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THE LEVELS OF IMMUNE COMPLEXES IN THE BLOOD SERA OF CALVES IN THE NEONATAL PERIOD AND IN ADULT CATTLE

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In this work immune complexes (IC) in the sera of healthy calves from birth to four months of age, heifers and cows were investigated. Polyethylen glycol (PEG) precipitation assay was applied after previous standardization. Optical density (OD) of redissolved precipitates, formed after adding PEG to serum samples, was a measure of IC level. The upper-limit values for normal IC levels were established using isolated cattle IgG in different amounts and aggregated by heat as an IC model in vitro, in the same assay. In newborn calves before colostrum intake, OD of 0.047±0.024 was registered in redissolved PEG precipitates, far below the values obtained for native, monomeric IgG. Therefore, these values can not be considered to refer to immune complexes. After colostrum intake, OD values for normal IC levels were higher, with significant interindividual differences: in calves at 6, 24 and 48 hours after birth the mean OD values of solubilized PEG precipitates were 0.069±0.025, 0.148±0.079, and 0.136±0.062 respectively. In the sera of calves 10 days after birth mean OD value was slightly lower, 0.089±0.053, whereas in the serum of 1, 2, 3 and 4 month old calves normal levels of IC rose to 0.165±0.067, 0.157±0.080, 0,231±0,124 and 0.261±0.092 respectively. OD values for IC levels for adult animals were found to be 0,456±0,150 for heifers, and 0,183±0,031 for cows. Together, the obtained data may be included in a reference values system for normal levels of IC at different ages, in relation to which the IC levels under pathological conditions could be estimated.

Key words: immune complexes, IgG, calves, PEG assay

INTRODUCTION

The formation of antigen-antibody complexes, or immune complexes (IC), in the circulation (CIC) is a normal physiological event in antigen elimination. CIC have been found in numerous conditions, either in health or in disease, both in man and in different animal species (Schifferli and Taylor, 1989; Aiello, 2003). The immune system of newborn calves encounters numerous antigens which can originate from the environment, food or commensal bacteria in the digestive system (Tizard, 1996). Early in their life, these animals are protected by maternal

immunoglobulins (antibodies) passively transferred from colostrum, and later by antibodies endogenously produced (Weaver *et al*., 2000). Irrespective of their origin, immunoglobulin isotypic variants, particularly IgG subclasses, are differently distributed in the calves sera (Butler, 1986), and their capacity to form immune complexes has not been appreciated yet. It, however, might be of relevance since immune complexes can act as stimulators or inhibitors of various immune reactions, by virtue of their capacities to interact (via immunoglobulin component in IC) with B and T lymphocytes, as well as with many other cells of the immune system (Theophilopoulos and Dixon, 1979; Abbas i Lichtman, 2002). Transfered from the colostrum to the calves' blood serum, immune complexes have been found to express immunosuppressive features (Kilshow and Slade, 1981; Brzezinska-Slebodzinska and Slebodzinski, 1982; Morgan *et al*., 1989).

The aim of this work was to investigate whether and when immune complexes appear in the blood sera of calves, from birth to four months of age. Heifers and cows were also included in the study. For IC detection a test was used (Haškova *et al*., 1978), which is based on selective precipitation of immune complexes in the presence of high-molecular polyethylene glycol (PEG test).

MATERIAL AND METHODS

Animals. Thirteen healthy Holstein-Friesian calves from birth to 4 months of age, nine heifers (12-18 months of age) and nine cows (one month after calving) from the farm “Kovilo 2”, PK Belgrade. were included in this study.

Blood samples. Blood was taken from the jugular vein of the calves immediately after birth, before colostrum intake (0 hrs), and then 6, 24, and 48 hours after birth, and at 10 days, 1, 2, 3 and 4 months of age. Blood was collected from heifers, 12-18 months old and from 9 cows one month after calving. Serum was separated after spontaneous coagulation of blood at room temperature, and centrifugation at 3000 rpm.

Immune complexes detection. Immune complexes were detected by polyethylene glycol (PEG) precipitation assay (Nikolić *et al*., 1981). PEG (MW 6000, 3% w/v) was added to the serum samples, and after 2 hours of incubation at 4^{o}C, the precipitated proteins were redissolved in distilled water. Optical density (OD) of the redissolved PEG precipitate was read at 350 nm, with a Ultrospec 3300 spectrophotometer (Amershem Bioscience).

Standardization of the procedure for IC detection. Heat aggregated bovine serum IgG was used as IC standard. IgG was isolated from a mixture of bovine sera by Rivanol/Ammonium sulphate method (Heide and Haupt, 1964). Rivanol soluble IgG in different concentrations (0.150-5.0 g/L of range) was aggregated by exposing the samples to a temperature of 63^{o}C for 10 minutes (Nikolić *et al*., 1983). Aggregates were precipitated by 3% PEG, under the conditions applied for IC detection. The optical density (OD) values of redissolved precipitates were used for the standard curve construction. Native isolated IgG in different concentrations served as a control.

Statistical analysis. The results are expressed as the mean and standard deviation, for each group of calves.

RESULTS

Standardization of PEG test system. The relationship between OD of redissolved PEG precipitates of heat aggregated IgG (AGG) and IgG concentration in the heated samples is shown in Figure 1. Higher concentration of IgG in the heated samples was shown to be followed by the higher levels of AGG precipitated by PEG. Such linearity was not observed in the native, non-aggregated IgG. The OD value of 0.605 (0.3 g/L AGG), calculated as the mean of all AGG and native IgG OD values, was considered as an upper limit value for normal serum IC level. The reference interval for normal level of IC was 15 μg/mL to 300 μg/mL of AGG equivalents.

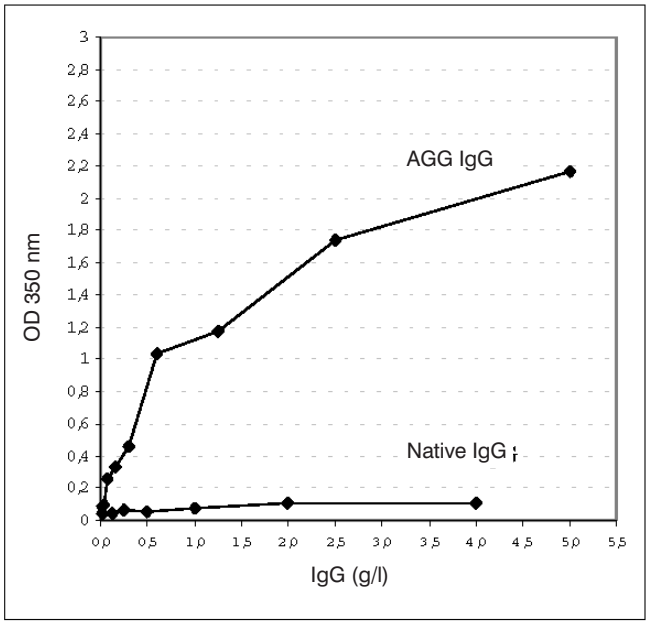

Figure 1. The effect of increasing amounts of IgG on IgG aggregates formation

PEG precipitated IC in calves sera. PEG precipitates were prepared from the sera of calves before colostrum intake (0 hour), and then at 6th, 24th, 48th hour, 10th day, 1, 2, 3 and 4 months of age. The results are shown in Figure 2. The OD values of redissolved PEG precipitates were extremely low for newborn calves before colostrum intake, and did not deviate from the values obtained for native, monomeric IgG. Therefore, the mean OD value (0.047±0.024) obtained for this group is not taken as to refer to immune complexes. In relation to this mean value, as well as to that of native IgG, the OD values of PEG precipitates were found to be higher for all other aged groups of calves. Although remained within the range of

AGG, OD values which reflect the normal levels of IC, protein content of PEG precipitates was shown to fluctuate: it increased slowly after colostrum intake, up to the 48th hour, fell on the 10th day, and then increased again, continuously, up to 4 months of age (Fig 2).

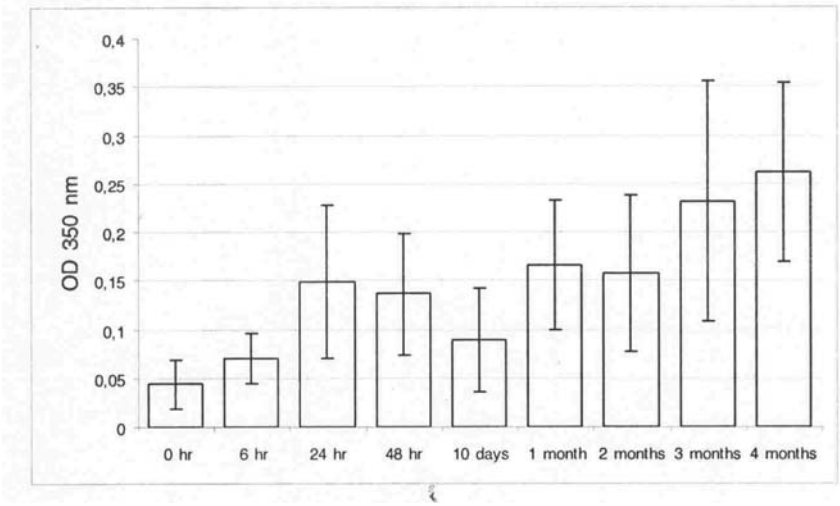

Figure 2. The level of IC, measured by the protein content of PEG precipitates, in the sera of calves from birth to four months of age

The highest level of PEG precipitable IC was detected in heifers, when compared with other groups. The IC levels in cow sera were shown to be lower than those found in heifers and calves of 1 to 4 months of age (Fig 3). The IC levels

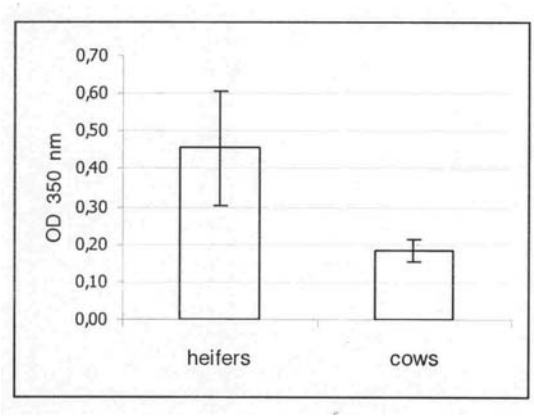

Figure 3. The level of IC, measured by the protein content of PEG precipitates, in the sera of heifers and cows

in both heifers and cows were below the values established as increased IC values in the control system. Thus, the obtained average values for the level of IC in the sera of adult animals can be considered as normal.

DISCUSSION

There is no single or universal method suitable for the identification of all sizes and all types of immunoglobulin components that make up the immune complexes participating in bovine immune reactions. We used PEG assay for the detection of IC in calves sera, because it allows both the quantification of CIC and the analyses of the composition of PEG precipitable material. PEG is known to precipitate high molecular weight IC, while only very small amounts, if any, of immunoglobulin monomers precipitate in the pellet. Due to the finding that calves' as well as cattle's IgG isolated from the serum and aggregated by heat were PEG precipitable, we considered PEG assay as adequate for the detection of immuno-complexed IgG, at different time points during the early life of calves, and in adult animals.

The data obtained in this work showed that immediately after birth calves had not IC in their sera. The OD values of solubilized PEG precipitates were below the values obtained for normal, non-aggregated IgG, and can not be considered as to refer to IC. The immunochemical analyses of PEG precipitates, which did not reveal any fraction reactive with anti-IgG antibodies (data not shown), confirm that newborn calves have not immuno-complexed IgG. Due to the fact that newborn calves are agammaglobulinemic, this finding seems to be logical. Neverthelees, the OD values of PEG precipitates obtained for this group of calves (mean$\pm$2SD) may be perhaps used as a parameter for comparison in a situation when increased values appear. Such values in calves' sera before colostrum intake can be viewed as a sign of intrauterine infection, and a parameter of clinical importance (Fratrić *et al*., 1999).

OD values of PEG precipitates increased readily after colostrum ingestion, and rose continuously up to the 48th hour, suggesting the possible appearance of the IC in the calves' sera. The finding that the changes in the level of PEG precipitable serum proteins coincided with colostrum intake may be due to IC passively transferred by milk (Kilshaw i Slade, 1981; Archambault *et al*., 1988, Sato *et al*., 1990; Prosser *et al*., 1992). The possible significance of IC aquired in such a way is unclear. It has been supposed that IC composed of milk proteins can have an immunoregulatory function acting as specific immunosuppressors. The transitory autoimmunity to milk proteins and the transfer of milk protein IC from mother to offspring are considered to be a normal process in cattle (Kilshow and Slade, 1981).

On the 10th day, IC level measured by the protein content of PEG precipitates fell to values below those achieved during the colostral period. The decrease in CIC levels may be due to the catabolism of immunoglobulins or to a limited transport of milk proteins through the gut, in this period (Prosser *et al*., 1992; Goldman, 1993; Korhonen *et al*., 2000). This may be as well related to bacterial passage through the gut wall (Logan *et al*., 1974). Hence, colostrum can

represent in calves a "mechanical" barrier causing early gut closure (Jochims *et al*., 1994; Arthington *et al*., 2000). A trend of continuous increase in IC levels was evident in calves between 1–4 months of age, with the highest reached values during the 4th month. However, those values were far below the values previously established as increased. Therefore, they can be considered as referent physiological values for IC levels in this age group of calves. The fact that these values were higher when compared with other age groups of calves is most probably due to the capability of the organism at that age to produce antibodies in response to different antigens. Our data (not shown) on the presence of IgG in PEG precipitates suggest that the obtained OD values are probably due to IC formed by immunoglobulins produced by the calves themselves. Others found a positive correlation between the level of IC and some of immunological parameters (serum immunoglobulin levels, autoantibodies etc) in the calves (Kishaw and Slade, 1981; Heckert *et al*., 1991).

The OD values for PEG precipitable IC in heifers and cows were below the values established as a lower limit value for increased IC level in the control system, and can be considered as physiological values for IC in these animals. These values were, however, significantly higher in heifers than in cows. Heifers have been found to have much higher levels of CIC than humans (Nikolić *et al*.,1981). The reason for this phenomenon is not clear. Hormonal changes which occur in this age period may affect the activity of the immune system, hence the possibility of a transient increase or drop of the immune reactivity. The level, size and composition of IC may change with time, following the distribution dynamics of different immunoglobulin isotypes, thus influencing the results of the assays used for IC detection.

There are a number of ruminant diseases (infectious, autoimmune, neoplastic) that could be associated with the elevated levels of pathological immune complexes in biological fluids. The detection of such complexes needs an assay practical for clinical laboratories. The results of our study indicate that PEG assay has a potential for detecting IC in the sera of calves and adult cattle. The assay enabled the reference intervals for IC (measured after precipitation by PEG) under physiological conditions to be established. However, due to the fact that PEG precipitates IC which have been found to be mainly heavy (>19S) or intermediate (8 to 19S) in size, smaller IC could remain out of the assay detection range.

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IMUNOKOMPLEKSI U KRVNOM SERUMU TELADI U NEONATALNOM PERIODU I KOD ODRASLIH GOVEDA

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SADRŽAJ

U radu je ispitivan nivo imunokompleksa (IC) u krvnom serumu teladi od rođenja do četiri meseca starosti, kao i kod junica i krava. Za analize je posle predhodne standardizacije korišćen polietilenglikol precipitacioni test (direktan PEG test). Meru nivoa IC predstavljale su vrednosti optičke gustine (OD) rastvorenog precipitata dobijenog nakon taloženja imunokompleksa iz seruma PEG-om. Gornja granica normalnih vrednosti za nivo IC utvrđena je prema vrednostima dobijenim za izolovane i toplotom agregirane kravlje IgG, u istom testu, gde su takvi IgG korišćeni kao modeli IC in vitro. U grupi novorođene teladi pre uzimanja kolostruma vrednosti OD su bile ekstremno niske (OD 0,047±0.024) i nisu mogle biti pripisane imunokompleksima. Vrednosti OD za normalan nivo IC u serumu teladi nakon uzimanja kolostruma su bile više: za telad 6, 24 i 48 sati nakon rođenja taj nivo je iznosio 0,069±0,025, 0,148±0,079 i 0,136±0,062 respektivno. U odnosu na ove vrednosti, nivo IC u serumu teladi 10 dana od rođenja je bio nešto niži i iznosio 0,089±0,053, da bi se, zatim, kontinuirano povećavao kod teladi uzrasta od 1 do 4 meseca i iznosio 0,165±0,067 za telad od 1-og meseca, 0,157±0,080 za telad od 2 meseca, 0,231±0,124 za telad od 3 meseca i 0,261±0,092 za telad od 4 meseca starosti. Nivo imunokompleksa u serumu odraslih goveda iznosio je kod junica 0,456±0,150 i krava 0,183±0,031. Dobijeni rezultati mogu biti uključeni u kontrolni sistem vrednosti za normalan nivo IC u raznim starosnim grupama goveda, koji se može koristiti pri određivanju nivoa IC PEG testom pod patološkim uslovima kod teladi, junica i krava.