



## Objective

**Identification** of 6 different species of *Eimeria* infection in the peri-weaned dairy calves with molecular approaches and **determination** of their prevalence.

## Materials and Methods

- Study conducted from May 2024 to February 2025.
- 665 faecal samples** collected from dairy calves in peri-weaned period.
- DNA extraction was performed using QIAmp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instruction.
- Application of conventional PCR-PCRc (**Table 1 and 2**) for the detection of *Eimeria*-positive faecal samples (**Fig 1**) and multiplex PCR-PCRm (**Table 1 and 2**) for the differentiation of 6 different *Eimeria* spp. among the *Eimeria*-positive faecal samples (**Fig 2**), aiming to the first internal transcribed spacer (ITS 1) region of 18s rRNA.

### Working Conditions

Step	Cycles	Temperature	Time
Initial denaturation		95 °C	5 minutes
Denaturation		95 °C	30 seconds
Annealing	30	58 °C	30 seconds
Extension		72 °C	45 seconds
Final elongation		72 °C	5 minutes

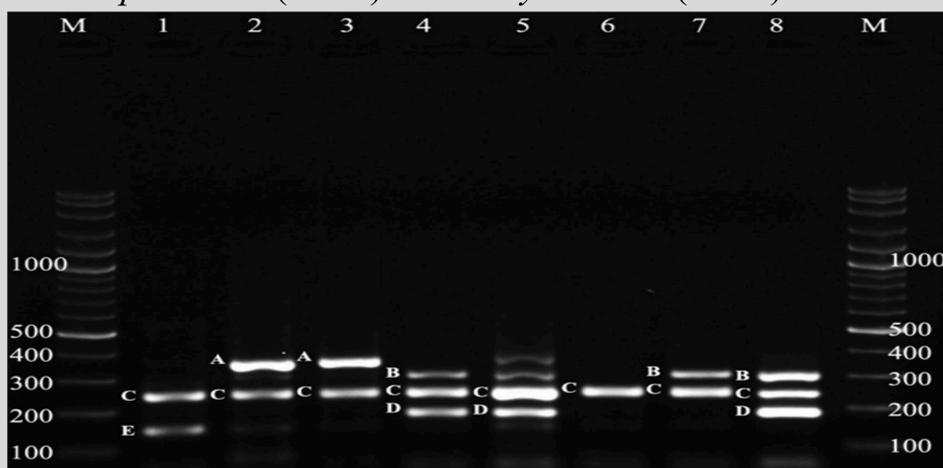
**Table 2.** Working conditions for PCRc/m

Species	Expected product size (bp)	Forward primer	Reverse primer
Common genus	348–546	5'-GCAAAAGTCGTAACACGGTTTCCG-3'	5'-CTGCAATTCACAATGCGTATCGC-3'
<i>Eimeria alabamensis</i>	184	5'-CATTACACATTGTTCTTTTCAG-3'	5'-GCTTCCAAACTAATGTTCTG-3'
<i>Eimeria auburnensis</i>	295	5'-TAAATTGGTGCGATGAGGGA-3'	5'-GCAATGAGAGAAAGATTTAATA-3'
<i>Eimeria bovis</i>	238	5'-TCATAAAACATCACCTCCAA-3'	5'-ATAATTGCGATAAGGGAGACA-3'
<i>Eimeria cylindrica</i>	304	5'-GACATTTAAAAAACCGATTGGT-3'	5'-GGCTGCAATAAGATAGACATA-3'
<i>Eimeria ellipsoidalis</i>	148	5'-CAACGTTTTTCCTTTTCCTATCA-3'	5'-ACTGCGATGAGAGAGAGCG-3'
<i>Eimeria zuernii</i>	344	5'-AACATGTTTCTACCCACTAC-3'	5'-CGATAAGGAGGAGGACAAC-3'

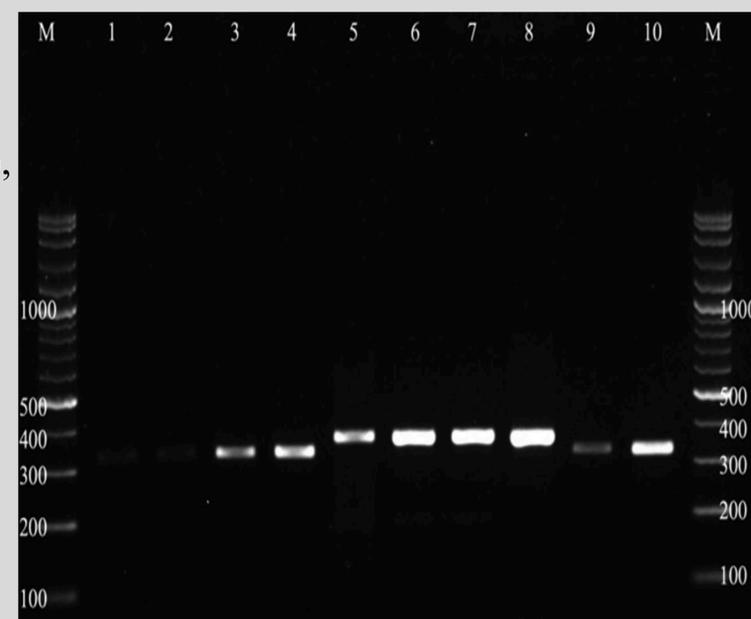
**Table 1.** Primers used and expected product size for PCRc/m

## Results

- Total prevalence:** 308 out of 665 (46.3%) faecal samples were *Eimeria* - positive.
- Single infection prevalence:** 151 out of 308 (49.0%) *Eimeria*-positive faecal samples.
- Mixed infection prevalence:** 157 out of 308 (51.0%) *Eimeria*-positive faecal samples.
- 5 identified species:** *Eimeria bovis* (24.7%), *E. zuernii* (14.0%), *E. alabamensis* (5.8%), *E. ellipsoidalis* (3.6%) and *E. cylindrica* (1.0%). No *E. auburnensis* was detected.



**Fig 2.** Electrophoresis of *Eimeria* spp. positive samples. M: Marker, 1-8: positive samples A=*Eimeria zuernii*, B=*E. cylindrica*, C=*E. bovis*, D=*E. alabamensis* and E=*E. ellipsoidalis*



**Fig 1.** Electrophoresis of *Eimeria*-positive samples. M: Marker, 1-10: positive samples at 348-546 bp

## Conclusions

- The molecular approach applied **confirmed** the presence of *Eimeria* spp. among peri-weaned dairy calves at a **high** percentage.
- E. bovis* and *E. zuernii* were the most **predominant** species in our study and the most **pathogenic**, according to literature review.
- These data could provide epidemiological information for developing future **strategies** to **control bovine coccidiosis**.