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Anthelmintic Resistance in Livestock: From Diagnosis to Sustainable Parasite Management

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Introduction

Anthelmintic resistance (AR) is defined as the heritable ability of parasites to survive therapeutic doses of an anthelmintic that would normally eliminate susceptible individuals of the same species. It is one of the greatest threats to sustainable livestock production worldwide, affecting cattle, sheep, goats, buffaloes, and other grazing animals. The widespread and often indiscriminate use of anthelmintics has accelerated the selection of resistant parasite populations, resulting in reduced treatment efficacy, production losses, increased veterinary costs, and compromised animal welfare (Kaplan, 2004; Sutherland and Leathwick, 2011).

The most important gastrointestinal nematodes exhibiting resistance include *Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus spp., Cooperia spp., Ostertagia ostertagi*, and *Nematodirus spp.* Resistance has been reported against all major anthelmintic classes, including benzimidazoles, imidazothiazoles, macrocyclic lactones, amino-acetonitrile derivatives, and spiroindoles (Kotze et al., 2020).

Diagnosis of Anthelmintic Resistance

Early detection is essential for implementing effective parasite control programs and delaying further development of resistance. Diagnosis can be performed using in vivo, in vitro, and molecular methods.

Clinical Indicators

Although not definitive, resistance should be suspected when:

- Persistent parasitic disease despite recent deworming
- Continued high faecal egg counts after treatment
- Poor weight gain or weight loss
- Anaemia in small ruminants due to *Haemonchus contortus*
- Reduced milk production
- Increased mortality in young animals
- Frequent need for repeat treatments

However, treatment failure may also result from incorrect dosing, poor drug quality, improper administration, or reinfection.

I. In vivo Diagnostic Methods

1. Faecal Egg Count Reduction Test (FECRT)

The Faecal Egg Count Reduction Test (FECRT) is the gold standard for field diagnosis of anthelmintic resistance (Coles et al., 1992; Kaplan, 2020).

Principle

The test compares faecal egg counts before and after treatment.

Procedure

- Select at least 10–15 animals per treatment group.
- Collect faecal samples on Day 0.
- Determine eggs per gram (EPG) using the McMaster technique.
- Administer anthelmintic accurately according to body weight.

- Collect faeces again:
  - 10–14 days after benzimidazoles and levamisole
  - 14–17 days after macrocyclic lactones
- Calculate percentage egg count reduction.

treatment EPG×100

Interpretation (WAAVP Guidelines)

FECR (%)	Lower 95% Confidence Limit	Interpretation
≥95%	>90%	Susceptible
<95%	<90%	Resistant
Borderline	One criterion not met	Suspected resistance

Advantages

- Inexpensive
- Practical under field conditions
- Internationally accepted
- Detects resistance in naturally infected animals

Limitations

- Time consuming
- Requires adequate egg counts
- Low sensitivity for early resistance
- Cannot identify parasite species responsible

2. Controlled Efficacy Test (CET)

The Controlled Efficacy Test is considered the definitive method.

Procedure

- Animals are treated.
- After an appropriate interval they are humanely euthanized.
- Worms remaining in the gastrointestinal tract are counted.

Advantages

- Highest accuracy
- Species-specific assessment
- Detects low levels of resistance

Limitations

- Expensive
- Ethical concerns
- Mainly used in research

II. In vitro Diagnostic Methods

1. Egg Hatch Assay (EHA)

Mainly detects resistance to benzimidazoles (Van Wyk et al., 1989).

Principle

Eggs from resistant worms hatch despite increasing concentrations of thiabendazole.

Advantages

- Sensitive
- Early detection
- No animal sacrifice

Limitations

- Only useful for benzimidazole resistance.

2. Larval Development Assay (LDA)

Measures the ability of larvae to develop in different concentrations of anthelmintics.

Detects resistance against

- Benzimidazoles
- Levamisole
- Macrocyclic lactones

Advantages

- Multi-drug evaluation
- Sensitive

Limitations

- Laboratory expertise required

3. Larval Migration Inhibition Test (LMIT)

Evaluates the ability of larvae to migrate after drug exposure.

Commonly used for:

- Levamisole
- Macrocyclic lactones

4. Adult Motility Assay

Adult worms collected from animals are exposed to drugs in vitro.

Resistance is indicated by continued motility despite drug exposure.

III. Molecular Diagnosis

Molecular techniques detect genetic mutations associated with resistance before clinical failure becomes evident.

1. PCR-based Detection methods:

Benzimidazole Resistance:

Mutations occur in the β-tubulin isotype-1 gene.

Major mutations:

- F200Y
- F167Y
- E198A
- E198L

Detection methods include:

- Conventional PCR
- PCR-RFLP
- Allele-specific PCR
- Real-time PCR

These mutations prevent benzimidazole binding to tubulin (Ghisi et al., 2007).

Quantitative PCR (qPCR):

Advantages:

- High sensitivity
- Quantifies resistant allele frequency
- Suitable for surveillance

Pyrosequencing:

Determines exact frequencies of resistant alleles within parasite populations.

Useful for monitoring emergence of resistance.

Digital PCR:

Provides absolute quantification of resistance-associated mutations.

Increasingly used in research.

2. **Next Generation Sequencing (NGS):**

Allows simultaneous analysis of multiple resistance genes (Kotze et al., 2020).

Applications include:

- Population genetics
- Detection of emerging resistance
- Species identification
- Surveillance

Management of Anthelmintic Resistance:

Successful management depends upon integrated parasite control, reducing selection pressure while maintaining parasite populations in refugia.

1. Strategic Anthelmintic Treatment:

Treat animals only when necessary.

Avoid routine calendar-based deworming.

Treatment should be based on:

- Faecal egg counts
- Clinical signs
- Seasonal epidemiology
- Pasture contamination

2. Targeted Selective Treatment (TST):

Only animals with high parasite burdens receive treatment (Kenyon et al., 2009).

Selection criteria include:

- FAMACHA© score
- Body condition score
- Weight gain
- Faecal egg counts
- Packed cell volume

Advantages:

- Preserves refugia
- Slows resistance
- Reduces drug costs.

3. Maintain Refugia:

Refugia refers to parasite populations not exposed to anthelmintics (Van Wyk, 2001).

Sources include:

- Untreated animals
- Larvae on pasture
- Arrested larvae

Importance:

- Dilutes resistant genes
- Maintains susceptible parasite populations
- Delays resistance evolution

4. Correct Drug Administration:

- Estimate body weight accurately
- Dose according to the heaviest animal in the group
- Calibrate drenching equipment regularly
- Avoid underdosing
- Ensure complete swallowing of oral drenches

Underdosing is a major driver of resistance.

5. Rotation of Drug Classes:

Rotate between different drug classes rather than repeated use of one class.

Major classes include:

- Benzimidazoles
- Imidazothiazoles
- Macrocyclic lactones
- Amino-acetonitrile derivatives
- Spiroindoles

Rotation should be based on efficacy testing rather than arbitrary schedules.

6. Combination Anthelmintic Therapy:

Use drugs from different classes simultaneously (Leathwick et al., 2012).

Advantages:

- Improves efficacy
- Kills resistant worms surviving one drug
- Delays resistance development

Widely recommended in sheep and goats.

7. Grazing Management:

Rotational Grazing

Interrupts parasite life cycle.

Alternate Grazing

Use cattle and sheep sequentially because many parasites are host-specific.

Mixed Grazing

Different livestock species reduce pasture contamination.

Safe Pastures

Use:

- Hay fields
- Silage aftermath
- Newly established pasture
- Crop residues

8. Nutritional Management:

Good nutrition improves host immunity.

Important nutrients include

- Protein
- Copper
- Zinc
- Selenium
- Vitamins A and E

Protein supplementation particularly enhances resistance against *Haemonchus contortus*.

9. Genetic Selection:

Breed animals naturally resistant to parasites.

Examples

- Red Maasai sheep
- Gulf Coast Native sheep
- Indigenous goat breeds

Selection criteria include

- Low faecal egg count
- High packed cell volume
- Good production under parasite challenge

10. Biological Control:

Nematophagous Fungi:

Duddingtonia flagrans produces trapping structures that destroy infective larvae in dung (Larsen, 2000)

Benefits:

- Reduces pasture contamination
- Environmentally safe
- Compatible with integrated parasite management

11. Botanical Anthelmintics:

Several medicinal plants possess anthelmintic activity.

Examples:

- *Azadirachta indica* (Neem)
- *Calotropis gigantea*
- *Ficus benghalensis*
- *Artemisia annua*
- *Allium sativum*
- *Carica papaya*

Plant bioactive compounds include:

- Condensed tannins
- Flavonoids
- Alkaloids
- Terpenoids
- Saponins

These are promising alternatives but require further standardization before routine field use.

12. Vaccination:

Vaccines reduce reliance on anthelmintics.

Example:

- Barbervax® against *Haemonchus contortus* in sheep.

Although effective, availability remains limited.

Integrated Parasite Management (IPM)

A sustainable parasite control programme combines:

- Regular faecal egg count monitoring
- FECRT to assess drug efficacy
- Targeted selective treatment
- Maintenance of refugia
- Rotational grazing
- Mixed-species grazing
- Adequate nutrition
- Genetic selection for resistant animals
- Biological control (*Duddingtonia flagrans*)
- Judicious use of effective anthelmintics
- Periodic surveillance using molecular diagnostics

Conclusion

Anthelmintic resistance has become a major constraint to profitable livestock

production worldwide. Early diagnosis through Faecal Egg Count Reduction Test (FECRT), supported by in vitro assays and molecular techniques, enables timely detection of resistance before widespread treatment failure occurs. Sustainable management requires an integrated approach combining targeted selective treatment, maintenance of refugia, accurate dosing, drug class rotation, combination therapy, improved grazing management, genetic selection, biological control, and enhanced nutrition. Adoption of integrated parasite management strategies will help preserve the efficacy of existing anthelmintics, improve animal health and productivity, and reduce the economic burden associated with parasitic diseases.

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