

Characterization of F5(K99) and F41 fimbria present on *E. coli* used in a multivalent cattle vaccine and its associated immunological responses in pregnant cows.

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INTRODUCTION

Neonatal calf diarrhea is an important cause of morbidity and mortality worldwide. Enterotoxigenic *Escherichia coli* (ETEC), rotavirus, coronavirus, and *Cryptosporidium* are the four major pathogens associated with neonatal calf diarrhea. Almost all ETEC bacteria are known to adhere to receptors on the small intestinal epithelium by their fimbriae. The most observed fimbriae on ETEC from calves with diarrhea are F5(K99) and F41¹. These two fimbriae often occur together but may be present independently²⁻⁴.

OBJECTIVE

Characterization of the *E. coli* antigen used in a commercial neonatal calf diarrhea vaccine (Bovilis[®] Rotavec[®] Corona, MSD Animal Health). Study the presence of the F41 fimbriae on top of the already characterized F5(K99) fimbria and the associated immunological responses in serum and colostrum of vaccinated cows.

MATERIALS AND METHODS

In vitro characterization: The purified cell free *E. coli* antigen was analyzed using Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS) after digestion of the denatured, reduced, and alkylated proteins using trypsin. The reported peptides were identified using published *E. coli* protein sequences. Relative protein quantification was performed by calculating the sum of the intensities of the three most intense peptides of 13 independently produced batches of the *E. coli* antigen.

In vivo characterization (immunological responses in cows): One group of 21 pregnant cows was vaccinated with (Bovilis[®] Rotavec[®] Corona, MSD Animal Health) and one group of 19 pregnant cows served as unvaccinated control. All animals were blood sampled before vaccination and six weeks later. Colostrum samples were collected within 4 hours after calving (M1) and one day later (M2). Antibody titers in serum samples (D0 & D42) and colostrum (M1 & M2) were assessed by Elisa (in-house or BioX, K-295).

Purified cell free *E. coli* antigen in the multivalent enteric vaccine (Bovilis[®] Rotavec[®] Corona, MSD Animal Health) expresses both *E. coli* F5(K99) and F41 fimbriae. The ratio of both fimbriae was found constant in different antigen batches. After immunization of pregnant cows, a strong antibody response against both F5 and F41 is measured in the serum and colostrum of vaccinated animals.



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RESULTS

Identification of fimbriae and their reactivity in control and vaccinated animals

Ratio (0.7) of identified F5/F41 fimbriae is constant in different *E. coli* K99 antigen batches used for the formulation of the Bovilis[®] Rotavec[®] Corona vaccine. This is in line with published ratio of 0.5⁵.

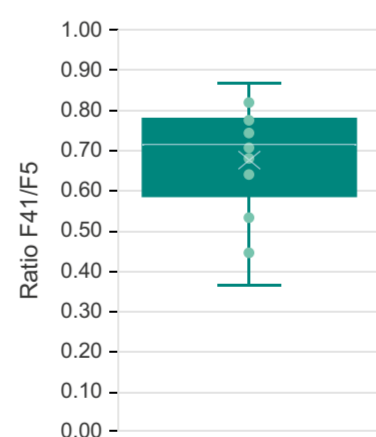
Six weeks (D42) after vaccination, serum antibody titers remained low / decreased in control animals, but significantly increased in the vaccinated animals.

Colostrum (M1 & M2) antibody titers against F5 and F41 were low in the control animals and were significantly higher in the vaccinated animals.

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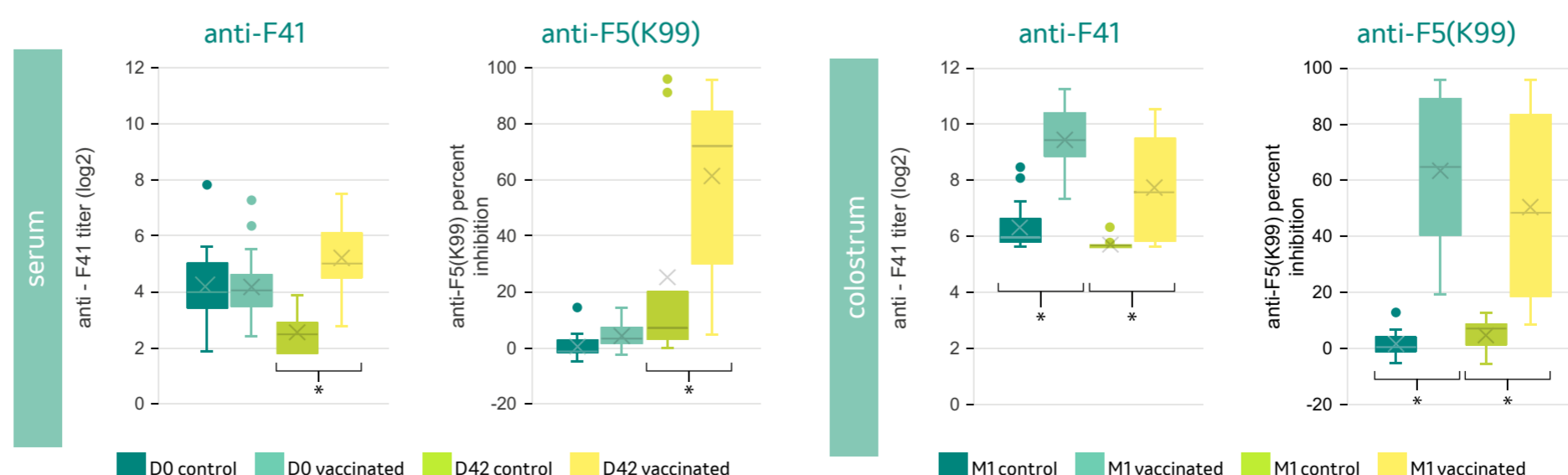
LC-MS/MS ratio F41/F5 of tested batches.



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F41 and F5(K99) antibody titers in serum and colostrum of vaccinated and control animals.



* Statistically significant (P<0.0001).