In vivo efficacy

Multiple parameters were monitored *in vivo*, i.e. number of treatment rounds until all animals at a given farm had a mite count of zero (nTR), reduction in MC after first treatment round (RedMC), CI reduction after two treatment rounds (RedCI) and the proportion of the animals with zero mite counts after the first treatment round.

RedMC is defined as

$$\text{RedMC (\%)} = 100 \left(1 - \frac{\overline{\text{MCA}}}{\overline{\text{MCB}}} \right)$$

where $\overline{\text{MCB}}$ and $\overline{\text{MCA}}$ is the mean MC value of a number of cows before and after the first treatment round, respectively, with its variance approximated by the delta method as

$$\text{Var(RedMC)} V = \left(\frac{\overline{\text{MCA}}}{\overline{\text{MCB}}} \right)^2 \left(\frac{\text{Var}(\overline{\text{MCA}})}{\overline{\text{MCA}}} + \frac{\text{Var}(\overline{\text{MCB}})}{\overline{\text{MCB}}} - 2 \frac{\text{Corr}(\overline{\text{MCA}},\overline{\text{MCB}})\sqrt{\text{Var}(\overline{\text{MCA}})\,\text{Var}(\overline{\text{MCB}})}}{\overline{\text{MCA}}\,\overline{\text{MCB}}} \right)$$

Assuming that $100\text{-Red}_{\text{MC}}$ follows a gamma distribution with shape parameter γ and scale parameter θ given by

$$\gamma = \frac{(1 - \text{RedMC})^2 n}{\text{Var(RedMC)}}, \qquad \theta = \frac{\text{Var(RedMC)}}{(1 - \text{RedMC})^2 \, n}$$

the lower limit (LL) and upper limit (UL) of the 95% confidence interval correspond to the 2.5th and the 97.5th quantile of the gamma distribution with shape parameter γ and scale parameter θ.

The RedMC and its LL and UL where then calculated for each farm. Calculations were done with the Paradrug tool® (www.starworms.org/tools). Mite isolates of a farm were then categorized according to the methodology used for the faecal egg count reduction test in gastrointestinal nematodes, with the highest sensitivity [22]. The mite population on a farm was considered sensitive when RedMC $\geq$ 95% and LL $\geq$ 90%, suspected of resistance when RedMC $<$ 95% or LL $<$ 90% and resistant when RedMC $<$ 95% and LL $<$ 90%.

The proportion of the animals with negative mite counts was the fraction that was successfully treated (MC = 0) after the first treatment round.

In vitro sensitivity

The lethal dose 50 (LD_{50}) values for each farm population was calculated for the survival at 24 h and 120 h and knockdown dose 50 (KD_{50}) values at 24 h using a mixed logistic regression model with mortality and knockdown as binary response variable, drug concentration as continuous independent variable and farm as random effect.

Spearman's rank correlations were calculated between nTR, RedMC, RedCI, mite free proportion and $\log(LD_{50})$. Regression analysis was done by fitting a linear model with nTR, mean Red_{MC}, mean Red_{CI} or the proportion of the animals with negative mite counts as independent variable and $\log(LD_{50})$ as dependent variable. All calculations were done in R (version 3.5.1.) [23]