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Immunoglobulins in nasal secretions of dog puppies from birth to six weeks of age

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Abstract

In order to investigate local immune defence mechanisms in the dog, the concentration of immunoglobulins (Ig) G, A and M in nasal secretions (NS) and serum of 42 healthy, neonatal Rottweiler puppies was determined. Ig were measured with a commercially available, dog-specific ELISA during the first six weeks of life. On average, IgG was the predominant Ig isotype during the first three days of life. The IgA:IgG ratio changed between weeks 1 and 3 due to markedly decreasing IgG concentrations. Between the fourth and sixth week, IgG predominated again. During the first week, only 21–39% of puppies had measurable amounts of IgM in NS, in week 2, this percentage increased to 69%. Marked differences between litters and between individual puppies within litters were found. No puppy diseased during the observation period and all developed normally.

Keywords: Dog puppy; Immunoglobulins; Nasal secretions; Serum

1. Introduction

Quantitative analysis of immunoglobulins (Ig) in NS has not been performed in neonatal puppies before. The Ig isotypes have different functions and development of effective vaccines requires to understand the dynamics of endogenous Ig production and knowledge about which antigen stimulates the production of which Ig isotype.

In piglets, IgG is the predominant Ig in NS from the second week of life on. The predominance of IgA in NS during the first week apparently is caused by passively acquired and selectively transported Ig. In the piglet, mucosal IgA synthesis starts six weeks after birth. The predominance of IgG between weeks two and six is believed to be advantageous for the local defence against pseudorabies infections (Morgan and Bourne, 1981). In foals, IgGa and IgGb are the major Ig isotypes in NS during the first two weeks of life, whereas no IgA is present during this time period. It has been suggested that selective transfer of IgA from serum to NS does not take place, because mainly the monomeric form without secretory component is absorbed. In the foal, IgA is supposed to be synthesized locally after day 42, which is comparable to neonatal piglets (Sheoran et al., 2000). Concentrations of IgM in NS were not investigated, although IgM is the first antibody produced in the course of an immune reaction (Done, 1988). Canine secretory IgM (sIgM) has been isolated from bronchial secretions and colostrum of adult dogs (Thompson and Reynolds, 1977), but the concentrations in NS of neonatal dog puppies during the first six weeks of life have not yet been measured.

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In puppies, mortality during the first three weeks of life ranges between 7% and 34%, mainly due to septicaemia of the neonates during the first three days after birth (Mosier, 1981; Nelson and Couto, 1998; Dumon, 1998). Information about the Ig concentrations in the nasal mucosa of healthy puppies will help to understand immunological reactions during specific infections and may contribute to the design of effective vaccines. It was the aim of the present study to determine the distribution of IgG, IgA and IgM in NS of neonatal puppies from birth to six weeks of age, and to investigate the physiological development of local immune defence mechanisms.

2. Materials and methods

2.1. Animals and sampling

A total of 42 neonatal Rottweiler puppies from six litters were examined. All animals were from one kennel and bitches were aged between 2 and 5 years. One week before mating, bitches were clinically checked and vaccinated against distemper, infectious hepatitis, leptospirosis, and parvovirosis. Two weeks before expected parturition, they were transferred to the whelping boxes. The boxes were equally prepared and located in one room, with standardized room temperature and humidity (22 °C, 70%). Special consideration was given that disturbances and air draught were avoided, and no other dogs were introduced.

At 24, 48, 72 h and once weekly until the sixth week after birth, all puppies were checked clinically and milk samples from the bitches, as well as serum and swabs with NS from the puppies, were taken. As a direction of the military working dog breeding unit, serum from the puppies was collected only once at 24 or 48 h after parturition, during routine crop of the animals' tails. A plastic tube was used to collect several blood drops. Colostrum and milk samples were taken as mixed samples from all teats of each bitch, milked into a plastic tube. Mammary secretions after day three postpartum were considered milk. Serum was produced by centrifugation of blood at 1500*g* for 10 min. Milk without preservative and serum was frozen at −18 °C until analysis. NS were taken from the neonates' nostrils with sterile urethra swabs (Bacteriette®, Copan, Italy) by introducing them as far as possible into each nostril and gently rotating once. Swabs were placed into 500 µl of 0.9% sodium chloride immediately afterwards for 24 h at +4 °C. To recollect all fluids during centrifugation (1500*g*, 10 min), the swabs were removed from the fluids and fixed with the cap of the plastic tube before. The supernatant was frozen until analysis.

2.2. Laboratory analysis

Total protein (TP) and albumin concentrations in milk were measured by dry chemistry (Kodak Ektachem DT 60, Kodak, USA; analyzer range for TP: 20–110 mg/ml, for albumin: 10–60 mg/ml). In some of the NS samples, TP, U-CSF protein and albumin were measured by wet chemistry (Hitachi 911 Roche Diagnostics, Austria; analyzer range for TP in serum: 2–150 mg/ml, for albumin in serum: 10–70 mg/ml, for U-CSF protein in urine: 0.06–2.00 mg/ml).

Before ELISA measurements, milk was thawed at room temperature, diluted 1:100 with ELISA-sample diluent and ultracentrifuged at 200,000*g* for 30 min (Vaerman and Heremans, 1969). The fat-free whey was removed and further diluted with the same diluent. Serum and NS were thawed and diluted with ELISA-sample diluent (Whey:IgG: 1:1000, 1:10,000, 1:50,000, 1:100,000; IgA: 1:10,000, 1:40,000; IgM: 1:5000, 1:10,000; Serum: 1:10,000, 1:100,000 and NS: 1:5, 1:10, for all isotypes).

IgG, IgA and IgM concentrations in milk, serum and NS were measured with a commercially available ELISA (Dog IgA-, IgG- and IgM-Quantitation Kits, Bethyl Lab. Inc., Montgomery, TX, USA). Sensitivity was 15.6–500 ng/ml for IgG, 62.5–1000 ng/ml for IgA and 31–1000 ng/ml for IgM.

ELISA plates (96-well, Nunc-Immuno™ Plates, MaxiSorp, Cat. No 446612) were coated overnight with 100 µl of diluted capture antibody (sheep anti dog IgG, goat anti dog IgA or goat anti dog IgM, affinity purified, heavy chain specific, 10 µg/ml, +4 °C), then washed automatically with washing buffer (50 mM Tris buffered saline, 0.14 M sodium chloride, 0.05% Tween 20, pH 8.0; washer: Sunrise, Tecan, Grödig, Austria). A volume of 200 µl ELISA post coat solution (50 mM Tris buffered saline, 0.15 M sodium chloride, 1% bovine serum albumin, pH 8.0) was loaded in each well and the plates incubated for 1 h at 21 °C. After two washes, all standards and samples were titrated in duplicate and incubated for 1 h at 21 °C. After washing, each well was filled with conjugate (HRP-conjugated sheep anti dog IgG, goat anti dog IgA or goat anti dog IgM, heavy chain specific, working dilutions 1:50,000, 1:40,000 and 1:60,000 with 0.05% Tween 20 in post coat solution, pH 8.0; incubation: 1 h, 21 °C). To achieve an optimal standard curve, the concentration of the IgA-HRP conjugate had to be doubled (2 µl/5 ml conjugate diluent) in comparison to the manufacturer's recommendations (Fig. 1).

After two washings, 100 µl of TMB peroxidase substrate (Kirkegaard and Perry Lab., Gaithersburg, MD, USA) per well was left for 10 min, before the reaction was stopped with 100 µl of 2 M H_2SO_4. Optical density was measured with a spectrophotometer at 450 and 650 nm (Sunrise, Tecan, Grödig, Austria) and concentra-