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Monitoring the emergence of verotoxinogenic *Escherichia coli* (VTEC) during intraruminal infection of ewes with recombinant verotoxin-encoding bacteriophages

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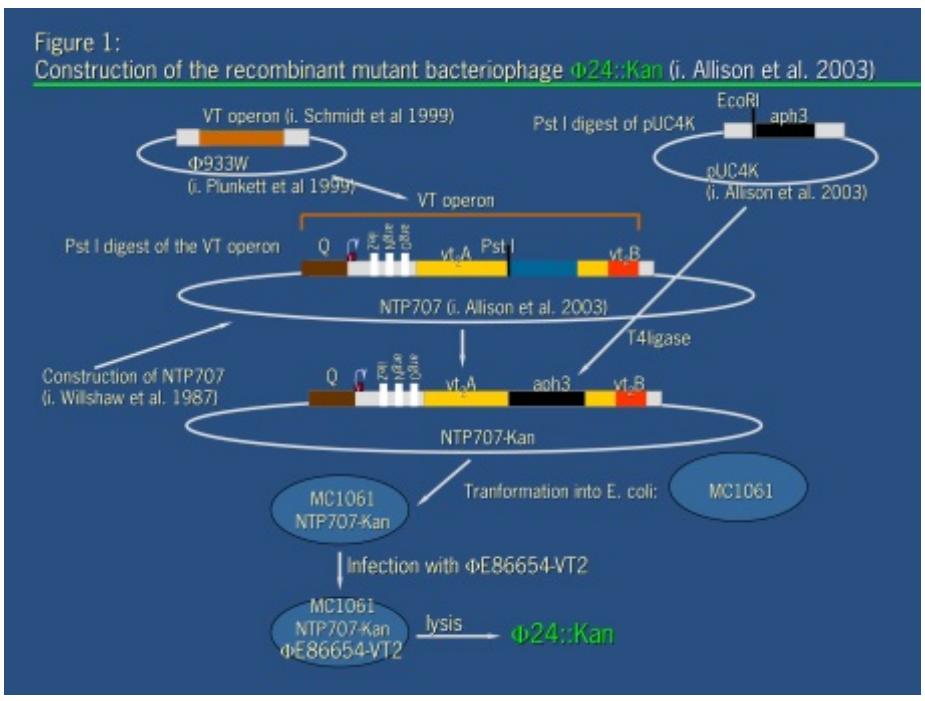
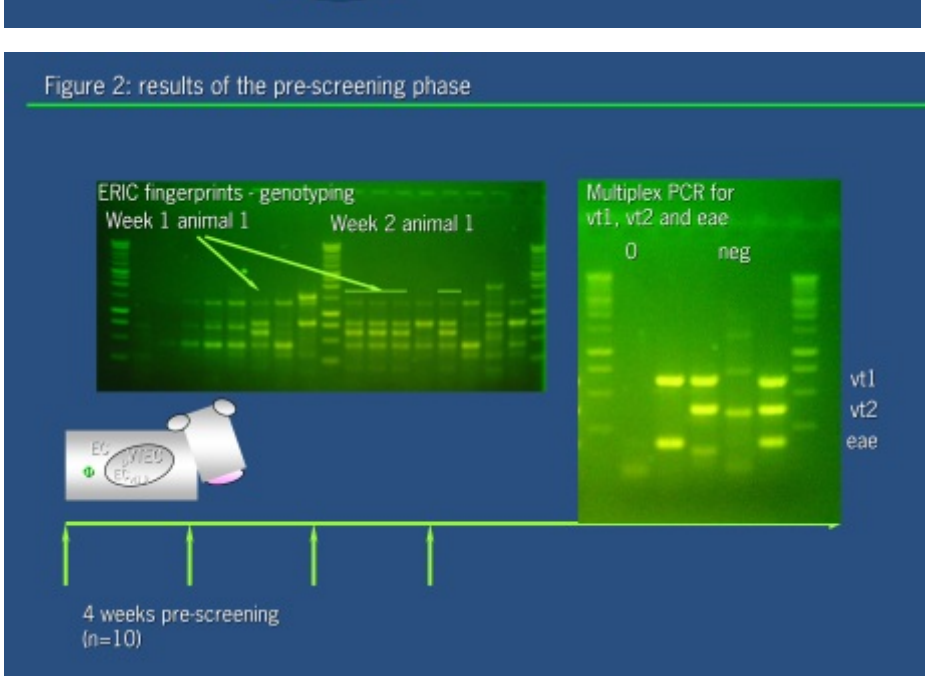
Introduction

Verotoxinogenic Escherichia coli (VTEC) cause outbreaks of gastrointestinal (GI) infections with (haemorrhagic) diarrhea in humans where 10% of cases can result in complications such as haemolytic uremic syndrom (HUS), thrombocytic thrombocytopenic purpura (TTP) and death. Ruminants are considered to be one of the main reservoirs of human pathogenic VTEC and bacteriophages are hypothesized to transfer verotoxin genes between bacteria in the ruminal fluid.

Materials and Methods

Ten ewes were monitored weekly for VTEC in faeces for four weeks and screened for kanamycin resistant E. coli, followed by intraruminal infection of 5 ewes using a recombinant verotoxin encoding bacteriophage Φ24::Kan (Allison et al. 2003, figure 1) at about 100 to 1000 phages/cfu ruminal E. coli. The infected sheep were sampled daily for 15 days post infection and 5 E. coli isolates per sample were genotyped using ERIC fingerprints. In addition, the five isolates were screened for verotoxin 1 and 2 (vt1 and vt2) and intimin (eae) genes using a multiplex PCR (v/d Giessen et al. 2003).

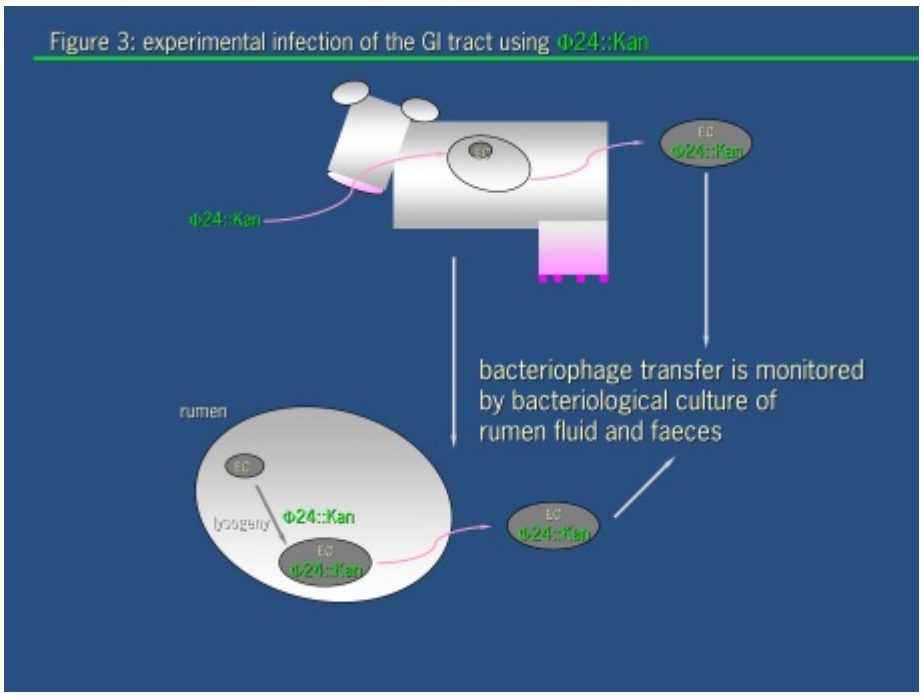


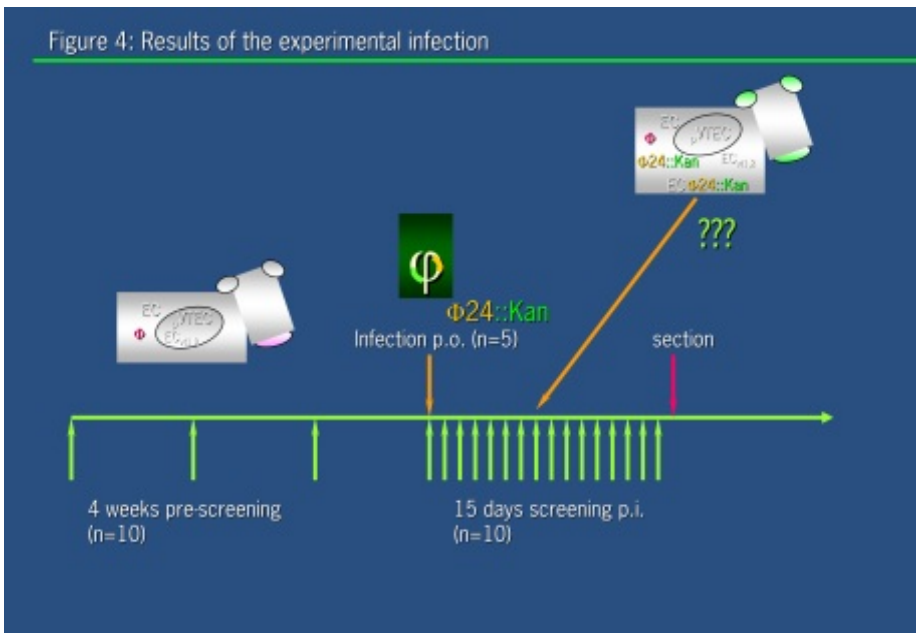


Results and Discussion

More than 1300 E. coli isolates were genotyped and screened for vt1, vt2 and eae during the experiment. Most animals had genotypes of E. coli and VTEC that could be isolated repeatedly from the ruminal fluid and/or the faeces over time (figure 2). If lysogeny of the bacteriophage genome into an E. coli had been detected, a kanamycin resistant vt2 encoding E. coli strain could have been isolated (figure 3). The 30 kanamycin resistant E. coli isolates detected in 1 ewe on day 11 of the experiment (3 days p.i.) were negative for wild-type vt2 and no more candidates for recombinant E. coli were detected after day 3 p.i. (figure 4). Those 30 isolates are in the process of being screened for the recombinant gene of phage Φ24::Kan. A failure to detect recombinant E. coli in either ruminal fluid nor faeces could be due to detection limits, too low dose of infection with recombinant bacteriophages or unstable lysogens in the GI tracts of the ewes.

Infection experiments with recombinant traceable bacteriophages will be continued with the aim of modelling the evolutionary dynamics of verotoxinogenic E. coli in the ruminant GI.





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