Deeper into knowledge of eprinomectin: pharmacokinetics, efficacy, cross-resistance

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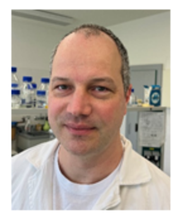

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Summary

- Microemulsion formulations significantly improved transdermal permeation of eprinomectin.
- Although pharmacokinetic profiles of eprinomectin (EPRI) pour-on formulations (F1, F2) were lower than the injectable reference, they still reached therapeutically relevant plasma concentrations.
- Both EPRI formulations demonstrated efficacy against *Trichostrongylus* sp. and *Teladorsagia* sp.
- Neither the optimized EPRI pour-on formulation (F2) nor the injectable EPRI reference effectively controlled ivermectin-resistant *Haemonchus contortus*.
- Real-time PCR effectively complemented fecal egg counts, enhancing parasite detection sensitivity.

Active substance	Formulation	Drug form	Nr. of animals	Dose of EPRI (mg/kg)	Cmax (ng/ml)	Tmax (hours)	AUCt (ng*hours/ml)	MRTt (hod)
eprinomektin	microemulsion F1	pour-on	5	1	4.4	36	667	114.2
eprinomektin	microemulsion F2	pour-on	5	1	4.8	36	735	118.2
eprinomektin	Eprecis inj. - reference	injectable	5	0.2	32.6	8	1764	58.3

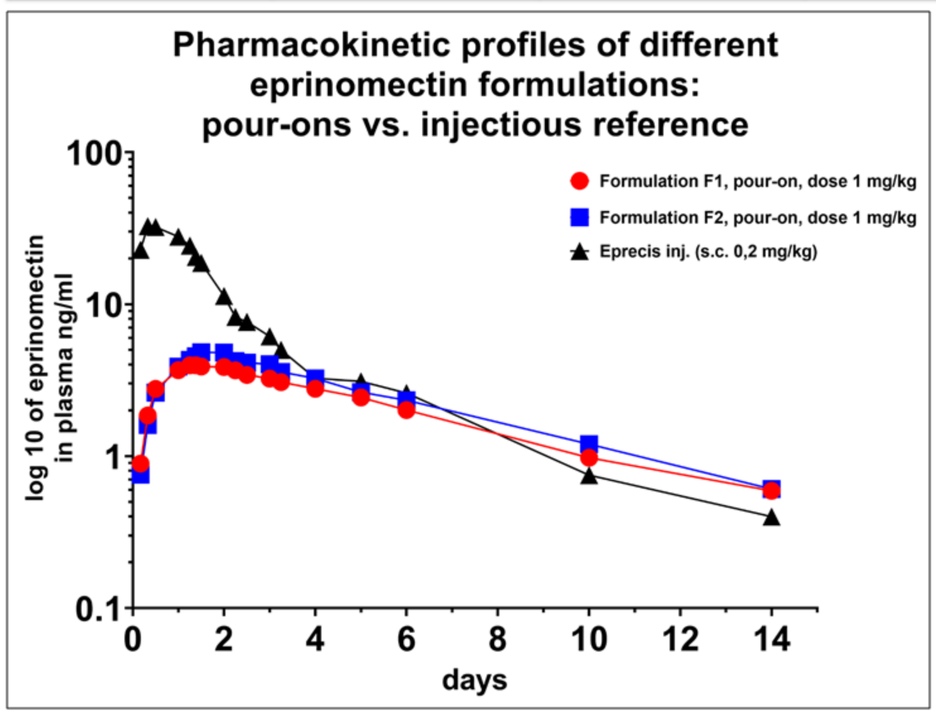
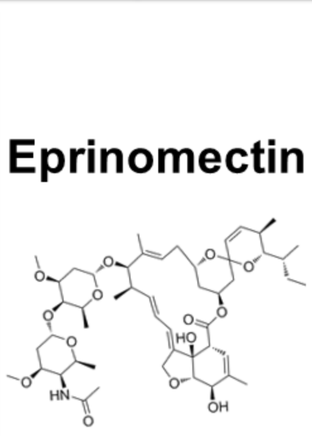


Fig 1. Pharmacokinetic profiles of eprinomectin formulations in lambs, illustrating plasma concentrations over a period of 336 hours post-treatment, demonstrating clear differences between pour-on and injectable administration routes.



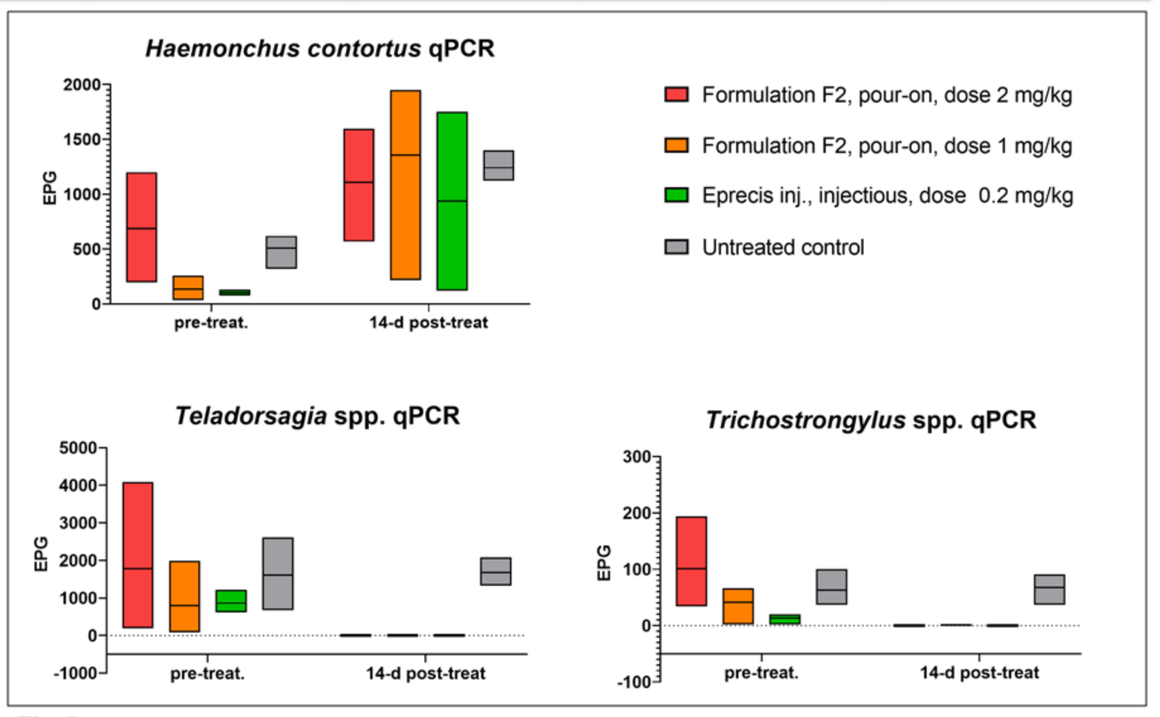


Fig 2. Real-time PCR quantification showing the efficacy of treatments against gastrointestinal nematode genera post-treatment. Results highlight the variable responses among parasite species, notably resistance in *H. contortus*.

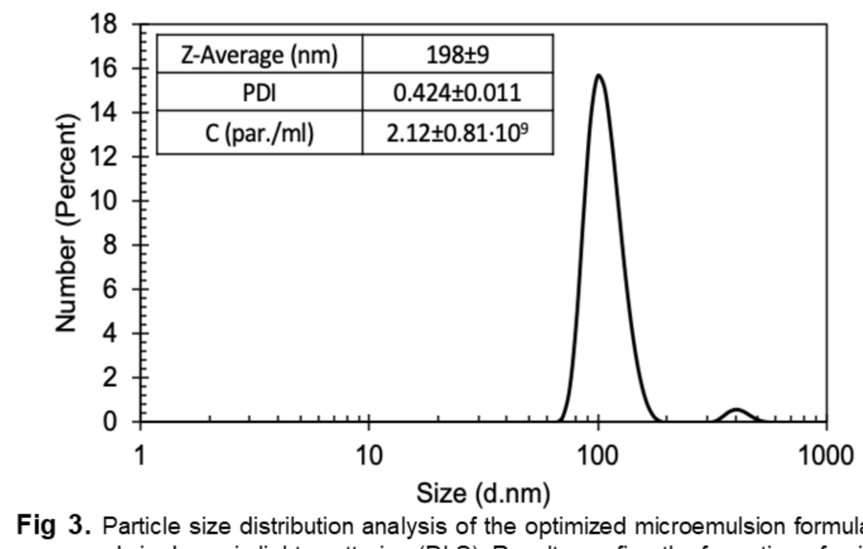


Fig 3. Particle size distribution analysis of the optimized microemulsion formulation measured via dynamic light scattering (DLS). Results confirm the formation of uniform nanoparticles (~200 nm) critical for enhanced transdermal delivery.

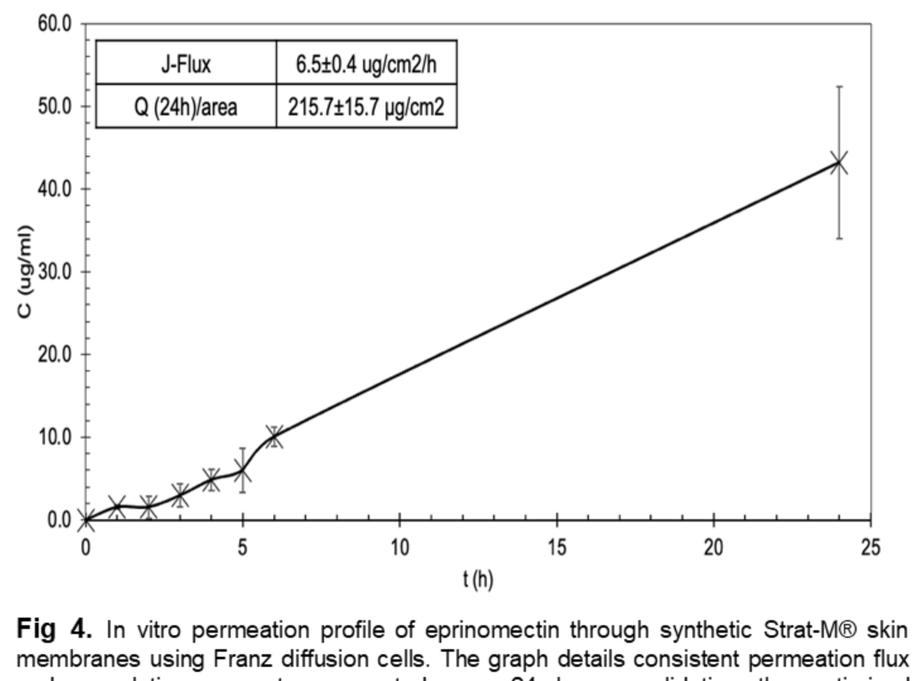


Fig 4. In vitro permeation profile of eprinomectin through synthetic Strat-M® skin membranes using Franz diffusion cells. The graph details consistent permeation flux and cumulative amounts permeated over 24 hours, validating the optimized microemulsion's transdermal efficacy.

Material and Methods

Seventeen different microemulsion systems were initially tested to optimize the transdermal delivery of eprinomectin in sheep. Each formulation consisted of a carefully balanced combination of surfactants, co-surfactants, penetration enhancers, and an oil phase. Formulations were homogenized and characterized to achieve optimal particle size (~200 nm) and stability, confirmed through dynamic light scattering (DLS) (Fig. 3).

In vitro permeability studies were conducted using Franz diffusion cells with synthetic Strat-M® skin membranes. The donor compartment contained the microemulsion formulation with 5 mg/ml eprinomectin, while the acceptor compartment contained an ethanol-phosphate buffer solution (EtOH/PBS, 50:50 v/v). Permeation was monitored for 24 hours at 32°C, mimicking skin conditions. The concentration of eprinomectin in the acceptor compartment was measured by UV-VIS spectroscopy. Permeation flux values averaged 6.5 ± 0.4 µg/cm²/h, indicating consistent transdermal delivery (Fig. 4). The cumulative amount permeated after 24 hours reached 215.7 ± 15.7 µg/cm², confirming the effectiveness of the optimized formulation.

In animal experiment 1, two optimized EPRI pour-on formulations (F1, F2) were selected for an *in vivo* pharmacokinetic study in sheep (n=15). Suffolk lambs (3 months old) were randomly allocated into three groups: group one received the novel pour-on eprinomectin F1 formulation at 1 mg/kg, group two received the F2 formulation at 1 mg/kg topically, and the reference group received injectable eprinomectin (Eprecis) at 0.2 mg/kg subcutaneously. Blood samples were collected at 0 (pre-dose), 4, 8, 12, 24, 30, 36, 48, 54, 60, 72, 78, 96, 120, 144, 240, and 336 hours post-treatment. Plasma concentrations of eprinomectin were analyzed using LC-MS/MS (Agilent 6410 Triple Quad, ESI positive mode).

In animal experiment 2, sixteen Suffolk lambs (3 months old) from a South Moravian farm (Czech Republic), naturally infected with mixed gastrointestinal nematodes, were enrolled. *Haemonchus contortus* resistant to ivermectin and benzimidazoles had been previously reported in the flock. Lambs were randomly allocated into four groups: group one was treated with pour-on formulation F2 at 1 mg/kg, group two with the same formulation at 2 mg/kg, group three received injectable reference Eprecis at 0.2 mg/kg subcutaneously, and two lambs served as untreated controls. Efficacy was evaluated using fecal egg counts (FEC) and real-time PCR on days 0 and 14 post-treatment. Real-time PCR was performed according to Reslova *et al.* (2021) with some modifications, utilizing six genus-specific primer pairs and hydrolysis probes targeting conserved regions of gastrointestinal nematode DNA (*Haemonchus*, *Teladorsagia*, *Trichostrongylus*, *Chabertia*, *Nematodirus*, and *Ashworthius*). PCR conditions included initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Results were interpreted based on cycle threshold (Ct) values, and plasmid copy numbers were converted to eggs per gram (EPG) using a calibration curve.

Results

Pharmacokinetic analysis revealed Cmax values of 4.4 ng/ml (F1) and 4.8 ng/ml (F2), with AUC values of 667 and 735 ng·h/ml, respectively (Table and Fig. 1). The injectable reference exhibited up to 6.5 times higher Cmax but only 2.4 times higher AUC compared to the pour-on formulation F2. However, both pour-on F2 and injectable reference failed to eliminate naturally established populations of *H. contortus* in lambs (Animal experiment 2). Conversely, both EPRI pour-on formulations and injectable reference successfully controlled *Trichostrongylus* sp. and *Teladorsagia* sp., as confirmed by real-time PCR (Fig. 2).

References

Reslova, N., Skorpikova, L., Kyrianova, I.A. Vadlejch J., Höglund J., Skuce P., Kasny M. The identification and semi-quantitative assessment of gastrointestinal nematodes in faecal samples using multiplex real-time PCR assays. Parasites & Vectors 14, 391 (2021). http://doi.org/10.1186/s13071-021-04882-4

Acknowledgements

This research was supported by Technology Agency of Czechia, project Nr. **TN02010017**, and by MZe Czechia, project Nr. RO0523