

# WP3 - Standardisation of diagnostic tests for resistance to enable pooling of results from different laboratories for surveys.

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# WP3 - Objectives

1. Produce standardised Faecal Egg Count Reduction Tests (FECRT) for sheep and cattle.
2. Ring Test four in vitro tests and produce SOPs
3. Provide recommendations for conducting field surveys.

# Faecal Egg Count reduction Test (Sheep and goats)

- Collect faeces.
- Weigh and dose
- Resample after X days according to the anthelmintic
- Updated guidelines produced incorporating practices developed in New Zealand e.g.
  - Pooled samples
  - FECPAK
  - BootStreat software to calculate efficacy

# FECRT in cattle 2006 & 2007

- Studies performed according to current WAAVP guidelines
  - Limited number of farms
  - Confounding factors low
  - Less than 95% reduction and LCL <90%

# FECRT in cattle

## 2006 & 2007

country	Farms	Farms with ≥95% efficacy	Farms with <95% efficacy & <90% LCL
Germany	14	4	10
Belgium	10	3	7
Sweden	9	1	8
<b>Total</b>	<b>33</b>	<b>8 (24%)</b>	<b>25 (76%)</b>

Additionally 13 farms were evaluated using oral BZ in 2007 (Germany & Sweden)  
100% efficacy was observed on all tested farms

# Faecal Egg Count reduction Test (cattle)

- Test still need to be improved
- Issues to be clarified:
  - threshold for pre-treatment EPG
  - Sensitivity of egg counts
  - Interval between samplings (ML)
  - Application of ML's
  - Number of animals

# Efficacy survey 2008

- Treatment done by farmer/veterinarian
- Sampling (rectal/ground) by farmer
- Anthelmintics chosen by vet/farmer

# Efficacy survey in cattle in EU

country	Farms surveyed	Farms included	Farms with ML efficacy <95%
Belgium	81	63	27
Germany	28	12	4
UK	34	17	15
Sweden	22	2	1

## Confounding factors

- Formulation & dosage of anthelmintics
- Collection of samples
- Low infection level pre treatment

## WP3.2 Ring test Egg Hatch Test.

- If nematode eggs hatch in above 0.1  $\mu\text{g}/\text{ml}$  thiabendazole, they are resistant
- Successfully standardised for nematodes (*C. oncophora*, *O. ostertagi* & *H. contortus*)
- Test sensitivity can be enhanced by using a discriminating dose ( $\text{EC}_{99}$ )
- Manuscript submitted which includes standard operating procedure.

# WP3.2 Ring test Larval Development Test (LDT)

- Eggs are incubated with food source and anthelmintic until they develop to L3 larvae. Resistant larvae will grow and susceptible ones will not.
- Overgrowth of bacteria occurred with shipped *Haemonchus* samples
- Suspect that anaerobic transport of eggs may be affecting results. *Pseudomonas aeruginosa* present in wells of test plates.
- So no SOP produced

# WP3.4 Ring test Larval Migration Inhibition Test

- Based on migration of L3 through a small sieve.
- Development undertaken in Germany.
- Testing conducted with samples sent from Germany.
- Species specific EC50 values
- Low resistance factor in tested IVM-resistant *C. oncophora* isolate
- Not yet suitable for use with mixed species of nematodes.

# WP3.5 Ring test PCR for BZ resistance in *H. contortus*.

% 'resistant' alleles	Bath	Moredun	Hannover	Uppsala
Isolate 1	40%	37%	47%	50%
Isolate 2	74%	86%	86%	87%

# WP3.6 Produce Standard Operating Procedures for evaluated tests

- Standard operating procedure - detailed instructions on how exactly to perform a test.
- Been produced and published for the Egg Hatch Test.
- Larval Development Test not reached the stage where SOP can be produced.
- Larval migration and PCR tests are now useful research tools but require adapting for field samples with multiple species of nematodes.

## WP3.7 Recommendations for conducting field tests.

- With finalisation of new Faecal Egg Count Reduction Test for anthelmintic resistance in ovine nematodes, recommendations for conducting surveys are being drawn up.
- Agreed protocol for FECRT for cattle required before improved recommendations for anthelmintic resistance tests can be prepared for use in cattle.

# WP3 - Conclusion

- FECRT with cattle suggest reduced anthelmintic efficacy must be taken very seriously in the EU.
- Egg Hatch Test useful for benzimidazole resistance in sheep and cattle.
- Larval Development Test can be useful but contamination problems remain.
- Other tests require further development for use with mixed species found in field samples.

# WP3 - Recommendations

- Further research is required to optimize FECRT in cattle nematodes
- Use Egg Hatch Test for benzimidazole resistance
- Further evaluation of the LMIT with characterized isolates of *C. oncophora* & *O. ostertagi*
- Use molecular tests for benzimidazole resistance in *H. contortus*