Serum and colostrum/milk alkaline phosphatase activities in the determination of passive transfer status in healthy lambs

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SUMMARY

The aim of this study was to evaluate the importance of serum and colostrum/milk alkaline phosphatase (ALP) enzyme activity in the determination of passive transfer status in healthy lambs. Thirty Akkaraman sheep (3-6 years old) which had normal pregnancy period and their 0 to 15 days old lambs (n=30) were used.

Blood and colostrum/milk samples were collected from sheep and lambs after birth, before suckling (0 Day) and at the 1st, 3rd, 7th and 15th day. Serum IgG concentration was determined by the use of Single Radial Immunodiffusion (SRID) method. Serum ALP activity was measured, using a colorimetric kit. Correlations were carried out between immunoglobulin concentrations and ALP activities. Regression models (simple and multiple) were calculated.

In lambs, although positive correlations were obtained between ALP activities and IgG concentrations in serum at Days 1, 3 and 7 (r = 0.689, p < 0.01, r = 0.464, p < 0.05 and r = 0.413, p < 0.05 respectively), the variations of the 2 parameters showed marked discrepancies during the experiment: the ALP activity was maximum at day 0 whereas IgG concentrations were very low and from day 0 to day 15, ALP activity rose up when IgG concentrations continued to decrease. From day 0 to day 7, ALP activities decreased in colostrum/milk whereas IgG concentrations were stable. Positive correlations between these 2 variables were only found on days 1 and 3 (r = 0.404, p < 0.05 and r = 0.580, p < 0.01 respectively).

Consequently, ALP activities were not strictly correlated with IgG concentrations nor in colostrum/milk either in lamb serum, and multiple regression models were not really suitable to calculate IgG concentrations. ALP activity is not suitable for accurately predicting lamb IgG status and eventual failure for immune passive transfer.

KEY-WORDS : IgG, ALP, lamb, milk/colostrum, serum.

RÉSUMÉ

Activités des phosphatases alcalines dans le sérum, le colostrum et le lait et détermination du transfert d’immunité passive chez les agneaux sains. Par M. MADEN, FM. BIRDANE, V. ALTUNOK et S. DERE.

Le but de cette étude était d’évaluer la pertinence de la mesure des activités enzymatiques des phosphatases alcalines (PAL) dans le sérum et le colostrum ou le lait pour apprécier la transmission passive de l’immunité chez des agneaux sains. Trente brebis Akkaraman âgées de 3 à 6 ans qui ont présenté une gestation normale et leurs agneaux de 0 à 15 jours ont été utilisés dans cette étude. Les échantillons sanguins et lactés ont été recueillis chez les brebis et chez les agneaux après la naissance, juste avant la première tétée (J0) et aux 1er, 3ème, 7ème et 15ème jours. Les concentrations sériques d’IgG ont été déterminées par une méthode d’immunodiffusion radiale simple (SRID) et les activités PAL ont été mesurées par colorimétrie. Les corrélations ont été établies entre les concentrations d’IgG et les activités enzymatiques et les modèles de régression correspondants ont été calculés.

Chez les agneaux, bien que les activités PAL et les concentrations d’IgG aient montré des corrélations significatives positives dans le sérum à J1, J3 et J7 (r = 0.689, p < 0.01, r = 0.464, p < 0.05 et r = 0.413, p < 0.05 respectivement), les cinétiques de ces 2 paramètres présentaient des différences notables au cours de l’expérimentation : les activités PAL étaient maximales à J0 alors que les concentrations d’IgG étaient très basses et du 7ème au 15ème jour, les activités PAL remontaient tandis que les concentrations d’IgG continuaient à diminuer. De J0 à J7, les activités PAL diminuaient dans le colostrum et le lait tandis que les concentrations d’IgG restaient stables. Des corrélations positives (r = 0.404, p < 0.05 et r = 0.580, p < 0.01) n’ont été obtenues entre ces 2 variables qu’à J1 et J3.

En conséquence, les activités PAL n’étaient pas strictement corrélées avec les concentrations d’IgG ni dans les échantillons lactés, ni dans le sérum des agneaux, et les modèles de régression multiple n’ont pas permis de calculer les concentrations d’IgG. L’activité PAL n’est donc pas suffisamment fiable pour prévoir avec précision le statut en IgG des agneaux et pour déceler une éventuelle insuffisance du transfert d’immunité passive.

MOTS-CLÉS : IgG, PAL, agneau, lait/colostrum, sérum.

Introduction

The importance of colostral passive transfer of immunoglobulin is well established for ruminants. Ruminants are characterized by the possession of a thick syndesmochorial placentation that prevents the in utero transfer of large molecular weight immunoglobulins. These species are essentially agammaglobulinemic at birth and require for passive defense ingestion and subsequent absorption of colostrum that is rich in antibodies and non antibody immune factors [1-3, 8].

Consequently, serum immunoglobulin concentration is an important indicator of failure of passive transfer (FPT).

Several methods are available to detect FPT in calves and lambs. These tests include evaluation of total serum protein concentrations by refractometry, zinc sulfate and sodium sulfite turbidity assays, quantification of serum IgG concentration by radial immunodiffusion, and a commercially available serum gluteraldehyde coagulation test. All of these tests vary in accuracy, cost, and adaptability to veterinary practice [2, 3, 5-8, 13, 15-19].

Some studies [2, 9, 14-16] reported usefulness of serum gamma-glutamyl transferase (G GT) enzyme activity as an indicator of FPT in calves and lambs. It was suggested that serum GGT enzyme activities were 140 fold higher in lambs...
which suckled than in healthy adult sheep, and that GGT enzyme activity was 470 fold higher in colostrums than in adult sheep serum. Furthermore, there is a positive correlation between neonatal serum immunoglobulin concentrations and GGT enzyme activities [3], and GGT enzyme activities have been proposed to evaluate passive transfer status [9, 14, 16]. On the other hand, analysis of hepatic enzyme activities in sera from 1 to 3 days old pups revealed that alkaline phosphatase (ALP) activities and GGT activities were considerably higher than activities in healthy adult dog sera (30 fold and 100 fold respectively) [4]. Significant differences were found in serum GGT and ALP activities between colostrum-deprived and suckling pups before initial feeding. In addition, Lombardi and et al. [11] suggested that colostral enzymes can be used for the evaluation of buffalo colostrum quality.

The aim of this study was to evaluate the relationships between ALP enzyme activity and IgG concentration in blood and colostrum for the determination of passive transfer status in lambs.

Material and Methods

ANIMALS

In this study, 30 Akkaraman sheep (3-6 years old) and their lambs (n = 30, 0-15 days old, 11 female, 19 male lamb) were used. Their pregnancy period was normal and females were in average 2nd-5th gestations. All sheep were bred in stall in the same farm. The average size of the litter was 1-2 lambs by pregnant female in this farm.

SAMPLE COLLECTION

Blood and colostrum/milk samples were collected from sheep and lambs after birth prior to suckling (0 Day), and on the 1st, 3rd, 7th and 15th days of the experimental study. Blood samples were taken by jugular veni-puncture with vacutainer tube (Venoject®, Terumo Corp. Belgium), and colostrum/milk samples were taken to the sterile tube.

ANALYSIS OF BLOOD AND KOLOSTRUM/MILK SAMPLES

Blood and colostrum/milk samples were immediately centrifuged at 1559 g for 30 minutes (4°C) and blood serum was separated from the packed cells. After first centrifugation in colostrum/milk samples, fat and sediment were removed, supernatant was then centrifuged at 1559 g for 30 minute (+4°C) and colostrum/milk serum was separated. All serum samples were stored at -20°C until analysis. Blood and colostrum/milk immunoglobulin G (IgG) concentrations were measured by Single Radial Immunodiffusion (SRID) method according to manufacturer’s instructions (Bethyl Laboratories®, Inc.), and Alkaline phosphatase (ALP) enzyme activity were assayed spectrophotometrically (Shimadzu, UV-Vis 2100 Model) using a commercial enzyme kit (Biocon®).

STATISTICS

Data were analyzed by ANOVA and Tukey test (SPSS 8.0 for Windows, SPSS Inc., Illinois, USA) in order to detect statistically significant differences. Correlations were carried out between immunoglobulins and ALP activities. Regression models (simple and multiple) were calculated in statistically significant data. A probability value of < 0.05 was considered to be significant.

Results and Discussion

During the experiment, ALP activities (Table I, Figure 1) were always higher in lamb sera than in sheep sera (p < 0.05). Serum ALP activities were found to be decreased on the 3rd, 7th and 15th days of the study, in sheep. But these variations were not statistically significant from the enzyme activity at Day 0. On the contrary, decreases of serum ALP activities were significant in lambs on the 3rd and 7th days (p < 0.05). In colostrum/milk, ALP activities was significantly more elevated at Day 0 than in sera of sheep and lambs (p < 0.05), then the enzyme activity markedly decreased on 1st and 3rd days (p < 0.05), was minimum at Day 7 (p < 0.05) and rose up again (Day 15) but was always below the initial activity (p < 0.05).

IgG concentrations in lambs (Table I, Figure 2) were very low at Day 0, then drastically increased at Day 1 (p < 0.05) and after progressively decreased from the 3rd to 15th days of the experiment (p < 0.05). In sheep (Figure 2), the IgG concentrations seemed to be stable, although a small and not significant reduction was noticed at Day 1. From Day 0 to day 3, the IgG concentrations in colostrum/milk were elevated and were markedly higher than in sera of sheep or lambs (p < 0.05). These concentrations significantly decreased on the 7th and 15th days of the experiment (p < 0.05) and became lower than serum IgG concentrations (p < 0.05) (Figure 2).

Correlations between ALP activities and IgG concentrations, simple and multiple linear regression models were given in Table II. In lambs, significant positive correlations were found between serum ALP activities and serum IgG concentrations on 1st, 3rd and 7th days of the experiment (r = 0.689, p < 0.01; r = 0.464, p < 0.05 and r = 0.413, p < 0.05 respectively). Despite these correlations and although IgG concentrations and ALP activities were decreased in lamb sera on 3rd and 7th days of the experiment the variations of these parameters showed some marked differences. Indeed, ALP activities were maximum at Day 0 whereas the IgG concentrations were very low. Furthermore, on 7th and 15th days, the serum IgG concentrations continued to decrease, while in the same time, serum ALP activities began to rise up. When the multiple linear regression model was used (Table II), a negative correlation seemed to occur between lamb IgG concentrations and ALP activities in serum. In sheep, no correlation was found between serum IgG concentrations and ALP activities in serum or in colostrum/milk. Nevertheless, at Day 1 and at Day 3, a positive and significant link in colostrum/milk was noticed between IgG concentrations and enzyme activity (r = 0.404, p < 0.05 and
As far as colostrum/milk was concerned, ALP activities progressively decreased from Day 0 to day 7 whereas IgG concentrations appeared to be stable. Consequently, positive and significant correlations between these 2 parameters were only obtained on Day 1 and Day 3, and multiple regression model using colostrum/milk

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**Table 1.** — ALP enzyme activities and IgG concentrations in blood and colostrum/milk serum samples in sheep and lambs after birth before suckling and until the 15th day after birth. Results are expressed as means (x) ± standard deviations, extreme values are reported in parenthesis.

**Table 2.** — Correlations of serum and colostrum / milk (col/milk) ALP enzyme activities and IgG concentrations and regression models in sheep and lambs after birth before suckling and until the 15th day after birth.
ALP activity and days as variables was not really suited to calculate the IgG concentrations (Table II). Moreover, positive correlations between colostrum/milk ALP activities and lamb serum IgG concentrations were noticed only on Day 1 and Day 7 ($r = 0.481$, $p < 0.05$ and $r = 0.543$, $p < 0.01$ respectively). Consequently, colostrum/milk ALP activity was not predictive of the IgG status in lamb. No correlation was found between IgG concentrations in colostrum/milk and serum ALP activities, suggesting that enzyme activity in lamb serum did not strictly depend for the colostrum/milk IgG supply.

All these results show that ALP activity is not strictly correlated with IgG concentrations nor in lamb serum either in colostrum/milk and, as a consequence, the biochemical marker cannot be considered as an indicator of lamb IgG status, and cannot be used to detect a failure in passive immune transfer. Besides, lambs have presented a very strong ALP activity in serum on day 0 before suckling, suggesting that serum ALP activity in lambs was greatly affected by other factors than immune passive transfer and colostrum supply. These factors affected serum ALP enzyme activity in newborn lambs should be researched in next studies. On the contrary, serum GGT activity was reported to be useful to assess passive transfer status of lambs [2, 9, 14, 16]. This procedure is inexpensive, rapid and readily available to many practitioners [16]. In addition, it has been reported that there was a direct correlation between serum IgG concentration and GGT enzyme activity [3], and that serum GGT activity was a reasonable marker for diagnosis of failure of passive transfer in individual lambs [3, 13]. MADEN et al. [12] evaluated serum and colostrum GGT enzyme activity and IgG concentration in lambs after birth prior to suckling (0 day) and on the 1st day of the experimental study. They found dramatic increases of GGT activities in colostrum (10 026.5 (2 200-22 870) U/l on day 1 vs. 3 605.6 (1 000-6 865) U/l on day 0) and in serum (11 750.3 (790-25157) U/l on day 1 vs. 57.33 (23-93) U/l on day 0) whereas in sheep before and after parturition, serum GGT activities remained constant (60.50 (38-94) U/l and 64.04 (23-97) U/l respectively). These results suggested that GGT activity in lambs was strictly dependent of colostrum supply. In the same way, in 1 to 16 day old lambs, serum GGT activities and IgG concentrations ranged from 52 to 5 400 U/l and 64 to 4 936 U/l respectively [16].

Reference parameters of serum and colostrum ALP enzyme activities in lambs were limited. Normal values of serum ALP enzyme activity in sheep were 68-387 (178 ± 102) U/l [10]. GGT and ALP enzyme activities in goat colostrum reported in an another study [20] were 884 ± 395.8 U/l and 433.4 ± 260 U/l respectively. It was suggested that because of the occurrence of correlation between GGT and IgG in goat colostrum, GGT can be used as a marker for colostrum quality in goats. By contrast, no significant correlation was obtained between ALP enzyme activities and IgG concentrations in goat colostrum. Serum GGT and ALP enzyme activities were found to be higher in 1 to 3 day old pups than in healthy adult dogs [4]: significant increases in serum GGT and ALP activities were observed within 24 hours in suckling pups, but not in the colostrum-deprived pups. Consequently, these 2 enzyme activities could reflect immune status in suckling pups. But whereas GGT enzyme activity was reported to be also indicative of immune status of lambs, our results showed that ALP activity was not a good marker for evaluating immune status in lambs.

As a conclusion, ALP activity is not suitable for accurately predicting lamb IgG status and eventual failure for immune passive transfer. Regression models were not really suited to calculate IgG concentrations.

**References**


