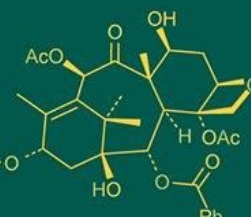
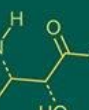
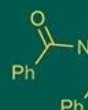


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## Development of anthelmintic resistance among non-descript goat population of southern Tamil Nadu, India

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### Abstract

A study was conducted on a non-descript goat population across four zones in Kanyakumari district, Tamil Nadu, to assess resistance to three anthelmintics (Oxfendazole, Levamisole, and Ivermectin) against nematodes (*Haemonchus*, *Trichostrongylus*, *Nematodirus*, and *Ostertagia*) from April 2024 to March 2025. The findings revealed an overall reduction in eggs per gram (EPG) ranging from 93% to 99.5%, indicating the effectiveness of these anthelmintics against nematodes in the studied area. The egg hatch test demonstrated a lethal concentration (LC50) of less than 0.1 µg/ml for oxfendazole, suggesting no resistance in eggs hatching to this drug. Furthermore, the results of the Egg Hatch Test corroborated those of the Faecal Egg Count Reduction Test. Qualitative analysis of faecal samples following coproculture identified four genera of gastrointestinal nematodes: *Haemonchus*, *Trichostrongylus*, *Nematodirus*, and *Ostertagia*.

**Keywords:** Sheep, anthelmintic resistance, oxfendazole, levamisole, ivermectin, *Haemonchus contortus*, *Trichostrongylus*, *Nematodirus*, *Ostertagia*

### Introduction

Over the past thirty years, controlling nematode populations has primarily depended on anthelmintics; however, their efficacy has diminished over time due to developing resistance. Reports indicate that anthelmintic resistance exists in various countries. Notably, early resistance to benzimidazoles against *Haemonchus contortus* was documented by George *et al.*, 2017 [5].

The geographical variation in climate, parasitic species prevalence, and treatment practices has influenced the rate at which anthelmintic resistance emerges within different regions. This phenomenon poses significant challenges for small ruminant production globally and raises concerns due to the lack of new therapeutic options (Xavier *et al.*, 2018) [15]. The present study aims to evaluate the development of resistance against commonly used anthelmintics among goat populations across four zones in Kanyakumari district.

### Materials and Methods

#### Sample Collection and Anthelmintics

The investigation targeted four distinct zones—Alancholai, Arukani, Chittar, and Katruva—in Kanyakumari district to examine anthelmintic resistance against gastrointestinal nematodes within a non-descript goat population. A total of 240 goats under one year old were randomly selected from each zone (60 animals per zone), ensuring they were naturally infected with gastrointestinal nematodes with a minimum count of 140 eggs per gram of faeces for efficacy evaluation.

Each zone was categorized into four groups (A-D), with fifteen animals per group: Groups A and B received Oxfendazole at a dosage of 4.53 mg/kg body weight and Levamisole at 7.5 mg/kg body weight orally; Group C was administered Ivermectin subcutaneously at a dose of 200 µg/kg body weight while Group D served as a control group. Dosages adhered strictly to manufacturer guidelines. Anthelmintic resistance detection involved conducting faecal egg count reduction tests alongside egg hatch assays using oxfendazole (Danish *et al.*, 1996) [1].

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### Faecal Egg Count Reduction Test

Five grams of faecal samples were obtained rectally from individual animals prior to treatment and again on day fourteen post-treatment. These samples were either examined immediately or stored at 4°C for later analysis as described by Kinger *et al.*, 1998 [9]. Identification of nematode species occurred via coproculture followed by microscopic examination for egg counting using modified McMaster technique. The percentage reduction in eggs was calculated using  $R\% = 100(1 - X_t/X_c)$ , where  $X_t$  represents treated groups and  $X_c$  denotes control groups' counts. A reduction below 95% or confidence levels falling below 90% indicated potential presence of resistant nematodes among treated subjects—fulfilling just one criterion suggested suspicion towards developing resistance.

### Egg Hatch Test

For this test, five grams from individual sheep's faeces were collected directly from the rectum before being thoroughly mixed into a composite sample for each flock labeled accordingly for identification purposes. After homogenization through sieving with wire mesh while adding saturated salt solution into shallow trays containing filtered faecal matter until floating eggs adhered onto plastic sheets placed atop them—these sheets were removed after thirty minutes allowing washed eggs into beakers for quantification via McMaster technique following standard protocols outlined by Finner (1986) [14].

One milliliter oxfendazole stock solution diluted in deionized water facilitated preparations yielding fifteen concentrations ranging from 0.001-22.65 µg/ml through two-fold serial dilutions utilizing saline solutions aimed at calculating doses necessary preventing fifty percent viable egg hatching (LC50). Each well within multi-well plates received one ml suspension along with specific concentrations according control wells receiving saline only before incubation at ambient temperature over forty-eight hours culminating adding lugol's iodine drops prior counting larvae hatched versus remaining unhatched under inverted microscope.

### Statistical Analysis

Logarithmic concentration values derived through log probit analysis (McSweeney, 2005) [10] indicated any LC value exceeding threshold limits relative indicating potential emergence signs pointing towards detectable anthelmintic resistances specifically regarding oxfendazole.

### Results

#### Faecal Egg Count

Table outlines percentage reductions observed concerning EPG post-treatment using varied anthelmintics showcasing notable efficacy across all zones studied revealing reductions between treatments reaching up-to seventy-seven percent decreased rates respectively attributed towards oxfendazole achieving decreases around ninety-five-ninety-seven percent along *H. contortus* whilst trichostrongylids followed suit reflecting similar trends across other genera like *Nematodirus* exhibiting comparable figures emphasizing broad effectiveness seen overall regardless contextually speaking about respective geographic differences.

**Table 1:** Mean faecal egg count (eggs/g) in non-descript goat population naturally infected with mixed species of nematodes before and after treatment with different anthelmintics

| Anthelmintics in four zones | Day 0 (n=15) | Day 14 (n=15) |
|-----------------------------|--------------|---------------|
| <b>Oxfendazole</b>          |              |               |
| Alancholai                  | 2592         | 59            |
| Arukani                     | 2635         | 76            |
| Chittar                     | 2758         | 96            |
| Katruva                     | 2864         | 112           |
| <b>Levamisole</b>           |              |               |
| Alancholai                  | 2423         | 51            |
| Arukani                     | 2591         | 69            |
| Chittar                     | 2650         | 90            |
| Katruva                     | 2741         | 101           |
| <b>Ivermectin</b>           |              |               |
| Alancholai                  | 1996         | 35            |
| Arukani                     | 2086         | 49            |
| Chittar                     | 2171         | 62            |
| Katruva                     | 2249         | 84            |

### Egg Hatching

Outcomes reported from egg hatch tests confirmed LC50 levels remained low beneath established thresholds confirming absence indications showing no evidence supporting hypotheses surrounding resistant strains emerging particularly related back towards responses noted earlier aligned consistently throughout observational periods documented during trials undertaken here reflecting consistency found across fecal evaluations corroborating initial results drawn forth upon first assessments conducted thereafter.

### Discussion

The findings indicate that *Haemonchus*, *Trichostrongylus*, *Nematodirus*, and *Ostertagia* exhibited a high sensitivity to oxfendazole, levamisole, and ivermectin across a non-specific goat population in all four zones. This aligns with the results reported by Dhankar *et al.*, (2019) [12], who documented a 100% efficacy of levamisole and fenbendazole against gastrointestinal parasites in Karakuk sheep in India. The LC50 determined from the egg hatching assay was recorded at 0.074±0.015 µg thiabendazole/ml. Similarly, Vishwesh *et al.*, (2016) [14] found anthelmintic effectiveness of the combination of these two drugs along with ivermectin in both sheep and goats.

In contrast, resistance was observed in *Haemonchus* and *Trichostrongylus* species within goat herds when treated with combinations of albendazole, tetramizole, and ivermectin. These observations are consistent with those of Harry *et al.*, (2017) [6], who found ivermectin to be fully effective against intestinal nematodes. Kailash *et al.*, (2019) [8] also noted a significant reduction in fecal egg counts of gastrointestinal nematodes in sheep following ivermectin treatment.

Dheeraj *et al.*, (2019) [3] reported that ivermectin demonstrated 99.1% efficacy against *Nematodirus spathiger*. Moreover, Namankar *et al.*, (2021) [12] indicated that sheep treated with ivermectin and closantel achieved a complete reduction in eggs per gram (EPG) for *H. contortus*, while treatments with benzimidazoles, levamisole, and morantel resulted in fecal egg count reductions ranging from 56% to 81%. These outcomes suggest the presence of multiple drug resistance against *Haemonchus contortus*.

Miraiz (2019) <sup>[11]</sup> showed that *Haemonchus* and *Trichostrongylus* displayed resistance to oxfendazole which proved to be less effective. Vihari (2020) <sup>[13]</sup> observed widespread resistance to albendazole and levamisole across all examined sheep farms, differing from this present study which did not find any resistance to ivermectin. Jaffer *et al.*, (2022) <sup>[7]</sup> affirmed instances of benzimidazole resistance against *H. contortus* particularly when fecal egg count reductions fell below 95%, maintaining confidence levels under 90%, thus establishing criteria indicating suspected instances where only one criterion fulfilled could denote potential resistance occurrence.

## Conclusion

There was marked percent reduction in eggs per gram (EPG) of faeces after treatment with different anthelmintics. All the three anthelmintics Oxfendazole, Levamisole and Ivermectin seem to be very effective against *Haemonchus*, *Trichostrongylus*, *Nematodirus* and *Ostertagia* in goats of all 4 zones. Egg hatch test confirmed that no resistance was found in the eggs to oxfendazole. Hence, it is important to apply the available anthelmintics in a manner that reduces the effect of anthelmintic resistance such as sensible and integrated implementation of anthelmintics and decreasing usage of same anthelmintics. Proper evaluation and surveillance of anthelmintic resistance development is also pivotal.

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