Evaluation of the effect of paromomycin dosed at 150 mg/kg on commensal gut microbiota of healthy calves

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Objectives

The objective of this study was to assess the impact of paromomycin administered orally for 5 days, on the development of resistance in intestinal commensal flora of healthy calves.

Material and methods

Twenty-five healthy calves originating from 7 different French farms and aged 12-21 days at the start of the study were enrolled. Calves were housed collectively, fed with milk replacers twice a day and randomly allocated to treatment (Gabbrovet Multi®, Ceva Santé Animale, 150 mg/kg once a day for 5 days; n=23) or untreated control group (n=2) on Day 0. Calves' health was monitored daily based on faeces status, depression score, and appetite (0-2 scorings). At Day+37, calves were euthanized. Faecal samples before, during and after treatment with paromomycin were collected to trace the evolution of paromomycin susceptibility of commensal intestinal *Escherichia coli.* For this, fresh faeces of the animals were collected at Day-1, Day+4, Day+20 and Day+36 in a sterile 50 ml sample container then immediately stored at -80°C and later transported frozen to a microbiology laboratory for isolation and purification of commensal *E. coli* strains. Twenty purified and randomly isolated colonies of *E. coli* were recovered from every faecal sample of each collection time point for further microbiological analysis. Because of the very high number of strains, clusters of epidemiologically related strains were identified by mean of mass spectrometry (MaldiTof Biotyper Compass explorer software), then subjected to paromomycin MIC determination by mean of a customized microdilution approach (UMIC) and an aminoglycoside antibiogram in accordance with CLSI guidelines. Based on the CA-SFM breakpoint of kanamycin for Enterobacteriaceae, the evolution o resistance to paromomycin of the *E. coli* strains was monitored for every strain/sample by comparing the results obtained individually for each animal at different times during the study with those obtained prior to paromomycin administration.

Results

A total number of 1780 *E. coli* were isolated for further individual analysis. Before any treatment administration (Day-1), the commensal *E. coli* flora consisted in both susceptible clones (69% observed in 16 out of 23 calves) and clones already exhibiting resistance (31% observed in 13 out of 23 calves) to paromomycin. Treatment with paromomycin did not result in the development of resistance in primarily susceptible *E. coli.* Indeed, during this study, no isolated clones identified before or after treatment have acquired paromomycin resistance. In fact, paromomycin treatment eliminated a high proportion of the susceptible commensal *E. coli*, thus allowing pre-existing resistant commensal *E. coli* and pathogenic *E. coli* to take over and predominate in the intestinal microbiota. This rearrangement was transitory, and the selection pressure exerted by paromomycin to select resistant clones strongly decreased after the end of treatment between Day+20 and Day+36. This was indeed demonstrated by a significant reappearance of the commensal *E. coli* clones susceptible to paromomycin (40 % at Day+36 compared to 7 % at Day+20; 10 % at Day+4 and 69 % at Day-1, respectively).

Conclusions

During this study, no acquisition of resistance at the clonal level was observed in *E. coli* following paromomycin treatment. The susceptibility profile tracked for each clone of *E. coli* did not show any shift of the MIC between the sampling points. The treatment as expected disrupted the flora by preferentially selecting preexisting resistant clones capable of surviving. A gradual reversion to the initial conditions of the colibacillary flora was observed after paromomycin treatment discontinuation. The curative bactericidal high dosage of paromomycin used in this study has certainly contributed in preserving the strains from acquiring resistance. Particular attention should be taken when lower dosage regimen is used (*i.e.* prophylactic usage) as these have been documented for inducing more readily resistance.