COMPARISON OF IMMUNE RESPONSES OF SHEEP AGAINST *E. coli* O157:H7 IN A RECTAL AND AN ORAL INOCULATION MODEL

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*Escherichia coli* (*E. coli*) O157:H7 causes haemorrhagic colitis and haemolytic uremic syndrome in humans. Ruminants are the main reservoir for this bacterium, excreting the bacteria sometimes for months without clinical symptoms. The reason for this persistence is still unclear, although it has been suggested that *E. coli* O157:H7 can suppress the immune system. To investigate the effects on the immune system of ruminants, a reliable infection model is needed that mimics the natural, long-term infection as it occurs in both sheep and cattle. Two models were developed in our lab: a rectal and an oral inoculation model. 10-week-old sheep were administrered a dose of $10^{10}$ CFU of a Shiga-toxin negative *E. coli* O157:H7 strain, on 2 consecutive days, either rectally via a sponge or orally via a syringe. Rectal inoculation led to a shedding pattern of 48-78 days, whereas oral inoculation resulted in shedding of 13-30 days. After cessation of shedding, the sheep were re-inoculated similarly (either rectally or orally) and faecal excretion was monitored again. After rectal re-inoculation, shedding occurred for a shorter period than after the first inoculation (9-33 days), whereas after oral re-inoculation shedding persisted longer (28-69 days). To determine the site of persistence of bacteria after both inoculation routes, one sheep was euthanized for each experiment. After rectal inoculation, the inoculated strain was found only in colon, caecum and rectum. In contrast, after oral inoculation, the strain was detected in all the samples, from rumen to rectum. It is clear that both inoculation routes lead to a persistent infection, however with a different pattern. In both models, the immune response against 3 virulence factors of *E. coli* O157:H7 ( intimine, EspA and EspB) was analysed. Since the rectal re-inoculation resulted in a shorter shedding pattern, we hypothesized that this could be due to acquired immunity. However, a clear serum antibody response after rectal inoculation or re-inoculation could not be shown. In contrast after oral inoculation, a clear immune response occurred with serum antibody levels first increasing, but later on decreasing as shedding disappeared. After oral re-inoculation antibodies increased again. Also a cellular immune response could be seen after oral infection and re-infection as evidenced by lymphocyte proliferation. Furthermore, IFN-γ was quantified by ELISA. Results indicated a negative correlation between IFN-γ production and the antigen-specific antibody responses.

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