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Development of a sandwich ELISA for quantification of immunoglobulin G in mink blood

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Abstract

A major concern amongst the Danish mink farmers is the incidence of the syndrome pre-weaning diarrhea. The syndrome causes major management issues and decreases the welfare of the mink and increases mortality in the pre-weaning period. The etiology of the syndrome is considered multifactorial as a specific cause is not fully established or understood. Adding to an increased risk of developing pre-weaning diarrhea is the fact that the mink kits are born with very low levels of circulating immunoglobulins. Rapid achievement of high levels of immunoglobulins in the bloodstream is essential for the kits early immunity and thus their resistance against pathogenic agents found in the environment.

This study describes a sandwich ELISA for quantification of the concentration of total immunoglobulin G in mink blood. The ELISA was validated with serum samples from females (n=8) and their kits (litters of 4-12). Preliminary results show that the IgG concentration among kits from the same litter was similar, while litter to litter variation was high.

Keywords: mink serum IgG, ELISA, validation.

Introduction

The role of maternal immunity with regard to pre-weaning diarrhea has not been fully elucidated. One approach to getting closer to an understanding of the importance of the female mink immune system and its effect on the immune system of the mink kits is to take a closer look at the concentrations of total IgG in the kit serum. The mink kits are born with very low levels of IgG (Coe and Race, 1978). In this study we designed and validated an immunoglobulin class specific ELISA for mink IgG in order to quantify the levels in mink blood. As there are no commercially available mink ELISA kits or antibodies towards mink IgG we developed and validated a sandwich ELISA based on a commercially available goat anti ferret IgG antibody, which cross-reacts with Ig from mink (Martel and Aasted, 2009).

Material and methods

Animals:

8 female minks (*Neovision vision*) and their litters (n=4-12) were obtained from two commercial mink farms in Zeeland, Denmark. They were housed in separate cages and fed a standard mink diet.

Sample collection:

Peripheral blood samples were taken from the mink dams and kits. Clotted blood was centrifuged at 4000 G for 15 min at 4 °C. Serum was collected and stored at -20 °C prior to ELISA.

IgG purification

10 ml of the mink serum pool were passed through a column packed with 4 ml of Protein G Sepharose High Performance (GE Healthcare, Bio-Sciences, Uppsala, Sweden). The column was washed extensively with washing buffer (0.2 M NaOH, pH 8.8) and eluted with 0.1 M glycine/HCl, pH 2.8. Absorbance at 280 nm was determined on a Nanodrop

spectrophotometer and used to estimate the protein concentration of the eluted fractions, which were pooled and dialyzed against PBS overnight at 4°C. The resulting IgG pool was analyzed by SDS-PAGE (12 % Bis-Tris NuPAGE, Invitrogen, Carlsbad, California, United States) and stored at -20 °C prior to ELISA.

ELISA:

The optimal dilutions of the catching antibody and detection antibody were determined by checkerboard titration. The catching antibody was a commercially available goat anti-ferret IgG (Sigma-Aldrich), which cross-reacts with mink IgG (Martel and Aasted, 2009). This was diluted in 0.05 M carbonate buffer (pH 9.6) and then coated overnight at 4°C on a Maxisorp plate (Nunc, Roskilde, Denmark) at a concentration of 0.5 µg/ml. Wells were then emptied and washed 4 times and blocked with 1% Bovine Serum Albumin (BSA, Sigma-Aldrich, St. Louis, Missouri, United States) in PBST (PBS with 0.05% Tween 20) for 1 hour at room temperature with shaking. After 4 washes the serum samples and 2-fold dilutions of the mink IgG pool (used as standard and prepared as described above, 0.5 µg/ml) were diluted in acetate buffer (0.05 M, pH 5.5). The diluted samples and standard were added to the wells and incubated with shaking at room temperature for 1 hour prior to 4 washes. Horseradish peroxidase (HRP) conjugated goat anti-ferret IgG (Sigma-Aldrich, St. Louis, Missouri, United States) diluted in 1% BSA + PBST to the concentration of 1.25 µg/ml was added to the wells and incubated for 1 hour at room temperature with shaking and then washed 4 times. Then substrate TMB (Kem-En-Tec, Taastrup, Denmark) was added to the wells and when a suitable color development was observed the reaction was stopped using 0.5 M sulfuric acid. The optical density (OD) of wells was read at 450 nm, and unspecific coloration was subtracted at 650 nm using an automatic plate reader (Thermo Multiskan Ex spectrophotometer, Thermo Scientific, Waltham, MA, USA). All samples including standard were analyzed in duplicates. Sample values were calculated from the curve fitted to the

readings of the standard (using Ascent software v. 2.6, Thermo Scientific, Waltham, MA, USA).

SDS-PAGE

The eluted IgG fractions from the protein G purification was analyzed using SDS-PAGE on NuPAGE 12% Bis-Tris gels (Invitrogen, Carlsbad, California, United States) and the samples/bands were visualized using silver staining.

Results

Protein G Sepharose affinity chromatography was used to purify serum IgG (see materials and methods). The purity of this mink IgG preparation was demonstrated by SDS-PAGE comparing it to an existing purified mink IgG preparation (a kind gift from Bent Aasted, University of Copenhagen). We confirmed the molecular weight of mink IgG heavy chain and light chain to be 54 kDa and 25 kDa, respectively. The ELISA was thoroughly validated and had a lower limit of quantification at 0.008 µg/mL, a good reproducibility with low intra- and inter-assay variability, and was linear for serum samples within a relevant dynamic range. Our results using this sandwich ELISA indicated a within litter effect on the serum concentrations of IgG in mink kits.

Discussion

There are no commercially available mink IgG ELISA kits and no commercially available reagents with defined specificities against mink immunoglobulins. This study describes the development, validation and optimization of a sandwich ELISA for the quantification of the concentrations of total IgG in mink serum. Quantification of total IgG in serum will be very useful for estimating the immunological status of the mink dam and kits with respect to availability and transfer of maternal antibodies during the suckling period. The preliminary finding of within litter clustering of IgG concentrations indicates that within a litter all kits

obtain the same IgG serum concentrations, which may suggest that the maternal supply of IgG may be the determining factor in the efficiency of transfer of IgG from the mink female to her kits. Future uses of the ELISA include its use as a tool for predicting which female will be able to let their kits attain optimal serum IgG concentration within an optimal time window.

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