Methods to detect Anthelmintic Resistance

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Learning Objectives

- *In vivo and In vitro* methods
- Fecal Egg Count Reduction Test (FECRT)
- Egg Hatch Assay
- Larval Development Assay
- Larval Motility Assay
- Larval Migration Inhibition Assay
- Polymerase Chain Reaction

Detection Anthelmintic Resistance

- In vivo Methods

1. Fecal Egg count reduction test (FECRT)

- In vitro Methods

1. Egg Hatch Assay(EHA)
2. Larval Development Assay (LDA)
3. Larval Motility Assay (LMA)
4. Polymerase Chain Reaction (PCR)

FECRT

- Standard and widely used method
- Egg count done before and after anthelmintic treatment
- Percent reduction in egg count is calculated
- FECRT less than 95 per cent signifies AR
- Day post treatment egg count varies depending upon anthelmintic drugs because these drugs can stop eggs being laid by adult worms without killing them

-Benzimidazoles 10-14 days

-Tetrahydropyrimidines: 3-7 days

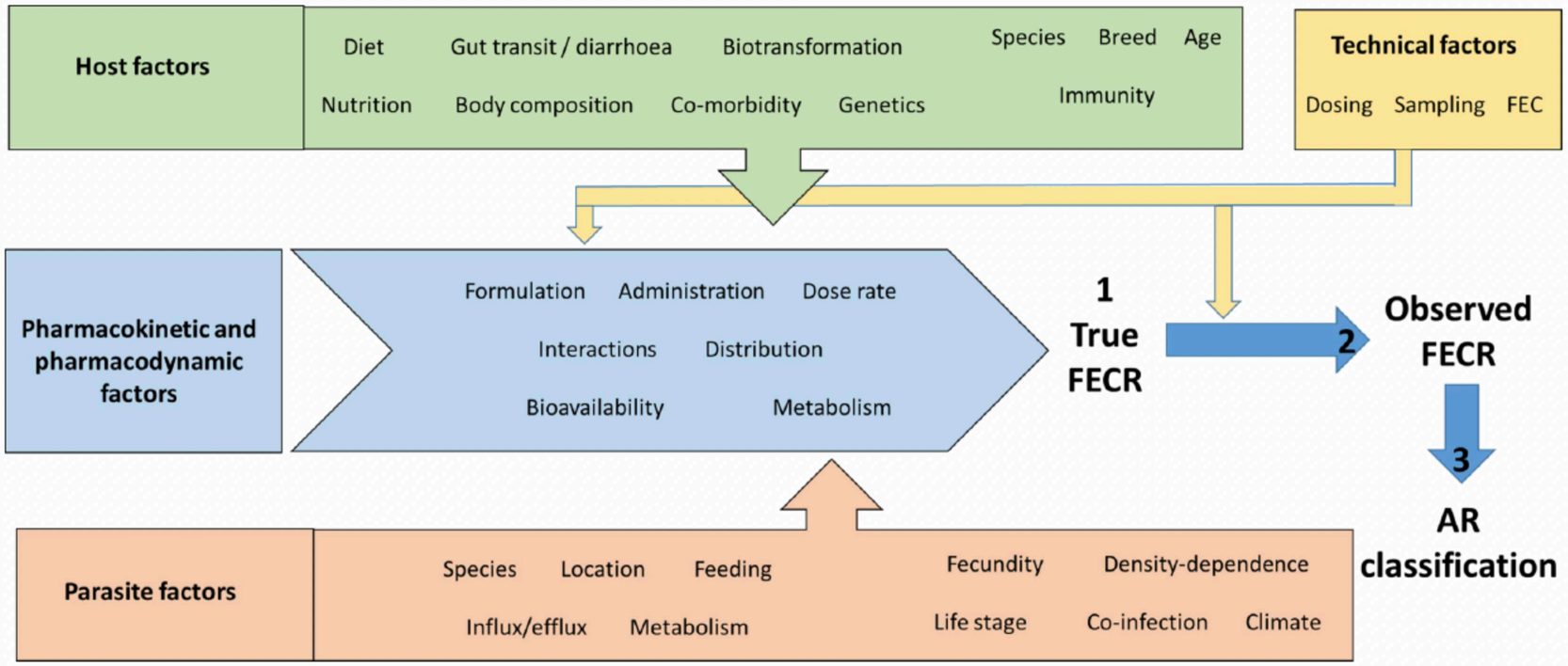
-Imidazothiazoles: 3-7 days

-Macrocyclic lactones: 14-17 days

FECRT

- Accurate but time consuming and expensive
- Sheep/ Goats to be randomly assigned into treatment and control groups
- Minimum 10-15 animals per group
- Ex. Two anthelmintics- 3 Groups (2 treatment & 1 control)
- Fecal samples should be taken rectally from each animal on the day of treatment
- All animals graze together
- All Animals should be sampled again on day 7 days (Levamosole) or on day 14 (Benzimidazoles or Ivermectin)
- Compare post treatment and control groups
  (Control EPG - Treatment EPG/ Control EPG) X 100
- Anthelmintic resistance is indicated if reduction in EPG is < 95%.

Confounding factors influencing faecal egg count reduction (FECR) following anthelmintic treatment



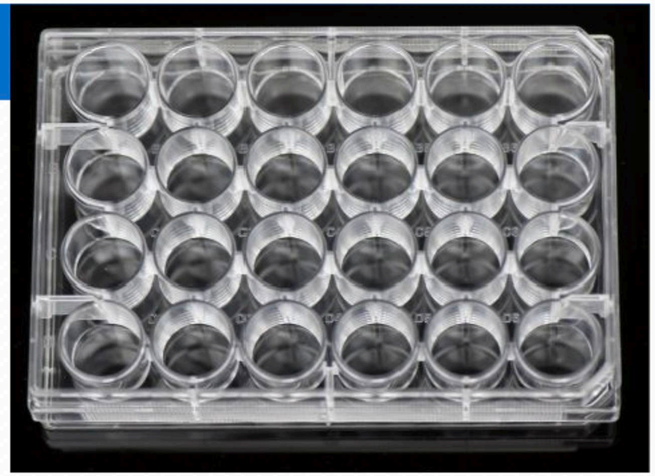
(Morgan et al., 2022)

According to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, anthelmintic resistance (AR) is considered to be present in small ruminant nematodes when the efficacy of an anthelmintic drug is below 95% and the lower 95% confidence interval (CI) is below 90%, whereas AR is suspected when only one criteria is met (Coles et al., 1992).

Egg Hatch Assay (EHA)

- EHA is the measure of drugs ability to prevent embryonation hence it is useful to assess AR against ovicidal anthelmintics
- This test is useful for BZ drugs as they prevent embryonation and hatching of nematode eggs
- EHA is not suitable for Tetrahydropyrimidines, imidazothiazoles and macrocyclic lactones because they are not ovicidal.

Egg Hatch Assay



- Add approximately 30 fresh eggs per well in 200 µl medium in 24 multiwell plates
- Add the different concentrations of BZ drugs and incubate for 15 h at 20 °C in a BOD incubator.
- At the end of the incubation, the percentage of hatched and unhatched eggs and the L1 larvae will be determined for each of the different drug concentrations by light microscopy.
- LD_{50} values will be calculated

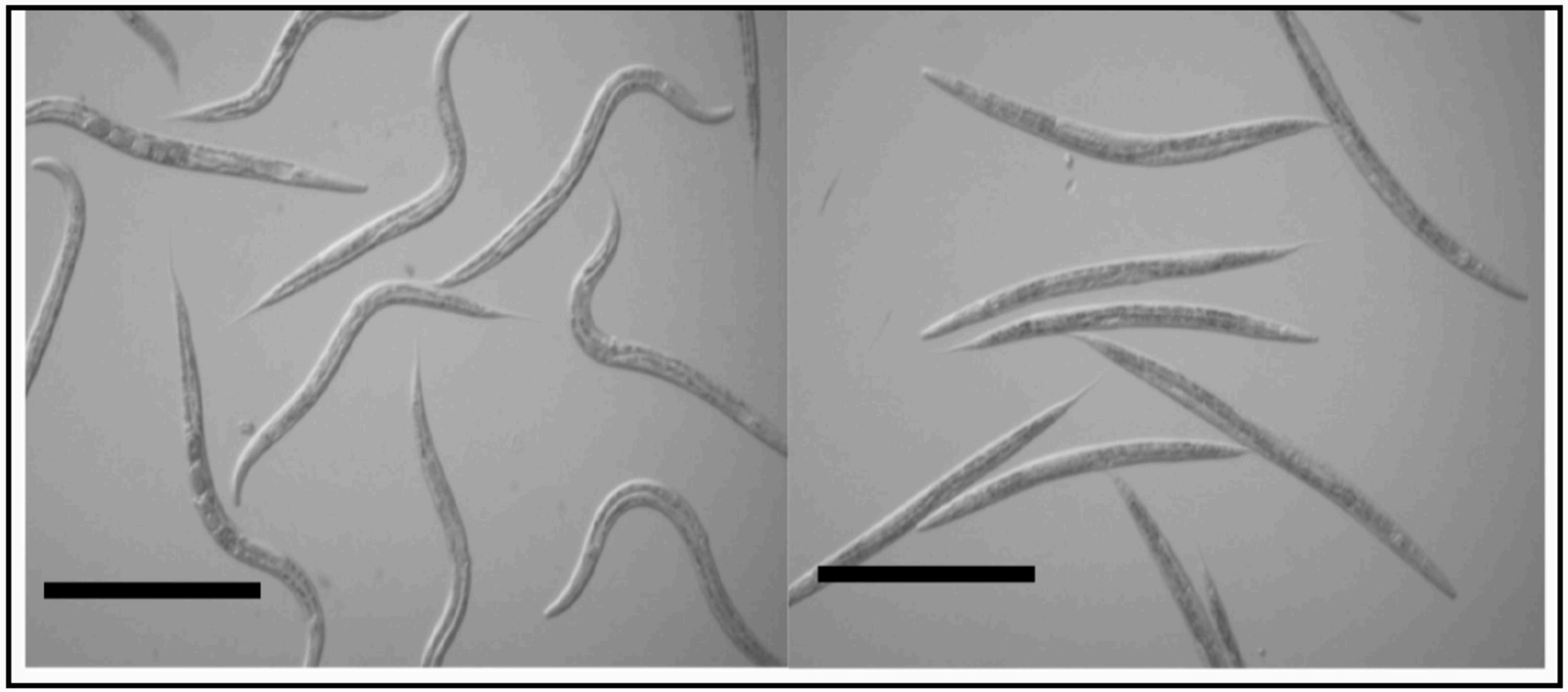
Larval Development Assay

- The Larval Development assay (LDA) measures the effects of drugs on the development of eggs to infective third-stage larvae
- This test is based upon the capacity of the larvae to survive and develop to infective stage in varied concentrations of anthelmintic drugs
- This test is mainly done for testing AR for major anthelmintic classes.
- LD_{50} is calculated to test the AR

Larval Motility Assay

- This assay is used to assess the ability of the anthelmintic drugs to immobilize the larvae.
- Larvae are incubated at room temperature for 24 hrs in various concentrations of anthelmintic drugs in darkness
- Then exposed to light for 20 min to stimulate motility.
- Number of immotile larvae in proportion of total larvae are calculated and interpreted for AR.

- The use of a micromotility metre to measure movement of L3 stage larvae
- The scoring of larvae as motile or nonmotile by observation after a period of exposure to the drug



Larval Migration Inhibition Test

Larval Migration Inhibition Test

- The separation of drug-affected and nonaffected larvae based on their ability to migrate through a mesh (Demeler et al., 2010)
- 24 well plate containing anthelmintic drugs at various concentrations
- Incubate L3 larva for 24h in drug followed by24h migration through 24 micrometer mesh

Larval Migration Inhibition Test

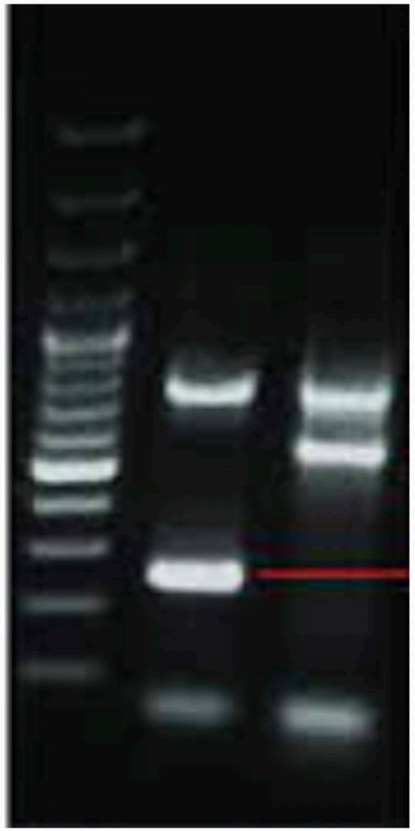
- Migration out of an agar block (d'Assonville et al., 1996)
- Migration through soft agar, followed by migration through a mesh (Kotze et al., 2006)
- Calculate % L3 not migrating (L3 left in sieve /Total L3)
- Anthelmintic activity relates to inhibition of migration

Polymerase Chain Reaction (PCR)

- PCR is a molecular test done for genotyping larvae or worms for the presence of resistance or susceptible genes
- Genotyping can identify resistant (rr) or susceptible (rS and SS) genes in adult worms or larvae
- Resistance against BZ drugs is tested by employing four primers in same reaction mixture
- Worms are genotyped for the mutation on β-tubulin residue at position 200 (phenylalanine to tyrosine)

Allele specific PCR for Benzimidazole Resistance

T. colubriformis
T. cincumcincta



H. contortus
750 bp and 203 bp- Resistant

(Bihaqi et al., 2020)

Other Molecular Tests are…

- **Restriction fragment length polymorphism-PCR** (Ghisi et al., 2007, Tiwari et al., 2006)
- **Real- Time PCR** (Walsh et al., 2007)
- **Pyrosequencing** (von Samson-Himmelstjerna et al., 2009).
- Pyrosequencing assays for H. *contortus codons 167*, 198 and 200 of β-tubulin isotypes 1 and 2.
- The method proved able to assess the BZ resistance status of a number of H. *contortus* isolates, indicating that it may be suitable for routine diagnosis of resistance in this species.

Thank you