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# Anthelmintic resistance and multidrug resistance in sheep gastro-intestinal nematodes in France, Greece and Italy

Thomas Geurden[a,*], Herve Hoste[b,1], Philippe Jacquiet[b,1], Donato Traversa[c,1], Smaragda Sotiraki[d,1], Antonio Frangipane di Regalbono[e], Nikolaos Tzanidakis[d], Despoina Kostopoulou[d], Christie Gaillac[b], Simon Privat[b], Annunziata Giangaspero[f], Claudia Zanardello[g], Laura Noé[a], Bindu Vanimisetti[h], David Bartram[i]

[a] *Zoetis, Veterinary Medicine Research and Development, Mercuriusstraat 20, 1930 Zaventem, Belgium*
[b] *Institut National de la Recherche Agronomique/UMT Maîtrise de la Santé des Troupeaux de Petits Ruminants/Ecole Nationale Vétérinaire de Toulouse, Toulouse cedex, France*
[c] *University of Teramo, Faculty of Veterinary Medicine, Teramo, Italy*
[d] *Veterinary Research Institute – Hellenic Agricultural Organization-Demeter (former NAGREF), Thessaloniki, Greece*
[e] *University of Padua, Department of Animal Medicine, Production and Health, Legnaro, Italy*
[f] *University of Foggia, Department of Science of Agriculture, Food and Environment, Foggia, Italy*
[g] *Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy*
[h] *Zoetis, Veterinary Medicine Research and Development, Kalamazoo, MI, USA*
[i] *Zoetis, 23/25 avenue du Docteur Lannelongue, 75668 Paris Cedex 14, France*

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ABSTRACT

Anthelmintic resistance (AR) in ovine gastro-intestinal nematodes has been reported to affect the health and productivity of sheep globally. The objective of the present study was to evaluate the efficacy of commonly used oral drenches in sheep in France, Greece and Italy. In each country, 10 farms were selected. On each farm, 50 animals were blocked based on the pre-treatment faecal egg count (FEC). Within each block, animals were randomly allocated to one of 5 treatment groups. In addition to an untreated control group, there were 4 groups treated per oral route: moxidectin (MOX) and ivermectin (IVM), both at 0.2 mg/kg bodyweight, levamisole (LEV; at 7.5 mg/kg bodyweight) and a benzimidazole (BZ; at 3.75–5 mg/kg bodyweight). In France, animals were not treated with LEV, but with netobimin (NET; at 7.5 mg/kg bodyweight). The FEC was monitored using a modified McMaster technique. Two weeks after treatment, individual faecal samples were taken from all animals and efficacy was calculated as the difference between arithmetic mean FEC of the control group versus each respective treatment group. The results of the present study indicate the high efficacy of treatment with oral formulations of MOX (99–100%) and IVM (98–100%) on all farms, except on 1 farm in Greece. On this farm, multi drug resistance (MDR) was identified involving 4 anthelmintics (efficacy MOX: 91%; IVM: 0%; BZ: 58% and LEV: 87%). In Greece and Italy, AR against LEV and BZ was observed on some farms, with MDR involving both anthelmintics on 3 farms in Greece and on 2 farms in Italy. In France, AR against BZ and NET was observed on all 10 farms included. In all countries, *Teladorsagia* sp. was the most common nematode larva identified after treatment, followed by

* Corresponding author. Tel.: +32 2 746 80 93.
*E-mail addresses:* thomas.geurden@zoetis.com, thomasgeurden@yahoo.com (T. Geurden).
[1] These authors contributed equally to the study.

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*Haemonchus* sp. and *Trichostrongylus* sp., with differences among farms and treatments. The current study confirms the high efficacy of oral treatments with MOX and IVM, even on farms with worm populations resistant to BZ, LEV or NET. This study also reports MDR against 4 anthelmintics on one farm in Greece.



## 1. Introduction

Anthelmintic resistance (AR) in sheep gastro-intestinal nematodes (GIN) occurs globally. Since the 1990s, the increasing number of reports has elevated the issue of AR, including multidrug resistance (MDR), from only an academic interest to a major threat to sheep production (Kaplan, 2004). The situation is at such a level that some authors contend that AR in sheep is the new standard, and no longer the exception. It is clear that the increased mortality and morbidity, and the economic losses associated with the inability to control GIN are detrimental for many sheep farmers. Furthermore, the occurrence of MDR threatens the health, welfare and production of small ruminants, to the point that farm closures or culling of entire flocks are becoming a reality (Kaplan and Vidyashankar, 2012; Miller et al., 2012).

In all main sheep markets in Europe, AR to benzimidazoles (BZs) has been described. Especially in the UK, the level of AR to BZ compounds is high (Taylor et al., 2009), but also in Greece (Papadopoulos et al., 2012), Northern Ireland (McMahon et al., 2013), Norway (Domke et al., 2012), France (Chartier et al., 1998) and Spain (Martínez-Valladares et al., 2013) BZ resistance has been reported. Next to BZ resistance, AR to levamisole (LEV) and ivermectin (IVM) has been reported in the UK (Sargison et al., 2007; Taylor et al., 2009; Mitchell et al., 2010), as well as in other European countries (Chartier et al., 1998; Traversa et al., 2007; Domke et al., 2012; Good et al., 2012; Papadopoulos et al., 2012; Martínez-Valladares et al., 2013; McMahon et al., 2013). Furthermore, MDR to these three anthelmintics has been described (Bartley et al., 2004), as well as failure of moxidectin (MOX) to control these MDR strains (Sargison et al., 2005, 2010). The occurrence of MDR is of particular concern, as MDR limits to a large extent the options for treatment when AR occurs on a farm.

In the UK, the predominant nematode species involved in AR is *Teladorsagia circumcincta*, with strains found to be resistant to BZ, LEV and macrocyclic lactones (MLs). Additionally, *Trichostrongylus* sp. and *Nematodirus* sp. have been identified to be resistant (Bartley et al., 2004; Sargison et al., 2010; Richards, 2011). Reports from other European countries suggest that the most important genera of sheep nematodes resistant to drug treatment are *T. circumcinta*., *Haemonchus* sp. and *Trichostrongylus* sp., and to a lesser extent *Cooperia* sp. and *Nematodirus* sp. (Chartier et al., 1998; Papadopoulos et al., 2012).

Although several studies have recently reported MDR in the UK and other European sheep markets, there is still a lack of data to understand the occurrence of AR and MDR in some of the major key areas for sheep industry. The objective of the present study was to evaluate the efficacy of 4 commonly used oral drenches in sheep on farms with a potential for development of AR in the following EU markets: Italy, France and Greece.

## 2. Materials and methods

### 2.1. Study design

In each country, 10 farms were selected based on the potential of AR occurring on the farm, as indicated by farm history, knowledge of intensive treatment regimens on the farm and reduction in production parameters despite treatment. Only farms with a sufficient number of animals older than 3 months of age were considered, in order to be able to include in the study at least 50 healthy yet nematode positive animals. Both young and adult animals were included, if positive. In France, 5 farms were selected in the region of Aveyron (South France) and five farms in the region of Pyrénées Atlantiques (South-West France). All farms were dairy sheep farms for cheese production. In Greece, 3 farms were included on the island of Crete and 7 farms were in mainland Greece in the regions of Central Macedonia (specifically Chalkidiki, Imathia, Thessaloniki, Pieria) and Thrace (Rodopi) covering different climatic and management conditions. In Italy, a total of 10 farms were included selected in the municipalities of Cosenza, Catanzaro, Padova, Isernia and Foggia, covering the Northern, Central and Southern parts of the Italian peninsula.

The diagnostic technique used to monitor the faecal egg excretion was a modified McMaster technique (MAFF, 1986), with a sensitivity of 50 eggs per gram of faeces (epg) in Greece and Italy, and 15 epg in France. Larval identification was performed before and after treatment, on bulk faecal samples (MAFF, 1986).

Prior to the collection of individual faecal samples used for the allocation, bulk faecal samples were collected at fortnightly intervals and examined to estimate the infection status on each farm. Fresh faecal samples from at least three different animals were included in each bulk sample. When the Faecal Egg Count (FEC) in the bulk samples was higher than 200 epg, the flock was deemed suitable for the study. In order to have 50 strongyle positive animals for treatment, at each farm approximately 100 healthy animals older than 3 months and grazing the same pasture were selected, and an individual faecal sample from each animal was collected. If more than 50 animals with a positive strongyle FEC were available for inclusion, the 50 animals with the highest strongyle FECs were selected.

This study was a negatively controlled efficacy study with the assessment of efficacy based on individual FEC 14 days after treatment. The study used a randomised complete block design for each study site (=farm), with the individual animal as the experimental unit. Blocking was based on the pre-treatment individual FEC. Before