

Immunoglobulin-G status of camels during six months *post-natum*

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Key words

Dromedary - Young animal - Immunoglobulin - Colostrum - Kenya.

Summary

The immunoglobulin-G (IgG) status of camel calves from birth until six months of age was investigated in a natural herding situation on a livestock ranch in Kenya. Camel IgG was quantified *post-natum* (pn) up to six months of age by indirect antibody ELISA in serum of 68 camel calves. First colostrum intake was observed on average at 3.6 ± 3.0 h pn and the average precolostral serum IgG concentration was 0.26 ± 0.23 mg/ml (range 0-1.07 mg/ml). The average maximum IgG concentration (IgG peak) in calf serum was 21.1 ± 11.7 mg/ml. Most calves had IgG peaks between 18 and 30 h pn, but 19% of the calves had later peaks (30-66 h pn) with significantly lower concentrations. After the peak, serum IgG concentration declined, the half-life of maternally derived IgG in the newborn's circulation being 16.3 ± 8.5 d. The average minimum IgG concentration was 8.1 ± 3.3 mg/ml (range of 1.6-15.1 mg/ml) and was observed on average at 27.6 ± 21.3 d pn. Following onset of own IgG synthesis, IgG concentration increased and reached a plateau on average of 24.5 ± 8.8 mg IgG/ml around 120 d pn, indicating that the immune system had matured. Two types of IgG profiles were observed, one with "immediate" concentration increase (above 10 mg/ml already at 30-40 d pn), the other with "delayed" concentration increase (below 10 mg/ml until around 70 d pn). Calves with delayed increase attained significantly lower IgG plateau values (19.0 ± 6.5 vs. 29.6 ± 7.3 mg IgG/ml). There is a tendency where early colostrum intake results in earlier and higher IgG peaks, but substantial IgG transfer is possible well after 24 h pn if calves are fasting until first suckling. Low serum IgG concentrations should be expected between two weeks pn and two months pn. Considering these findings in health care programs will contribute to improved camel calf rearing.

■ INTRODUCTION

High mortality of newborn camels during their first weeks of life is a frequently reported constraint to camel husbandry (1, 4, 11). Not only does newborn mortality mean the loss of the animal itself, but in most cases the lactation of its mother ceases as well. A high level of newborn mortality represents a loss of valuable animal products produced under difficult conditions and is an unnecessary drain on scarce feed resources.

Transfer of antibodies from the mother to the newborn via the colostrum is of major importance for health and survival of neonates of livestock species with epitheliochorial placentation, i.e. cattle (6), sheep (10), goats (5), horses (12) and New World Camelids (9). Failure of transfer of antibodies (FTA) leaves the neonate at high risk of infection and death. As the placentation in camels is also epitheliochorial (13), FTA could likewise be a reason for the reported high newborn mortality in this species.

Transfer of antibodies and development of the newborn's own antibody syntheses in camels are not yet documented in the literature. The present study investigates the immunoglobulin-G (IgG) status of camel calves from birth until six months of age in a natural herding situation in East Africa.

■ MATERIALS AND METHODS

The study was carried out from September 1994 to January 1996 on Ol Maisor Ranch in semiarid Northern Kenya. Blood sampling and quantification of IgG concentration in blood serum were accomplished for 68 camel calves born during the study period. Blood samples were taken in Greiner Vacurette® evacuated blood collecting systems immediately after birth before first colostrum intake, at 3, 6, 12, 18, 24, 36 and 48 h *post-natum* (pn), then every other day until 14 d pn, and thereafter in weekly intervals until six months of age. For 65 out of the 68 calves, blood samples were obtained from their mothers (dams) on the day of parturition. Blood samples were allowed to clot at +4°C, and serum was secured after centrifugation and stored at -20°C until assay.

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Camel IgG (camIgG) was quantified in serum by enzyme-linked immunosorbent assay (ELISA). The assay was designed as indirect antibody ELISA carried out in 96-well microtiterplates. The required anti-camIgG-antibodies were raised by immunization of layer hens with camIgG, and were subsequently extracted from the egg yolk by the polyethylenglycol-ethanol precipitation method of Polson *et al.* (7, 8). The assay had an upper level of detection of 40 mg camIgG/ml. The inter-assay coefficients of variation were 14.3% for a standard of 10 mg camIgG/ml and 15.4% for a standard of 30 mg camIgG/ml (3). Total amount of camIgG circulating in the animals' vascular system was calculated as:

$$\text{Total circ. IgG[g]} = \text{IgG serum conc.} * (\text{BW} * 0.09 * (1-\text{PCV}))$$

assuming that blood volume is 9% of live weight and serum volume is blood volume multiplied by 1-PCV (packed cell volume).

RESULTS AND DISCUSSION

Maternal serum IgG concentration at parturition

The average serum IgG concentration of the mother camels on the day of parturition was 23.9 ± 7.9 mg/ml (average plus/minus standard deviation) with a range from 10.7 to over 40 mg/ml. Heifers had significantly lower concentrations than multiparous females (16.8 ± 4.6 vs. 24.7 ± 7.9 mg/ml, $p = 0.01$).

Serum IgG concentrations before first suckling

Blood samples from 51 calves were obtained before first colostrum intake. These calves were on average 2.2 ± 1.7 h old at first sampling. Time of first suckling could be observed in 42 calves as being on average 3.64 ± 2.97 h pn (range 0.95-13.5 h). Precolostral serum IgG concentration was 0.26 ± 0.232 mg/ml with a range of 0-1.073 mg/ml. The total amount of camIgG circulating in the vascular system before first colostrum intake was calculated as $0.59 \text{ g} \pm 0.573 \text{ g}$ with a range of 0-2.772 g.

Time of IgG peak

Following colostrum intake, calves' serum IgG concentrations rose to a peak, defined as the local maximum in the IgG profile, which

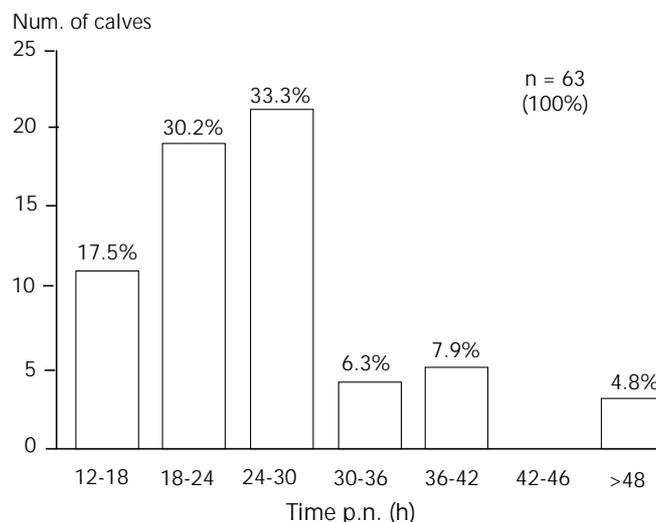


Figure 1: Postnatal time (p.n.) of peak serum IgG concentration.

was preceded and followed by a lower IgG concentration. Peak concentration was measured on average at 25.11 ± 8.34 h pn (median = 24.85h). The frequency distribution in figure 1 shows that 11 out of 63 calves (17.5%) had an early peak between 12 h and 18 h pn, but the majority attained their peak concentration either at 18-24 h (30.2%) or at 24-30 h (33.3%), while only 19% of the animals had their peak thereafter. In these latter animals, IgG concentration was still below 2 mg/ml at 12 h pn and started to rise only thereafter.

IgG peak concentration

The average IgG peak concentration for all calves was 21.1 ± 11.7 mg/ml. Three calves had very low IgG concentrations of 0.47 ± 0.35 mg/ml, while the remaining calves had peaks distributed evenly over the classes of higher concentration (figure 2, left).

No significant correlation was found between age at first suckling and peak IgG concentration, or between age at first suckling and time of peak ($r = 0.404$, $p = 0.008$).

The calculated maximum circulating amount of IgG attained was on average 50.6 ± 33.1 g ($n = 62$; one calf with no record of birth

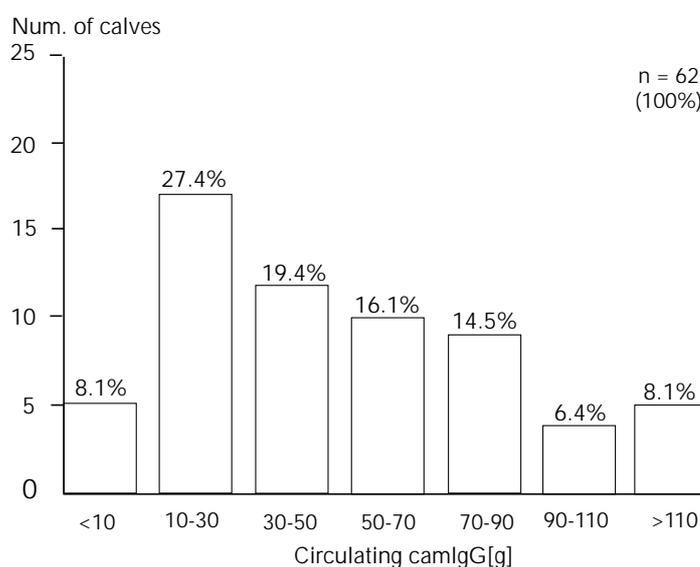
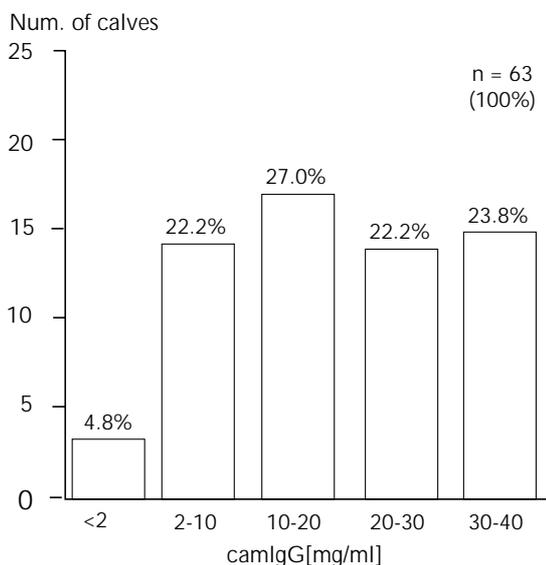


Figure 2: Frequency distribution of peak IgG concentration (left) and maximum circulating IgG amount (right) in camel calves.

weight was excluded from calculation). The frequency distribution in figure 2 (right) shows that five calves (8.1%) had maximum circulating IgG amounts below 10 g, and the majority of calves (27.4%) had amounts between 10 and 30 g. The number of animals constantly decreases through the classes of higher amounts. In all 62 calves the maximum circulating amount of IgG was found at the same postnatal time as the peak IgG concentration.

In figure 3, peak IgG concentration (left ordinate) and maximum circulating IgG amount (right ordinate) are plotted over classes of time of postnatal peak. Both peak concentration and maximum circulating amount are highest in animals with peaks between 18 and 24 h pn.

While the differences between the 18-24 h class and the 24-30 h class are significant at $p < 0.05$, they are not significant between all other neighboring classes.

There was no significant correlation between postnatal peak time and either peak IgG concentration or maximum circulating IgG amount. Significant though weak positive correlations were found between the serum IgG concentration of dams at parturition and both IgG peak concentration ($r = 0.31$; $p < 0.05$) and maximum circulating IgG amount ($r = 0.32$; $p < 0.05$) in their calves.

Peak concentration was below 10 mg/ml in 17 calves (26.5% of the total). Out of these, five calves (7.8% of total) had peaks below 4 mg/ml. This implies that, using the critical diagnostic thresholds for FTA commonly used in bovines (10 mg/ml) and equines (4 mg/ml), 26.5 or 7.8%, respectively, all calves under study would have been considered positive for FTA.

Half-life of maternal IgG in newborns' circulation

After IgG peak was attained, both serum IgG concentration and IgG amount in newborns' circulation declined markedly due to the distribution of IgG into the extravascular body fluid compartments, IgG catabolism, immune responses mounted to invading antigen and growth of the animals. The amount of IgG that disappeared from the circulation ranged from 0.2 to 16.3 g/d. Half-life of maternally derived IgG in newborns' circulation was calculated based on the two-compartment half-life model (2, 14) and was 16.3 ± 8.5 d, similar to what is reported for other livestock species.

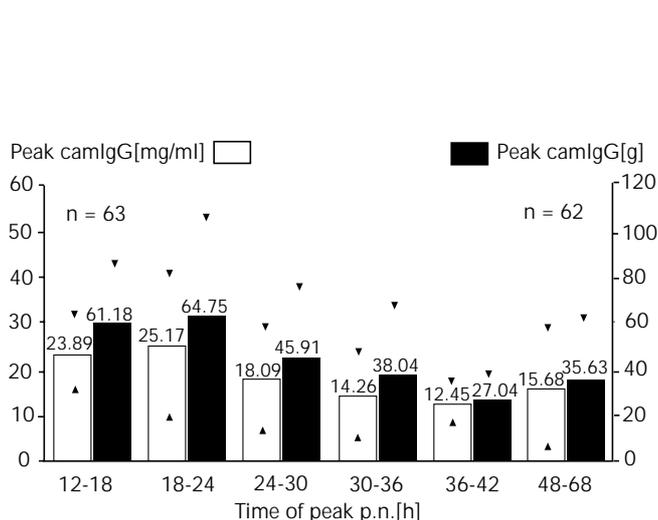


Figure 3: Peak IgG concentration and peak IgG amount (avg. ± std.) by time of peak.

Minimum IgG concentration and onset of calves' own IgG synthesis

Following the decline of maternally derived IgG in newborns' circulation, a minimum was observed from where the concentration started to rise again due to the calves' own antibody synthesis. Minimum IgG concentration was on average 8.1 ± 3.3 mg/ml (range 1.6-15.1 mg/ml). It was observed at 27.6 ± 21.3 d pn (median 25.7 d pn), but in 47.5% of the animals before day 15 pn.

As for IgG peak, the minimum circulating IgG amount was calculated and was on average 27.7 ± 13.6 g (range 4.6-57.4 g). The minimum amount was observed before 15 d pn (on average on day 11) in 89.5% of the calves.

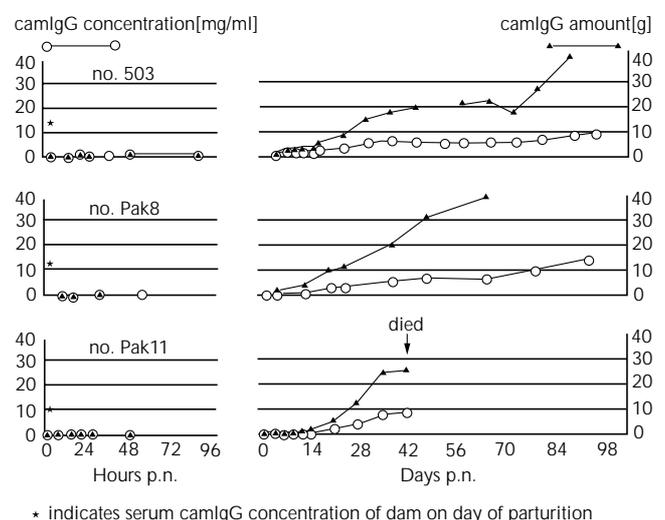
Thus, in 33 calves the circulating IgG amount had already increased, while the IgG concentration was still declining. This indicates that their own IgG synthesis had already started, but did not result in an IgG concentration rise. This can be attributed to their parallel body weight increase and the concurrent increase in blood volume, hence dilution of newly synthesized IgG. Minimum concentration in these calves was observed up to 35 days after the minimum amount.

Determining the exact onset of IgG production by the calf's immune system is difficult because it occurs parallel to the decline of maternally derived IgG, and the "maternal" and "own" IgG cannot be differentiated by the assay. Three calves had complete failure of transfer of antibodies (figure 4). The observed increase of the circulating camIgG amount in these calves from 14 d onwards indicates the onset of calves' own IgG production. This coincides with the observed 89.5% of calves having their minimum serum IgG concentration before day 15 pn.

Different IgG profile types

For each calf, an IgG profile was generated by plotting serum IgG concentration over time from birth until 180 d pn. Among these, two different types of profile were observed:

- Those in which serum IgG concentration increased immediately following minimum and attained values of above 10 mg/ml already at 30-40 d pn;



* indicates serum camIgG concentration of dam on day of parturition

Figure 4: IgG profiles (concentration and circulating amount) for camel calves without significant transfer of antibodies.

Post-natum IgG concentrations

- Those in which increase of serum IgG concentration was delayed and concentration remained below 10 mg/ml until around 70 d pn.

In figure 5, averages of several individual profiles plotted over time of calves which showed an immediate increase in IgG concentration following the IgG minimum are grouped according to peak height. In figure 6, the same grouping is shown for average profiles of those calves which showed a delayed increase in IgG concentration following the minimum.

Among the animals with immediate increase (figure 5), those with peaks below 10 mg/ml had a steady rise in IgG concentration from day 4 pn onwards, attained average concentrations above 10 mg/ml from around day 30 pn, and a plateau around day 120 pn. In animals with peaks between 10 and 20 mg/ml the concentration never fell considerably below 10 mg/ml, and from

about 42 d pn onwards it rose to a plateau. In animals with peaks over 20 mg/ml, the decline in concentration lasted until about day 40 pn from where it immediately rose again to the plateau. The common feature of the profiles in figure 5 is that between 30 and 40 d pn IgG concentration is already above 10 mg/ml, it then rises sharply to reach plateau concentrations at about day 120 pn.

In the profiles of figure 6, the minimum serum IgG concentration was below 10 mg/ml for all animals irrespective of peak height. The common feature of all three profiles in figure 6 is that after attaining the minimum, IgG concentrations remain below 10 mg/ml until around 70 d pn, only thereafter moderately rising to reach plateau concentrations at about day 120 pn. Calves with delayed increase (figure 6) attained significantly lower IgG plateau values than those with immediate increase (figure 5, 19.0 ± 6.5 mg IgG/ml vs. 29.6 ± 7.3 mg IgG/ml).

■ PHYSIOLOGIE

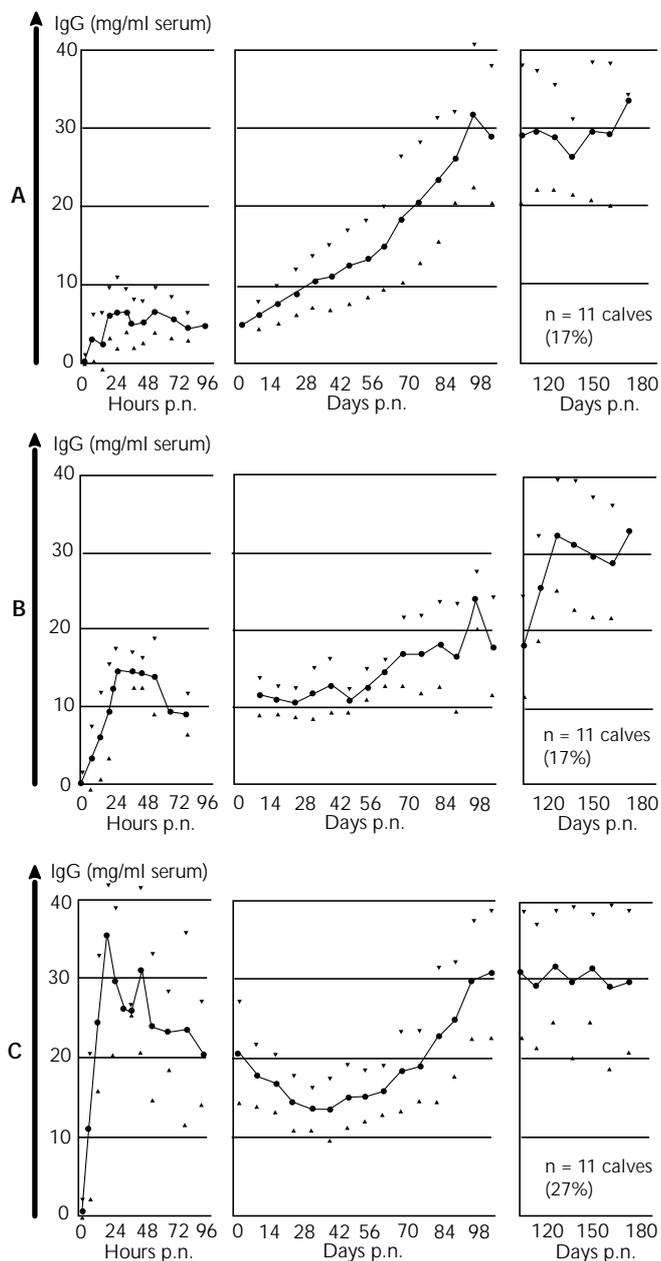


Figure 5: Average IgG profiles for calves with immediate increase of IgG concentration following the minimum (A: peak < 10 mg/ml; B: peak between 10-20 mg/ml; C: peak > 20 mg/ml).

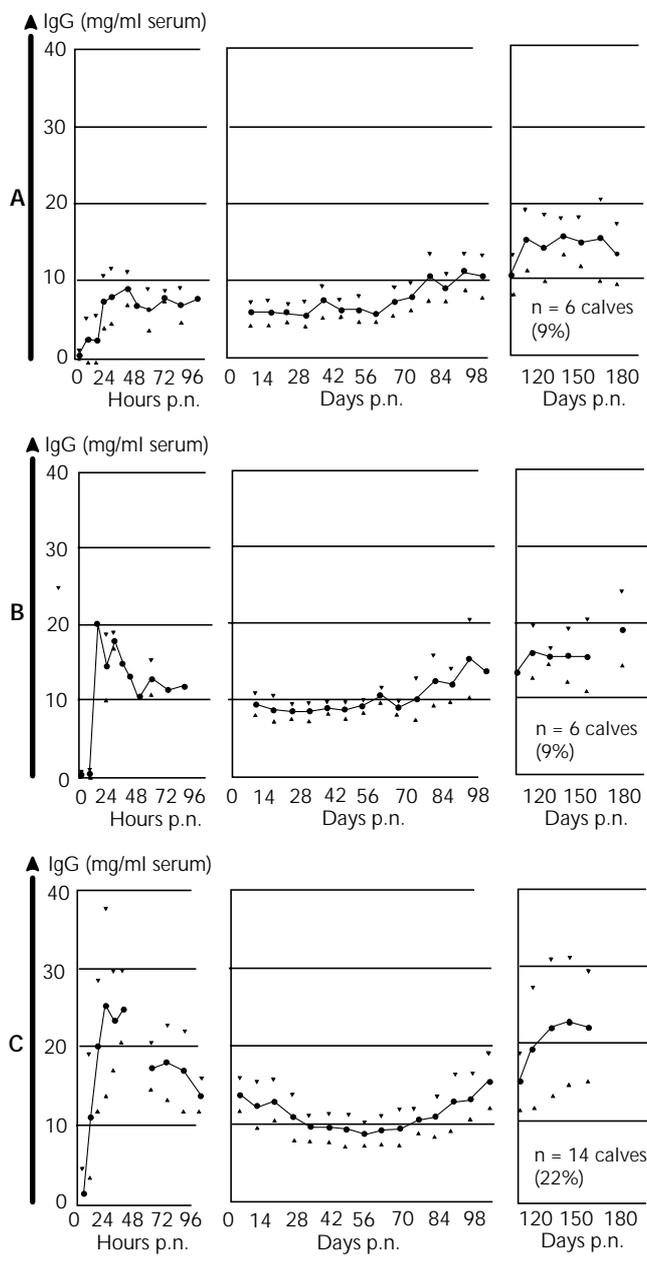


Figure 6: Average IgG profiles for calves with delayed increase of IgG concentration following the minimum (A: peak < 10 mg/ml; B: peak between 10-20 mg/ml; C: peak > 20 mg/ml).

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The factors breed of the calf, breed of the mother, parity of the mother, sex, season or month of birth, herdsman, body weight development and number of sick days did not contribute to the explanation of the different patterns of increase shown in figures 5 and 6.

Average IgG plateau concentrations between 150 and 180 d pn were significantly correlated with maternal serum IgG concentrations on the day of parturition ($r = 0.44$; $p < 0.001$).

■ CONCLUSION

There is a wide variation of naturally occurring IgG status in newborn camels under normal husbandry conditions. Although there is a tendency where early postnatal colostrum intake results in earlier IgG peaks with higher concentrations, there is evidence that intestinal closure is not yet complete and a substantial IgG transfer is possible after 24 h pn if calves are fasting until first suckling. Lowest serum IgG concentrations must be expected in camel calves from around two weeks *post-natum*, indicating that the protection of the newborn by maternally derived IgG is waning by that time. The calf's own antibody production starts around two weeks *post-natum*, but a marked increase in serum IgG concentration to above 10 mg/ml can be expected by one month *post-natum* at the earliest, but often only after two months *post-natum*. Factors contributing to a brisk development of young camels' own IgG synthesis following waning of the passively acquired maternal immune protection deserve further scientific attention. Serum IgG concentrations stabilize at a plateau around four months *post-natum*, indicating that the immune system has matured by that time. The present study identified those periods in the camels early life during which its immune status is low, and special attention should be paid to its health and general hygiene.

Acknowledgments

The author is indebted to J. and J. Evans and D. Atkins of Ol Maisor Ranch for access to their camel herd and for providing excellent working facilities. The author wishes to thank Eppendorf-Netheler-Hinz, Sartorius, Hettich, WTW, IKAWERK, Merck, Greiner, Brand, Unipath, Nunc, Kautex, Bürkle and Sebak for donating laboratory materials, and Eiselen Stiftung, Ulm, Germany, Deutscher Akademischer Austauschdienst DAAD and the Government of the Federal State Baden-Württemberg for financial support.

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Résumé

Hülsebusch C.G. Statut de l'immunoglobuline-G chez le chamelon pendant six mois *post-natum*

Le statut de l'immunoglobuline-G (IgG) du chamelon a été étudié de la naissance jusqu'à six mois dans des conditions naturelles d'élevage au sein d'un ranch au Kenya. L'IgG cameline a été quantifiée *post-natum* (pn) jusqu'à l'âge de six mois par la méthode de mesure indirecte des anticorps Elisa dans le sérum de 68 chamelons. La première prise de colostrum a été observée à $3,6 \pm 3,0$ h pn et la concentration moyenne en IgG sérique précolostrale a été de $0,26 \pm 0,23$ mg/ml (valeurs extrêmes 0-1,07 mg/ml). Les concentrations maximales moyennes d'IgG (pics) dans le sérum des chamelons a été de $21,1 \pm 11,7$ mg/ml. La plupart des pics sont survenus entre 18 et 30 h pn, mais pour 19% d'entre eux ils ont été plus tardifs, survenant entre 30 et 66 h pn, avec des concentrations significativement plus faibles. Après le pic, la concentration en IgG a diminué, la demi-vie des IgG d'origine maternelle dans le sang des chamelons ayant été de $16,3 \pm 8,5$ jours. La concentration minimale moyenne a été de $8,1 \pm 3,3$ mg/ml (valeurs extrêmes : 1,6-15,1 mg/ml) et a été observée en moyenne à $27,6 \pm 21,3$ jours pn. Après l'émergence des IgG chez le chamelon, la concentration a augmenté jusqu'à un plateau avec des valeurs moyennes de $24,5 \pm 8,8$ mg IgG/ml au 120^e jour pn environ, indiquant que le système immunitaire du jeune était désormais mature. Deux types de profil d'IgG ont été observés, l'un avec une augmentation immédiate de la concentration (au-dessus de 10 mg/ml dès le 30-40^e jour pn) et l'autre présentant une augmentation retardée (en dessous de 10 mg/ml aux environs du 70^e jour pn). Chez les chamelons où l'augmentation a été retardée, les valeurs d'IgG au plateau ont été significativement plus faibles ($19,0 \pm 6,5$ vs $29,6 \pm 7,3$ mg IgG/ml). Il semble qu'il y ait eu apparition d'un pic plus élevé et plus précoce lors de prises colostrales plus précoces, mais le transfert substantiel d'IgG était possible bien après 24 h pn si le chamelon était à jeun jusqu'à la première tétée. De faibles concentrations d'IgG sériques devraient être rencontrées entre la 2^e semaine et le 2^e mois *post-natum*. Ces résultats pris en compte dans des programmes de prévention sanitaire pourraient contribuer à l'amélioration de l'élevage du chamelon.

Mots-clés : Dromadaire - Jeune animal - Immunoglobuline - Colostrum - Kenya.

Resumen

Hülsebusch C.G. Estado de la inmunoglobulina G de los camellos durante los seis meses post nacimiento

Se investigó el estado de la inmunoglobulina G (IgG) en jóvenes camellos desde el nacimiento hasta seis meses de edad, bajo condiciones de hato naturales, en un rancho de ganado en Kenia. La IgG del camello se cuantificó post nacimiento (pn) hasta seis meses de edad mediante un ELISA de anticuerpos indirectos en 68 camellos jóvenes. La primera ingestión de calostro fue observada en promedio $3,6 \pm 3,0$ h pn y la concentración promedio de IgG sérica pre calostrual fue de $0,26 \pm 0,23$ mg/ml (rango 0-1,07 mg/ml). La concentración promedio máxima de IgG (pico de IgG) en el suero animal fue de $21,1 \pm 11,7$ mg/ml. La mayoría de los jóvenes presentaron picos de IgG entre 18 y 30 h pn, pero 19% de éstos presentaron picos tardíos (30-66 h pn), con concentraciones significativamente menores. Después del pico, la concentración de IgG sérica disminuyó, con una vida media de IgG materna en la circulación del recién nacido de $16,3 \pm 8,5$ d. El promedio mínimo de la concentración de IgG fue de $8,1 \pm 3,3$ mg/ml (rango 1,6-15,1 mg/ml) y se observó en promedio $27,6 \pm 21,3$ d pn. Después de este inicio, la concentración de IgG aumentó, alcanzando un tope de $24,5 \pm 8,8$ mg de IgG/ml en promedio, alrededor de 120 d pn, indicando que la madurez del sistema inmune. Se observaron dos tipos diferentes de perfiles de IgG, aquellos con un aumento "inmediato" de la concentración (por encima de 10 mg/ml a 30-40 d pn) y aquellos con un aumento "retardado" de la concentración (bajo 10 mg/ml hasta alrededor del día 70 pn). Los jóvenes con aumento retardado alcanzaron valores tope de IgG significativamente menores ($19,0 \pm 6,5$ vs. $29,6 \pm 7,3$ mg IgG/ml). Existe una tendencia de que una ingestión temprana de calostro resulte en picos de IgG mayores y más tempranos, pero una transferencia de IgG substancial es posible bastante después de 24 h en animales en ayuno hasta la primera mamada. Deben esperarse concentraciones séricas bajas de IgG entre dos semanas pn y dos meses pn. La toma en consideración de estos hallazgos en los programas de salud contribuiría al mejoramiento en la crianza del camello joven.

Palabras clave: Dromaderio - Animal joven - Inmunoglobulina - Calostro - Kenia.