



RESEARCH ARTICLE

Re-evaluation of the efficacy of Oxfendazole+Oxyclozanide combination against gastrointestinal nematodes in sheep, a long time after the introduction into the Turkish antiparasitic drug market

Onur Ceylan¹, Uğur Uslu², Ceylan Ceylan¹, Abdullah Küçükyağlıoğlu³, Bayram Şenlik⁴

¹Selcuk University, Veterinary Faculty, Department of Parasitology, Konya, Turkey

²Selcuk University, Medicine Faculty, Department of Medical Microbiology, Konya, Turkey

³Selcuklu Municipality, Department of Agriculture and Animal Services, Konya, Turkey

⁴Uludag University, Veterinary Faculty, Department of Parasitology, Bursa, Turkey

Received:05.03.2020, Accepted: 03.11.2020

*onurceylan@selcuk.edu.tr

Türkiye antiparaziter ilaç pazarına girdikten uzun bir süre sonra koyunlarda Oxfendazole+Oxyclozanide kombinasyonunun gastrointestinal nematodlara karşı etkinliğinin yeniden değerlendirilmesi

Eurasian J Vet Sci, 2020, 36, 4, 255-260

DOI: 10.15312/EurasianJVetSci.2020.306

Öz

Amaç: Bu çalışma, oksfendazol+oksiklozanid kombinasyonunun Türkiye antiparaziter ilaç pazarına girişinden otuz yıl sonra etkinliğini yeniden değerlendirmek için yapılmıştır.

Gereç ve Yöntem: Çalışmada, gastrointestinal nematodlarla enfekte otuz iki koyun kullanılmıştır. Koyunlar, dışkıda gram başına yumurta sayısına göre seçilmiş ve her biri 16 hayvandan oluşan iki gruba ayrılmıştır. Grup I'deki hayvanlar 0. günde, sırasıyla 7.5 mg/kg ve 15 mg/kg vücut ağırlığı dozunda oksfendazol+oksiklozanid kombinasyonu ile oral olarak tedavi edilmiştir. II. gruptaki hayvanlara tedavi yapılmayıp, kontrol grubu olarak ayrılmıştır. Her hayvandan dışkı örnekleri 0. gün (tedavi günü); tedavi sonrası 7., 14. ve 21. günlerde toplanmıştır. Antelmintik tedavinin etkinliğini belirlemek için McMaster metodu ile gram dışkıdaki yumurta sayımı ve dışkı yumurta sayımı azaltma testi uygulanmıştır.

Bulgular: Dışkı örneklerindeki yumurta sayılarında tedavi ve kontrol gruplarında 0. gün istatistiksel olarak fark tespit edilmemiştir. Tedavi sonrası 7. günde, sadece bir koyunun dışkı örneğinde çok az yumurta bulunmuş ve tedavi grubu için ortalama yumurta sayısı 66.7 ± 66.7 olarak belirlenmiştir. Tedaviden sonraki 14. ve 21. günlerde dışkıdaki yumurta sayıları, tedavi grubunda sıfıra kadar düştü ancak tedavi edilmeyen kontrol grubunda önemli bir değişiklik görülmemiştir. Tedavi grubunda, oksfendazol+oksiklozanid kombinasyonunun etkinliği tedaviden sonraki 14. ve 21. günlerde oldukça yüksek (% 100) bulunmuştur.

Öneri: Bu çalışma ile oksfendazol+oksiklozanid kombinasyonunun, çalışma alanındaki koyunların gastrointestinal nematodlarına karşı oldukça etkili olduğu ortaya konulmuştur.

Anahtar kelimeler: Nematod, oksfendazol, oksiklozanid, koyun, Trichostrongyloidea

Abstract

Aim: This study was performed to re-evaluate the efficacy of the combination oxfendazole+oxyclozanide, after thirty years of its introduction into the Turkish antiparasitic drug market.

Materials and Methods: In this study, 32 sheep infected with gastrointestinal nematodes were used. Sheep were selected on the basis of the number of eggs in one gram of their fecal sample. Sheep selected were divided into two groups: group I, sheep were treated orally at day zero, with oxfendazole+oxyclozanide combination at a dose rate of 7.5 mg/kg and 15 mg/kg of body weight, respectively; group II, sheep were used as untreated controls. Fecal samples were collected from each sheep on day 1 of their treatment, and on days 7, 14, and 21 of post-treatment. To determine the anthelmintic drug treatment's efficacy, individual sheep's fecal egg count per gram of feces was determined by performing McMaster method and fecal egg count reduction test.

Results: Fecal egg counts of these two groups were statistically not different on the day of treatment. At day 7 of post-treatment, few eggs were detected only in one sheep, and the mean of fecal egg counts of the treatment group was 66.7 ± 66.7 . On days 14 and 21 of post-treatment, fecal egg counts decreased to zero in the treatment group but significantly did not change in the control group. The efficacy of the combination was found to be 100% at day 14 and 21 of post-treatment.

Conclusion: This study revealed that the combination oxfendazole+oxyclozanide was highly effective against the gastrointestinal nematodes in sheep.

Keywords: Nematode, oxfendazole, oxyclozanide, sheep, Trichostrongyloidea





Introduction

Sheep are superior to other ruminants for having high adaptability to the environment, more prolificity, and more selectivity in grazing (Hastuti and Samsi 2010, Koseman and Seker 2015, Senlik 2017). Sheep also have significant importance for farmers having economic problems. Such farmers prefer to rear sheep as animals for cash income, milk, meat, fertilizer and employment of family members (Saddiqi et al 2006). Therefore, sheep are one of the ruminants that farmers like to rear; thus, sheep rearing has been of great significance in the rural economy throughout the world (Hastuti and Samsi 2010). Several factors affect the productivity of these animals. One such factor is a health problem caused by different types of parasitic infections and is one of the major constraints to increased ovine productivity in sheep (Senlik 2015, Senlik 2017). Nematodes are the major group of helminths affecting sheep in pasture-based production models. Gastrointestinal nematodes are encountered as the most common infectious agents and adversely affect animal production by causing significant economic losses worldwide (Gaba et al 2012, Seyoum et al 2017, Hamel et al 2018). The gastrointestinal nematodes cause infection ranging from sub-clinical to outbreak level, that can seriously affect the health and welfare of animals (Senlik 2015, Hamel et al 2018). Economic and production losses occurring during gastrointestinal nematode infection in sheep are caused due to reduced intake of amino acids and vitamins, retarded growth, and increased treatment expenses (Senlik 2013, Senlik 2015). This infection can lead to numerous problems, including loss of body weight, reduction in skeletal growth, low milk and wool yield, low birth weights, and difficulty in lambing, which can impose a significant economic burden on sheep production. Besides these losses, the sheep also become susceptible to other infectious diseases and metabolic disorders (Sahin et al 2009, Kumsa et al 2010, Mehmood et al 2013, Senlik 2015). Therefore, efficient control of ovine gastrointestinal nematodes is the main purpose of an economically sustainable sheep breeding (Hamel et al 2015, 2018). Till now, preventive measures against ovine nematodes, have frequently relied on the routine application of various anthelmintic drugs. Therefore, anthelmintic treatment has played an important role in the control of ovine internal parasitic infection throughout the world, and Turkey is no exception to it (Little et al 2011, Senlik 2015).

In Turkey, due to the lack of highly efficient helminth control programs for small ruminants, anthelmintics are intensively used to eliminate the adverse effects of nematode infection (Senlik 2013, Senlik 2015). Oxfendazole (OXF) and oxyclozanide (OCZ) were introduced over 30 years ago, and their combination OXF+OCZ is an anthelmintic drug formulation which is commonly used in Turkey, for the control and treatment of ovine nematode infection. The development of anthelmintic drug resistance is inevitable because of its ex-

tensive usage; hence, regular monitoring of the effectiveness of these drugs is necessary. Despite the extensive usage of OXF+OCZ combination produced by national and multinational companies with different trade names, only limited studies have been presented on the efficacy of these products (Tinar et al 1997, Yildirim et al 2008). Therefore, the aim of the current study was to re-evaluate the efficacy of the most commonly used anthelmintic combination (OXF+OCZ) against gastrointestinal nematodes in naturally infected sheep at field scale in Konya province, Turkey.

Material and Methods

Study area

The present study was conducted from June to July, 2018, in the province Konya, Turkey. Konya is the principal city of Turkey and has a great number of small ruminants (22,252,461 according to TÜİK). The study area is geographically situated between 36°41' and 39°16' N and 31°14' and 34°26' E.

Determination of infected flock

To determine sheep infected with gastrointestinal nematodes, a total of 500 fecal samples were collected and examined at different locations of Konya. Thereafter, a farm located in Kadinhanı, which had 180 sheep and willingness to participate, was selected. No anthelmintic was applied to the farm sheep, in the previous three months.

Group allotment

Fecal samples of total 100 sheep in the herd, were collected and analyzed for the number of eggs per gram (EPG) of feces. Thereafter, 32 Akkaraman sheep of different ages (1–3 years) and with the highest number of EPG were identified and divided into two groups (group I & group II) with 16 sheep in each. Anthelmintic was applied to the sheep in group I; however, group II was left untreated as it served as the control group.

Fecal samples

Fecal sample of each sheep was picked up directly from the rectum at day 0, 7, 14 and 21 of post-treatment. Collected fecal samples were put into individual plastic bags, labeled with the sheep's ear tag number, and carried in refrigerated vials to the parasitology laboratory (Veterinary Faculty, Selçuk University) for further analyses. All samples were stored at 4 °C until further parasitological examinations.

Laboratory procedures

To determine the parasitic infection, fecal samples collected from each sheep were screened for the presence of gastro-



intestinal nematode eggs, by using a saturated salt solution flotation technique. Next, the McMaster egg counting technique was performed to determine the EPG number for each positive sample (Taylor et al 2007). For McMaster technique, 3 g of the fecal sample was mixed in 42 mL of saturated NaCl solution and filled in McMaster slide with a sensitivity of 50 EPG. The number of eggs in the two chambers of the slide was counted, and the total egg number obtained was multiplied by 50 to obtain the number of EPG. The process is performed for each of the fecal samples.

Body weight determination and drug administration

Weighing balance was used to determine and record the bodyweight of each sheep. The anthelmintic combination (OXF+OCZ) used in this study was supplied by the retail markets of Konya. Sheep in the treatment group (group I) were treated with the OXF+OCZ combination, having 375 mg of oxfendazole and 370 mg of oxyclozanide, and sheep in the control group (group II) were left untreated. According to the manufacturer's recommendation, the combination (7.5 mg/kg OXF + 15 mg/kg OXC) was administered orally to sheep, considering their body weight.

Fecal egg count reduction (FECR) test

The efficacy of this anthelmintic combination was evaluated by the percentage reduction in the mean egg excretion number on days 7, 14 and 21 of post-treatment. The FECR test was performed and the reduction estimates were calculated using a method described by World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al 1992, Wood et al 1995). To calculate the efficacy of this anthelmintic drug combination, the formula:

$$\text{Efficacy\%} = 100 (a-b/a),$$

where a and b are arithmetic mean of fecal egg count (FEC) of the control and treatment groups, respectively, was used.

Data analysis

The data were analyzed using Minitab statistical software (Minitab Inc 2007). The mean FEC numbers of the treated and the control groups were significantly not different before treatment at day zero.

The homogeneity of egg counts in both groups was compared by the Mann-Whitney U test before treatment at day zero, and no significant difference was found between the treated and the control group.

Results

The mean of pre- and post-treatment egg counts for the treatment and control groups are illustrated in Table 1. Fecal samples taken from naturally infected sheep before treatment revealed that all were infected with a moderate number of trichostrongylid nematodes.

At day zero of the treatment, the average number of trichostrongyle EPG in sheep fecal samples of the treatment and control groups were 372 ± 207 and 221.9 ± 59.7 , respectively. The mean values of the FEC numbers for trichostrongylid in the sheep of the treatment and control groups were 66.7 ± 66.7 (only one sheep) and 408 ± 127 , at day 7 of post-treatment. While the mean of EPG numbers of the control group was 176.9 ± 32.8 , no egg was detected in the treatment group at day 14 of post-treatment. Similarly, on day 21 of post-treatment, the mean EPG number increased to 203.8 ± 32.3 for the control group, but no trichostrongyle egg was found in sheep of the treatment group.

The FECR test verified the results obtained using FECs, by exhibiting 100% efficacy at day 14 and 21 of post-treatment. Comparison of drug efficacy on different days revealed the consistent activity with a marked reduction in EPG of feces from days 7 to 21 of post-treatment.

Table 1. Efficacy of oxfendazole+oxyclozanide combination on the sheep naturally infected with the gastrointestinal nematodes

Groups	Sampling day	FEC (Egg per gram fecal sample)			Efficacy (%)
		Minimum	Maximum	Mean $\pm$ SE Mean	
CONTROL (n:16)	0	100	1000	221.9 ± 59.7	-
	7	100	1600	408 ± 127	-
	14	50	400	176.9 ± 32.8	-
	21	50	500	203.8 ± 32.3	-
TREATMENT (n:16)	0	100	3450	372 ± 207	-
	7	0	800	66.7 ± 66.7	83.65
	14	0	0	0 ± 0	100
	21	0	0	0 ± 0	100





Discussion

In Turkey, many kinds of anthelmintic drugs are commercially available, and well reputed national or multinational brand preparations are preferred by farmers, depending mostly on their purchasing power. Till present, one of the most frequently used drugs in Turkey, against nematode infection is OXF+OXC combination. However, there are limited studies on the assessment of the efficacy of this anthelmintic drug combination in sheep infected with trichostrongyle nematodes (Tinar et al 1997, Yildirim et al 2008). Therefore, the essential aim of the present study was to re-evaluate the efficacy of OXF+OXC combination at the dose rate of 7.5 mg/kg and 15 mg/kg body weight, respectively, against gastrointestinal nematode infection in sheep, after 30 years of introducing it into the veterinary antiparasitic drug market. In the current study, it is revealed that the efficacy of OXF+OXC combination is 100% based on the FEC numbers on both days 14 and 21 of post-treatment.

The efficacy of OXF and OXC has been evaluated as a separate or combined form, in some studies of Turkey and many countries (Himonas and Theodorides 1986, Tinar et al 1997, Saddiqi et al 2006, Yildirim et al 2008, Kakar et al 2015). Himonas and Theodorides (1986) determined that the efficacy of OXF was 100% against different genera of gastrointestinal nematodes, such as *Haemonchus*, *Ostertagia*, and *Trichostrongylus*. Kakar et al (2015) reported that FECR was 95–97% in four different breeds of sheep treated with OXF. In contrast to these authors, Saddiqi et al (2006) used three different preparations of OXF and found the efficacy to be between 56% and 83%. The effectiveness of OXF+OXC combination against gastrointestinal nematodes was recorded 100% in this current study, which is not different from those of Himonas and Theodorides (1986) but higher than that of Saddiqi et al (2006).

The combination of drugs that have different actions may develop a synergistic effect and slows down the development of resistance in the host. Therefore, to treat mixed helminth infections, combinations of anthelmintics are commonly used to control the parasitic diseases of sheep. In Turkey's anthelmintic drug market, there are nearly forty different trademarks of OXF+OXC combination. Hence, we decided to re-evaluate this combination, which has been used for many years in Turkey.

Previous field studies in Turkey, on the determination of the efficacy of OXF+OXC anthelmintic drug combination, showed variable results. The results obtained in this study are consistent with the results of Tinar et al (1997), who reported that the FECR was 100%. However, Yildirim et al (2008) in their study reported the efficacy of 94.2% and 96.2% on days 14 and 21, respectively, for the combination OXF+OXC. Besides, Gunes et al (2008) reported the tablet and paste formu-

lations were effective at rates 94.2% and 97.5% on day 14 post-treatment. The small difference between the results is attributed to the dose and formulation of the drugs used in the studies.

For the interpretation of the FECR test results, the international guidelines classify anthelmintics on the basis of their efficacy percentage: highly effective (> 98%), effective (90–98%), moderately effective (80–89%), and ineffective (< 80%) (Coles et al 1992, Wood et al 1995). According to these criteria, the present study has revealed the consistently high level of efficacy (100%) of OXF+OXC combination against ovine gastrointestinal nematodes in sheep, from Turkey.

Oxfendazole is a benzimidazole anthelmintic, whereas, OXC is a salicylanilide compound routinely used in ruminants. In the case of an overdose, some unfavorable side effects on intestinal functions and the central nervous system can be observed in sheep (Riviere and Papich 2009). In the present study, OXF+OXC combination orally administered to sheep was safe without causing any adverse reactions or health complications in treated sheep.

Moreover, the spectrum of nematodes determined in this study covers all economically important nematode species present in grazing sheep, including *Ostertagia*, which are known to be highly prevalent in Konya province (Guclu et al 1996).

Considering the data obtained in this study, it is concluded that the combination (OXF+OXC) therapy, introduced over 30 years ago into the Turkish antiparasitic drug market, is highly effective against gastrointestinal nematodes in naturally infected sheep. This combination is inexpensive and effective in a single dose, and can be available everywhere in Turkey. Hence in light of this overall discussion, oral administration of a combination of OXF and OXC is recommended to control and treat gastrointestinal nematode infection in sheep.

Conclusion

This study reveals that the combination OXF+OXC is still highly effective against gastrointestinal nematodes, with no adverse reactions in sheep of the research area during the study.

Acknowledgement

This research study is original and is not submitted to anywhere as full text or abstract.

Conflict of Interest

The authors did not report any conflict of interest or finan-





cial support.

Funding

This study was supported by the Scientific Research Projects Coordination Unit, Selcuk University (Project Number: 17401184).

References

- Coles G, Bauer C, Borgsteede F, Geerts S, et al., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol*, 44, 35–44.
- Gaba S, Cabaret J, Chylinski C, Sauv   C, et al., 2012. Can efficient management of sheep gastro-intestinal nematodes be based on random treatment? *Vet Parasitol*, 190, 178–184.
- Guclu F, Dik B, Kamburgil K, Sevinc F, 1996. Prevalence of gastro-intestinal nematodes in sheep around Konya and their seasonal fluctuation. *Veterinarium*, 7, 50–55.
- Gunes V, Inci A, Uyanik F, Yildirim A, et al., 2008. The effect of Oxfendazole+Oxyclozanide paste and tablet formulations on parasite burden and metabolic status of sheep. *J Anim Vet Adv*, 7 (5), 589–594.
- Hamel D, Bosco A, Rinaldi L, Cringoli G, et al., 2018. Eprinomectin pour-on (EPRINEX® Pour-on, Merial): efficacy against gastrointestinal and pulmonary nematodes and pharmacokinetics in sheep. *BMC Vet Res*, 13 (1), 148.
- Hamel D, Visser M, Mayr S, Rauh R, et al., 2015. Eprinomectin pour-on: prevention of gastrointestinal and pulmonary nematode infections in sheep. *Vet Parasitol*, 15 (264), 42–46.
- Hastuti S, Samsi M, 2010. Performance of Oxfendazole in Nematode-Infected Post-Weaning Lamb on Pasture. *Anim Prod*, 12 (1), 39–43.
- Himonas CA, Theodorides J, 1986. The comparative efficacy of oxfendazole administered as bolus and suspension to naturally infected sheep in Greece. *J Helminthol*, 60, 245–249.
- Kakar H, Lateef M, Maqbool A, Jabbar M, et al., 2015. Evaluation of commonly used anthelmintics resistance against nematodes infection in different breeds of sheep in Balochistan. *Pak J Zool*, 47 (4), 1077–1082.
- Koseman A, Seker I, 2015. Current status of cattle, sheep and goat breeding in Turkey. *Van Vet J*, 26, 111–117.
- Kumsa B, Tolera A, Nurfeta A, 2010. Comparative efficacy of seven brands of albendazole against naturally acquired gastrointestinal nematodes in sheep in Hawassa, southern Ethiopia. *Turk J Vet Anim Sci*, 34 (5), 417–425.
- Little PR, Hodge A, Maeder SJ, Wirthlerle NC, et al., 2011. Efficacy of a combined oral formulation of derquantel–abamectin against the adult and larval stages of nematodes in sheep, including anthelmintic-resistant strains. *Vet Parasitol*, 181, 180–193.
- Mehmood K, Ijaz M, Zameer Durrani A, et al., 2013. Infection rate and therapeutic trials on various gastrointestinal parasites in sheep and goats in and around Lahore, Pakistan. *Pak J Zool*, 45 (2), 489–494.
- Minitab Inc, 2007. Statistical Software. Minitab 15, State College, PA USA.
- Riviere JE, Papich MG, 2009. Veterinary Pharmacology & Therapeutics, Ninth edition. Wiley-Blackwell Ames (Iowa US), 1524.
- Saddiqi HA, Jabbar A, Iqbal Z, Babar W, et al., 2006. Comparative efficacy of five anthelmintics against trichostrongylid nematodes in sheep. *Can J Anim Sci*, 86, 471–477.
- Sahin A, Akkan HA, Gul A, Karaca M, et al., 2009. Trichostrongylidae spp. ile dođal enfekte koyunlarda moxidectin ve doramectinin etkinliđi. *YYU Vet Fak Derg*, 20 (1), 57–59.
- Senlik B, 2013. Koyun ve ke i s r lerinde helmint enfeksiyonlarında korunma ve kontrol. *Koyun ve Ke i Sađlıđı Sempozyumu*, Maritim Pine Beach Hotel, Belek-Antalya, 40–51.
- Senlik B, 2015. Koyun ve ke ilerde endoparazit m cadelesi. 2. Koyun ve Ke i Sađlıđı ve Y netimi Sempozyumu, Grand Yazıcı Turban Hotel, Marmaris, 114–131.
- Senlik B, 2017. Important parasitic disease in terms of flock health in sheep. *T rkiye Klinikleri J Vet Sci Intern Med-Special Topics*, 3 (1), 89–100.
- Seyoum Z, Demessie Y, Bogale B, Melaku A, 2017. Field evaluation of the efficacy of common anthelmintics used in the control of gastrointestinal nematodes of sheep in Dabat district, Northwest Ethiopia. *Irish Vet J*, 70, 18.
- Taylor MA, Coop RL, Wall R, 2007. Veterinary Parasitology. 3rd ed. Oxford: Blackwell Sciences Limited, 874.
- Tinar R, Akyol CV, Senlik B, 1997. Oxyclozanide+oxfendazole kombinasyonunun farklı dozlarda koyunların mide-barsak nematodlarına etkisi. *T rkiye Parazitolo Derg*, 21 (4), 423–425.
- Wood I, Amaral N, Bairden K, Duncan J, et al., 1995. World Association for the advancement of Veterinary Parasitology (WAAVP) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet Parasitol*, 58, 181–213.
- Yildirim A, Gunes V, Ica A, Sariozkan S, et al., 2008. Koyunlarda mide-bađırsak nematodlarına kar ı oxfendazole + oxyclozanide’nin tablet ve pasta form lasyonlarının kısa s reli etkinliđinin ara tırılması. *T rkiye Parazitolo Derg*, 32 (2), 134–138.

Author Contributions

Motivation / Concept: Onur Ceylan, Uđur Uslu

Design: Onur Ceylan

Control/Supervision: Onur Ceylan, Uđur Uslu, Bayram  enlik

Data Collection and / or Processing: Onur Ceylan, Ceylan

Ceylan, Abdullah K   kyađlıođlu

Analysis and / or Interpretation: Onur Ceylan, Uđur Uslu,

Bayram  enlik

Literature Review: Onur Ceylan, Ceylan Ceylan, Abdullah K   kyađlıođlu,

Bayram  enlik

Writing the Article : Onur Ceylan, Bayram  enlik





Critical Review: Onur Ceylan, Uğur Uslu, Bayram Şenlik

Ethical Approval

This study was carried out with the permission of the Selcuk University, Faculty of Veterinary Medicine, Experimental Animals Production and Research Center Ethics Committee (Decision Number: SUVDAMEK /2017/39) report.

CITE THIS ARTICLE: Ceylan O, Uslu U, Ceylan C, Küçükyavaşlıoğlu A, et al., 2020. Re-evaluation of the efficacy of Oxfendazole+Oxyclozanide combination against gastrointestinal nematodes in sheep, a long time after the introduction into the Turkish antiparasitic drug market. *Eurasian J Vet Sci*, 36, 4, 255-260.

