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Field evaluation of anticoccidial efficacy: A novel approach demonstrates reduced efficacy of toltrazuril against ovine *Eimeria* spp. in Norway

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ABSTRACT

Ovine *Eimeria* spp. infections cause reduced welfare, increased mortality, and substantial economic losses, and anticoccidials are crucial for their control. Recent reports of toltrazuril resistance in pigs, and anecdotal reports of reduced anticoccidial efficacy in lambs, necessitate evaluation of anticoccidial efficacy. Due to the substantial lifecycle differences between nematodes and coccidia, current WAAVP methods for assessing anthelmintic efficacy are not suitable for such evaluations. Faecal samples were collected from 8 pairs of twin lambs from 36 Norwegian sheep farms 6–8 days after turnout. One twin of each pair was then treated with 20 mg/kg toltrazuril and a second faecal sample from all lambs was collected 7–11 days later. Oocyst excretion rate in all samples was determined using McMasters. Suitability of treatment timing was investigated by evaluating the increase in mean log oocyst excretion in untreated lambs. Based on comparisons between groups, a threshold of ≥ 0.75 (13 farms) was used to identify farms where drug efficacy could be assessed with confidence, drug efficacy on farms with increases of ≥ 0.5 but < 0.75 (7 farms) were evaluated with caution, and drug efficacy on farms with increases of < 0.5 (16 farms) was not estimated. Reduction in oocyst excretion between samples from treated lambs compared with controls from the 20 farms with a threshold of ≥ 0.5 were then analysed using a generalised linear mixed model. The results were classified based on 95% CI obtained using parametric bootstrapping. Among these 20 farms, two exhibited reduced drug efficacy (upper 95% CI $<$ 95%), 13 had good efficacy (lower 95% CI $>$ 90%), and for 5 the results were inconclusive. This is the first evidence-based report of reduced anticoccidial efficacy in ovine *Eimeria* spp. Additionally, we highlight the problem of sub-optimal timing of treatment (16/36 farms), which could potentially result in incorrect conclusions being reached regarding lack of drug efficacy.

1. Introduction

Eimeria spp. are host-specific obligate intracellular protozoan parasites that infect fish, reptiles, birds and mammals (Walker et al., 2013). Of the 15 *Eimeria* spp. known to infect sheep, only two are regarded as major pathogens: *E. ovinoidalis* and *E. crandallis* (Catchpole et al., 1976; Catchpole and Gregory, 1985; Rommel, 2000; Joachim et al., 2018). *E. ahsata,* and occasionally *E. bakuensis,* are generally considered to be minor pathogens, which may cause clinical signs in heavily infected animals (Mahrt and Sherrick, 1965; Deplazes et al., 2016). In addition, infections with multiple species might also be important for the development of clinical signs, as described for calves (Enemark et al., 2013). Coccidiosis in lambs caused by pathogenic *Eimeria* spp. leads to reduced welfare, increased mortality and substantial economic losses in the sheep industry worldwide (Foreyt, 1990; Chartier and Paraud, 2012).

Pasture management and hygienic measures, e.g., cleaning water troughs and maintaining dry bedding, are considered important factors for reducing the infection pressure from *Eimeria* spp. (Taylor, 2000; Daugschies and Najdrowski, 2005). However, these measures are often

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labour intensive and can be difficult to implement, and chemoprophylaxis with anticoccidials is therefore frequently used, in addition to hygiene measures for control of clinical coccidiosis in sheep farms (Taylor and Kenny, 1988; Platzer et al., 2005; Saratsis et al., 2013; Odden et al., 2017). Metaphylactic administration of a single oral treatment with toltrazuril in the prepatent period has been shown to be effective at reducing clinical signs and maintaining adequate growth rates in different production systems (Gjerde and Helle, 1986, 1991; Taylor and Kenny, 1988; Le Sueur et al., 2009; Saratsis et al., 2013). In several European countries (e.g., Denmark, Sweden, and Norway) toltrazuril is the only anticoccidial available for use in sheep (Felleskatalogen, 2017; Läkemedelsverket, 2017; Veterinærmedicinsk Industriforening, 2017). In other countries, other treatments such as diclazuril and decoquinate are also available (Taylor, 2000; Diaferia et al., 2013). According to the Veterinary Medicines Directorate, 510,388 kg and 787,300 kg toltrazuril from products authorised for use in farm animals were sold in the UK in 2014 and 2015, respectively (Dr Gillian Diesel, Head of the Pharmacovigilance Team, personal communication). However, treatment of clinical coccidiosis is considered to be inefficient due to the extensive intestinal damage caused by the parasite (Mundt et al., 2003; Taylor et al., 2003).

Anticoccidial resistance (ACR) is a widely recognised problem in poultry production (Chapman, 1997; Stephan et al., 1997; Chapman and Jeffers, 2014; Lan et al., 2017), and has been reported for monensin, salinomycin, nicarbazin, halofuginone, robenidine, toltrazuril and diclazuril (McDougald, 1981; Chapman, 1997; Stephan et al., 1997). ACR in poultry production is generally considered to be the result of intensive use of anticoccidials, which has led to loss of sensitivity to these drugs (Peek and Landman, 2011). Testing for anticoccidial efficacy (ACE) in poultry production involves the use of histopathological observations and the combination of different indexes, such as oocyst index, body weight gain, relative weight gain, lesion scores, and anticoccidial index (Chapman, 1998). However, no such methods have been published for the evaluation of anticoccidial efficacy in other livestock, including sheep. One obvious practical requirement for a method to be useful in field situations is that it should not include euthanasia of large numbers of animals.

The controlled efficacy test (CET) is the gold standard method for the evaluation of anthelmintic efficacy (Wood et al., 1995; Coles et al., 2006). The CET is performed by infecting animals with a suspected resistant isolate, treating the animals with the drug under evaluation, and then euthanizing the animals before quantifying the parasite burden post mortem. This procedure has various difficulties for implementation, not only because of the ethical concerns associated with euthanasia of the animals, but also due to the requirement for a parasite-free environment in testing the suspected strain (Taylor et al., 1995; Wood et al., 1995). Similar problems relate to the assessment of anticoccidial efficacy against *Eimeria* spp. in poultry (Chapman, 1998) and *Cytosisospora suis* in pigs (Shrestha et al., 2017). Thus, evaluation of anthelmintic efficacy in animals is routinely assessed by the faecal egg count reduction test (FECRT), currently recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP), and involves comparison of faecal egg counts pre- and post-treatment (Coles et al., 1992). The advantage of the FECRT is its ability to assess a range of drugs under field conditions. However, analysis of the results for FECRTs can be difficult in cases where egg excretion rate is low, where the sensitivity of the counting methods is poor, for highly aggregated faecal egg counts, and when the sample size is small (Torgerson et al., 2005; Denwood et al., 2010; Dobson et al., 2012; Peña-Espinoza et al., 2014).

Different statistical models have been applied to improve the calculation of the estimated efficacy from FECRT results, including bootstrapping techniques, and Bayesian methods such as Markov chain Monte Carlo (Denwood et al., 2010; Torgerson et al., 2014; Peña-Espinoza et al., 2016). However, challenges remain regarding the use of faecal oocyst count reduction tests (FOCRT), the (coccidial) oocyst equivalent of FECRT, for the assessment of ACE, due to extreme variation in oocyst excretion rates compared with excretion of helminth eggs. This is, in general, a reflection of the more complicated biology and lifecycle of *Eimeria* spp., in which sexual reproduction of the parasite in the animal host is preceded by several rounds of intracellular asexual reproduction that occurs in waves (Walker et al., 2013). The maximum range of *Eimeria* oocyst excretion can differ from between 0 and 75,000 to between 0 and 2,000,000 oocyst per gram (OPG), with large inter-individual variation (Chapman, 1974). In contrast, helminth egg excretion usually does not exceed 20,000 eggs per gram (Sréter et al., 1994; Zaros et al., 2014). As toltrazuril acts against intracellular stages of the parasite, and extracellular stages are unaffected (Haberkorn and Stoltefuss, 1987; Harder and Haberkorn, 1989; Mehlhorn, 2008), oocyst counts immediately post treatment may not be zero. Detailed data concerning the efficacy of toltrazuril when the drug was first marketed are not available from the literature, but practical experience confirms that post-treatment oocyst counts are not always zero even when the observed clinical efficacy is good. Thus, any model for evaluation of ACE has to take into account that a reduction to zero is not always the case, even when highly efficacious anticoccidials are used (Taylor and Kenny, 1988; Gjerde and Helle, 1991).

The emergence of ACR in poultry and pig production systems (Lan et al., 2017; Shrestha et al., 2017), along with anecdotal reports of reduced ACE in Norwegian lambs (Odden et al., 2017), demonstrates the need for a FECRT-type method to evaluate drug efficacy in live animals. However, due to the reasons outlined above, the standard FECRT (Coles et al., 1992) currently recommended by WAAVP for evaluation of anthelmintic efficacy is unsuitable for use with coccidia. The aim of our study was therefore to develop a tool for field evaluation of ACE, based on oocyst counts in lambs, and use it in a preliminary investigation of ACE in Norwegian sheep farms.

2. Materials and methods

2.1. Study design

2.1.1. Inclusion criteria

Norwegian sheep farms (n = 80) were selected based on a previous questionnaire study performed in October 2015 (Odden et al., 2017). The inclusion criteria were: a) treatment with anticoccidials annually for at least four years, b) coccidiosis-related symptoms in lambs treated with an anticoccidial, and c) flock size of more than 60 winter-fed ewes. The geographical location of the farms was consistent with the population density of sheep farms in Norway (Supplementary data 1) (Statistics Norway, 2017). All 80 farmers, of whom 60 agreed to participate, were contacted via telephone during the winter of 2016. The 60 participating farmers received a detailed written sampling and treatment protocol, a 10 ml syringe for oral drenching, envelopes with pre-paid postage, and a “faecal spoon” to facilitate sampling of young lambs (Supplementary data 2). Farms with < 5 lambs per treatment group were excluded.

2.1.2. Timing of treatment and sampling

Most Norwegian ewes are winter housed, with indoor lambing in the spring i.e. March–May (Vatn, 2009). Turnout to spring pastures commonly occurs two to three weeks postpartum (Domke et al., 2011). Clinical signs due to coccidiosis are mainly seen at around turnout. Lambs may become infected before turnout, mainly due to oocyst excretion from older, already infected lambs (Taylor, 1995), or immediately after turnout, as the oocysts survive overwintering on permanent pastures (Helle, 1970; Gjerde and Helle, 1991). Current Norwegian recommendations against ovine coccidiosis consist of a single metaphylactic treatment with toltrazuril, either at turnout or around one week after turnout (Animalia, 2017).

Farmers enrolled in the study were instructed to identify 8 pairs of twin lambs from which they would twice collect faecal samples during