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# ITS-2 rDNA metabarcoding and amplicon sequencing enabled investigation of anthelmintic resistance in cattle gastrointestinal nematodes in Uruguay

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A prevalence study of anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) was conducted in beef cattle in Uruguay using a combination of fecal egg count reduction testing, nemabiome metabarcoding and deep amplicon sequencing.

**Methods**. 1. Assess drug efficacy with *in vivo* FECRT
2. Assess species composition using Nemabiome sequencing
3. Deep amplicon sequencing of benzimidazole resistance associated mutations in isotype-1 β-tubulin gene

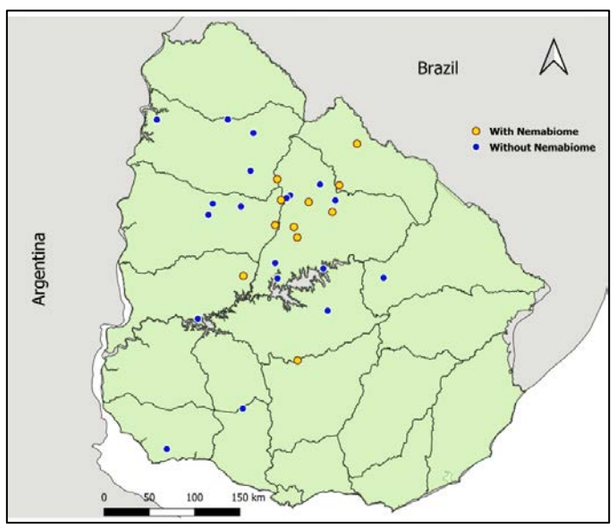
## Assess drug efficacy with *in vivo* FECRT

***Field work:***

- At each farm, on "day 0" pre-treatment,15 calves were assigned to one of the following five groups: Control, Ivermectine (IVM) 200µg/kg bodyweight, levamisole (LEV) 7.5mg/kg, ricobendazole (RBZ) 4mg/kg and fenbendazole (FBZ) 5mg/kg.
- Individual fecal samples were collected directly per rectum and the animals received the corresponding treatment except for the control group. In addition, individual fecal samples were collected from each calf on "day 14".

***Lab work:***

- Fecal egg counts estimated using a Mini-Flotac technique (lower detection limit = 5eggs/gr)

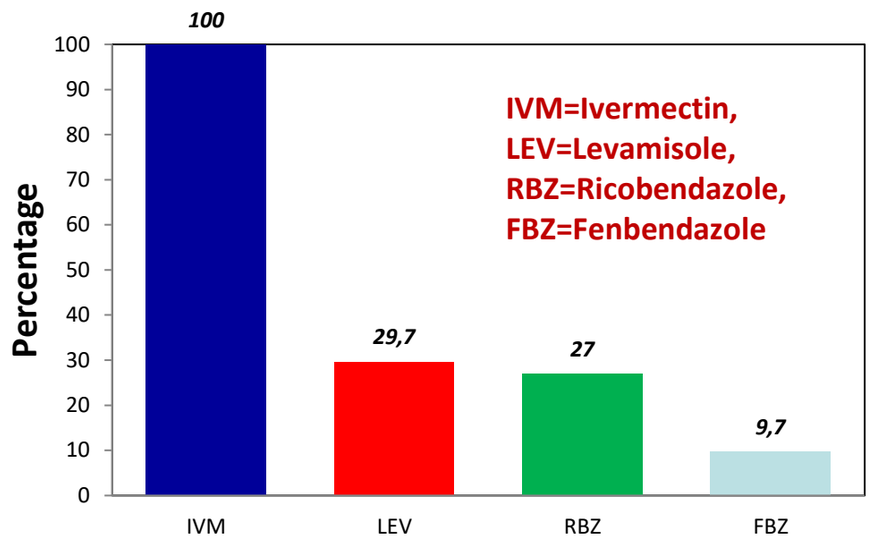



**FECR Estimation:**
**100x[1-(T2/T1)/(C1/C2)]**

**Mean efficacy of drugs tested**

| Drug | Mean Efficacy (%) | Range (%) |
|---|---|---|
| IVM | 28,2 | 0-80 |
| LEV | 89,8 | 0-100 |
| RBZ | 92,9 | 0 - 100 |
| FBZ | 92,8 | 0 - 100 |

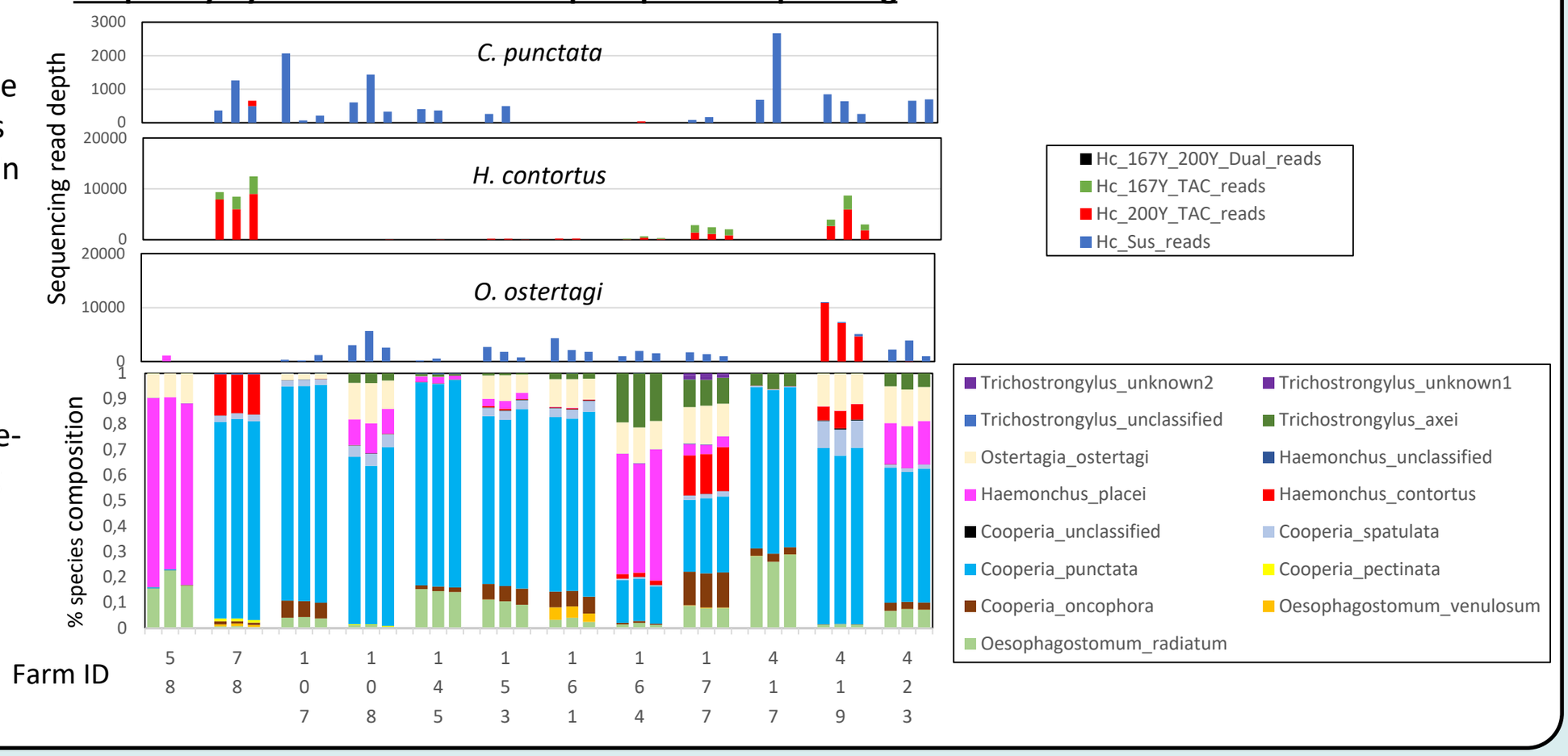
**Proportion of farms (n=37) where anthelmintic resistance was detected**




## Determination of GIN relative species abundance and BZ resistance mutation frequency by Nemabiome and deep amplicon sequencing

Frequency of Benzimidazole resistance associated snps in the Isotype-1 beta tubulin gene in pre-treatment samples

Species composition of pre-treatment samples using Nemabiome sequencing




## Conclusions:

- Anthelmintic resistance was detected on 100%, 29.7%, 27.0% and 17.0% of farms for IVM, LEV, RBZ and BZ, respectively
- Mixed GI nematode infections identified and *C. punctata* predominates
- Benzimidazole resistance associated mutations in Isotype-1 beta tubulin gene at codons 167Y and 200Y common in *H. contortus.*
- Benzimidazole resistance in *O. ostertagi* (codon 200Y of Isotype-1 beta tubulin gene) is emerging in Uruguay
- No Benzimidazole resistance identified in any of the other species.