, and

measured based on the reduction in cyst excretion for treatment group compared to those of control group. The reduction in cyst excretion was calculated using the Henderson–Tilton formula (Henderson and Tilton, 1955), involving mean cyst counts similar to what have beedn described previously (Geurden et al. 2011):

$$100 * [1 - \frac{Ta * Cb}{Tb * Ca}]$$

Ta and Tb; showed the geometric mean cyst count in 2 different secnidazole treatment groups before and after treatment, respectively; where as Ca and Cb; the geometric mean cyst count in the control animals before and after treatment (Presidente, 1985).

The Henderson–Tilton formula (Henderson and Tilton, 1955), is considered as the most appropriate method as described and used previously (Geurden et al., 2011).

Statistical Analyses

Statistical analyses were performed by use of SPSS 18 package program (SPSS, 2009) by one of the authors (DAU). Results for cyst counts involving faecal samples for both eprinomectin groups (GI and GII) and control group, were tested for normality via the Kolmogorov–Smirnov test. Even if the faecal cyst count was not normally distributed; the data regarding faecal cyst count were log-transformed to achieve near-normality. For comparing fecal cyst counts on D0 and D10 among the groups, an independent-samples t test was conducted. For group comparisons within the baseline cyst values, were made by use of t test for dependent measures. Probability ($P<0.05$) was suggested to indicate a statistically significant difference. Summarized data were shown as least square means and standard error.

RESULTS

Animal Management and Treatment Applications

Secnidazol treatment at 2 different dosage regime applied in the present study did not result in any observable and significant adverse reactions. All ewes in groups had clinical signs compatible with naturally occuring Giardiosis, involving mild diarrhea. No coccidosis nor *Cryptosporidium* infection were found. Thirty Sakiz ewes at the age of 2-4 years, that tested microscopically positive for *G. duodenalis*, were allocated into 3 groups. Ten of the animals were randomly assigned to the positive control group (Group III), with written owners consent and regarding the ethical guidelines. The remaining ewes were enrolled in secnidazol groups, as aforementioned above, receiving the latter drug (Flagentyl® Eczacıbaşı, 500 mg tablets) at a single dosage of 10 mg/kg or 30 mg/kg perorally. Due to ethical concerns and commercial value of the lambs, only a limited number of animals served as controls. Albeit at the end of the study all positive control animals were also treated with secnidazol at the same dosage to the previosuly treated animals.

Cyst Excretion

The results regarding the cyst counts were presented in Table 1. Throughout the study period ewes in control group III remained positive for Giardiasis, besides 3 out of 10 ewes presented an increase in cyst counts on day 10 (ranged 1300-241650) compared to the initial values (ranged between 5600-274600), albeit there was no statistical significance. The least square means and standard error of means of cyst excretion on D0 and D10 revealed that there was a significant reduction ($P<0.001$) in cyst reduction in GI and GII animals treated with different dose secnidazol. For both groups mean for cyst excretion was significantly decreased ($P<0.001$) after treatment.

Table 1. The *Giardia duodenalis* cyst excretion in the control (Group III) and in the secnidazole treated (Group I and II) groups at each sampling day (before treatment [day 0] and after treatment [day 10]). The least square means and standard errors of cyst excretion (D0 and D10).
Tablo 1. Her bir örnekleme gününde (sağaltım öncesi [0. gün] ve sağaltım sonrası [10. gün] kontrol grubu (Grup III) ve seknidazol sağaltım gruplarında (Grup I ve II) *Giardia duodenalis* kist ekskresyonu. 0. ve 10. günlerde kist saçılımına ait en küçük kareler ortalamaları ve standart hataları.

		D0			D10		
Groups	N	$\overline{X} \pm S_{\overline{X}}$	Min	Max	$\overline{X} \pm S_{\overline{X}}$	Min	Max
Group I	10	$55615.0 \pm 30702.23^{Aa}$	2000	320000	177.0 ± 169.36^{Bb}	0	177
Group II	10	$55959.0 \pm 27343.72^{Aa}$	8500	298000	175.0 ± 161.07^{Bb}	0	1620
Group III	10	$55575.0 \pm 24877.51^{Aa}$	5600	274600	$46395.0 \pm 22605.72^{Aa}$	1300	241650

A, B (Capital letter): Different among means of groups at the same columns indicated statistically significant difference (P<0.001); a,b (Small letter): Different among means (D0 and D10) at the same lines indicated statistically significant difference (P<0.001); D0: Before treatment; D10: After treatment; Group III: Control group

Milk Yield

The mean values of milk yield of G I ewes were 258.6 ± 14.99 ml, 354.1 ± 20.26 ml and 478.6 ± 32.37 ml for 0, 7 and 14 day, respectively. The mean values of milk yield of G II ewes were 380.6 ± 16.48 ml, 413.2 ± 34.89 and 582.2 ± 55.85 ml, respectively. The mean values of milk yield of G III ewes were 240.7 ± 20.95 ml, 213.8 ± 19.88 ml and 211.1 ± 19.62 ml, respectively. Both group GI and GII ewes produced significantly more milk than group GIII ones (P<0.001). The change of mean milk yield over time was statistically significantly (P<0.001) among group GI and GII ewes; besides a statistically significant (P<0.001) reduction in the mean milk yield of group Group III ewes was observed.

Table 2. The least square means and standard errors of of milk yield (ml) of ewes.
Tablo 2. Koyunlarda süt verimine ait en küçük kareler ortalamaları ve standart hataları.

	N	0. day $\overline{X} \pm S_{\overline{X}}$	7. day $\overline{X} \pm S_{\overline{X}}$	14. day $\overline{X} \pm S_{\overline{X}}$
Group I	10	258.6 ± 14.99^{Bc}	354.1 ± 20.26^{Ab}	478.6 ± 32.37^{Aa}
Group II	10	380.6 ± 16.48^{Ab}	413.2 ± 34.89^{Ab}	582.2 ± 55.85^{Aa}
Group III	10	240.7 ± 20.95^{Ba}	213.8 ± 19.88^{Ba}	211.1 ± 19.62^{Ba}

A, B (Capital letter): Different among means of groups at the same columns indicated statistically significant difference (P<0.001); a,b (Small letter): Different among means (D0 and D10) at the same lines indicated statistically significant difference (P<0.001); D0: Before treatment; D10: After treatment; Group III: Control group. The difference of mean values of groups were significant (P<0.001).

DISCUSSION and CONCLUSION

Giardia infestation may result in both clinical and subclinical forms of disease, inducing direct and indirect losses. The direct losses may be related to acute illness and death. Indirect losses accompany a decrease in the productivity potential of livestock including milk, meat and wool production (Imran et al., 2013).

In a prior study evaluating the effects of giardiasis on production and carcass quality, 6 week-old, specific-pathogen-free lambs were infected with Giardia trophozoites; followed by controlling clinical signs of infection, body weight, and feed intake (for 10 weeks) and carcass weight and quality were determined at slaughter weight of 45 kg. Giardia infection was related to a decreased weight gain and impairment in feed efficiency. Time for reaching slaughter weight was extended in infected lambs, and the carcass weight of Giardia-infected lambs was lower than that of control lambs. According to the

result of that study Giardiasis has a negative effect on domestic ruminant production (Olson et al., 1995).

Economic losses due to gastrointestinal helminthes and protozoan species may be controlled by use of regular devorming of the livestock. Fast and early detection of those aformentioned parasitic infections and therapy may have help in reducing losses in the terms of productivity (Imran et al., 2013).

Sheep are susceptible to the adverse effects of parasitism, similar to cattle (Radostits et al., 1994), furthermore this interference within the host animal's defense may prone clinical disease and/or productivity losses (Armour, 1989, Hawkins, 1993), as was also noticed above. The impact of parasitism and antiparasitic treatment on milk yield in cattle has been reported in detail (Little et al., 2000; Sithole et al., 2005; Mason et al., 2012).

Several researches have evidenced that appropriate anthelmintic treatment may result in a positive milk yield response, suggested as 0.35kg-0.63kg/cow per day for lactation period (Gross et al., 1999; Sanchez et al., 2004). Relevant studies have determined that eprinomectin therapy increase milk yield by more than 2 Litres per cow per day (Reist et al., 2002). As shown above eprinomectin treatment resulted in increased milk yield in cattle, where as no similar study has been performed in sheep with giardiasis, according to the authors knowledge.

The efficacy of secnidazol administered at a single dose of 10 mg/kg, orally, in 12 weeks of age lambs naturally infected with Giardiasis were discussed elsewhere. There was a high (99.98%) and significant reduction ($P<0.001$) in cyst excretion in the secnidazol treatment group in contrast to the positive control group on day 10, after therapy, suggesting the latter drug a highly effective treatment option (Ural et al., 2014).

In the present study secnidazol at both 10 mg/kg and 30 mg/kg dosages were found effective for the treatment of Giardiasis. On day 10, after secnidazol treatment, the reduction in mean cyst excretion was very high. For groups I and II, geometric mean for cyst excretion was significantly decreased ($P<0.001$) after treatment.

Treated ewes in GI and GII yielded significantly more milk than untreated control animals. The change of mean milk yield over time was statistically significantly ($P<0.001$) among group GI and GII ewes; besides a statistically significant ($P<0.001$) reduction in the mean milk yield of group Group III ewes was observed. Obtained findings indicate that Giardiasis adversely affected the milk production of the infected ewes Adverse effects caused by Giardiasis in sheep have been noticed, as aforementioned above, however the present researcher group was unaware of finding documented reports regarding milk yield and its relation with Giardiasis.

Altough the exact reasons for the detrimental effects are unclear, it may be suggested that the pathological alterations related to Giardiasis may be briefly involved. Given the infection resulted from G. duodenalis may cause epithelial barrier function loss, villus atrophy and crypt hyperplasia in the small intestine (Ruest et al., 1997; Geurden et al., 2011), resulting in intermittent and mucous diarrhea (Ruest et al., 1997), intestinal malabsorption and hypersecretion (Buret, 2008). Therefore alterations in association with the gastro-intestinal system finally accompany decreased weight gain and to an altered feed efficiency (Olson et al., 1995; Sweeny et al., 2010), resulting with decrased milk yield. It is therefore noteworthy that these findings further enhance the need for efficacous treatment of the disease, such as secnidazol used in this study.

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