ZEBRA FOUNATION PROJECT

EVALUATION OF FIELD TESTS FOR ASSESSING ALPACA COLOSTRUM

First published in BVZS *ZooMed Bulletin*, March 2009

Hanna Flodr
Royal Veterinary College of London
Hawkshead Lane, North Mymms
Hatfield
Hertfordshire AL9 7TA
United Kingdom

March 2007

Summary

The main reason for neonatal mortality and morbidity in alpaca crias is the failure of passive transfer (FPT) of maternal immunoglobulins. The prevalence of FPT is reported to be between 9-20.5%. Studies in other farm species have shown that the colostral IgG concentration is the main determinant of antibody levels in the young. It is therefore vital for the offspring to obtain sufficient good quality colostrum within the first 24 hours of life, as after this period of time the intestines are no longer able to absorb the colostrum specific antibodies. This study evaluates two different field methods for assessing alpaca colostrum. Samples were obtained from 77 alpacas and their corresponding crias on native pastures in Peru. Colostrum was collected from the dams immediately postpartum and pre-suckle. Blood was taken from the crias by jugular venipuncture 36-48 hours after parturition. Colostral viscosity was assessed visually and the colostral total solids were measured by sugar Brix refractometry. The serum IgG values of all crias and 26 of the colostral IgG concentrations were determined by radial immunodiffusion assay. The results were analysed using parametric correlation and un-paired t-test model statistics. The minimum mean cria serum IgG concentration was 2679 mg/dl (SD ± 603.4 mg/dl). Only one cria had serum levels below 1000 mg/dl. The prevalence of failure of passive transfer was therefore only 1.3 %. The Brix refractometer accurately measured the colostral IgG concentration ($P=0.0007$). However, there was a poor correlation between the % Brix refractometry readings and the cria serum IgG values ($P=0.338$), as well as between colostral IgG concentrations and the cria serum IgG levels ($P=0.15$). As opposed to other farm species, this suggests that cria serum IgG concentration is not dependent on colostrum IgG levels. As no low values of colostral IgG concentration were obtained, the usefulness of viscosity at detecting poor quality colostrum could not be determined. However, within the range examined, viscosity and colostral % Brix readings were highly correlated ($P<0.0001$), and warrants future studies examining the utility of differentiating between very poor to high quality colostrum. Sex and weight of cria, parity of dam and location showed to have no influence of passive transfer status.

Introduction

The newborn camelid is similar to the newborn of most large animal domestic species in that it is born with a very naive and immature immune system which is relatively unable to respond to pathogen exposure.[3, 8, 24] This is due, in part to the fact that the camelid placenta has a diffuse, microcotyledonary and epitheliochorial type structure which essentially prevents prepartum transfer of maternal antibodies (Ig) from the dam to the fetus.[2, 23, 25] The newborn camelid is therefore virtually devoid of serum antibodies at birth, and relies almost entirely on the acquisition of maternal antibody from ingested colostrum (the antibody-rich first milk) for immunological protection during the first weeks. Since the newborn intestine is only permeable to colostral antibody for the first few hours of life, an adequate intake of good quality colostrum within this period is vital for the immediate survival of the cria and provides resistance to disease and the ability to combat infectious agents for several weeks. [8, 23, 25]

Unfortunately the production of antibody-rich colostrum, and its ingestion by the newborn alpaca, is not always optimal. The condition following sub-optimal acquisition of colostral antobodies is called failure of passive transfer (FPT) and is thought to occur when the cria has an antibody level of less than 1000 mg/dl at 48 hours of age.[7, 23, 24, 25] It is a well recognized problem and a major factor in neonatal mortality in alpacas. One study of alpacas in Peru indicated that approximately 9 % of newborn crias had FPT [7]. The overall mortality in this study was 12% and in crias with FPT it was as high as 78%. Another study of alpacas in the United States reported an prevalence of FPT as high as 20.5%, however the mortality in this study was low due to superior husbandry conditions. [23] It has also long been known that the incidence of infectious disease conditions often is a significant and important cause of immediate and longer-term suffering in animals with FPT. [1, 9, 12, 13, 14, 25]

The frustrating aspect of these statistics is that FPT is relative easy to prevent with good husbandry practices. However, the current methods available are only able to assess passive transfer status by evaluating the serum immunoglobulin levels when the cria is 36-48 hours old, at which stage the intestinal wall is no longer absorptive and prophylactic colostral feeding has limited utility.[8] Once identified, treatment of FPT can be quite laborious, even involving a plasma transfusion of 20-40ml/kg body weight.[25] The gold standard for evaluating serum Ig status is the radial immunodiffusion test, but is quite unsuitable as a rapid field test for FPT as the results require an incubation of 24 hours.[3, 24] Other methods include measuring the total serum proteins and globulins by a modified biuret procedure on a automated analyzer, and a commercially available sodium sulfite turbidity test. Serum total solids measured by a hand held refractometer have also shown to correlate significantly with serum IgG levels, however no endpoint had sufficient sensitivity and specificity for the refractometer to be considered for routine clinical use. [24] As mentioned previously, all the above methods are only able to assess passive transfer status when the cria is too old to benefit from prophylactic colostral feeding and treatment.

Several studies in other farm species, and in particular horses, have shown that the IgG concentration and specific gravity of dam colostrum correlate significantly. [1, 5, 9, 10, 11, 13, 21, 22] In horses there is also a high association between foal serum IgG levels and mare colostral specific gravity.[9, 10, 11, 15, 19] This has led to the useage of a sugar refractometer as a predictor of colostral status and thus, an indication of which foals run the risk of FPT: The sugar refractometer measures the concentration of dissolved solids in a solution and as immunoglobulins make up a major part of colostrum the sugar refractometer has proven to be a quick, cheap and valuabel "on farm" method with a an acceptable level of sensitivity in the detection of poor colostral quality.[4, 5] This allows prophylactic measures to be taken prior to the onset on clinical disease.

To the best of the author's knowledge, there has been no previous studies focusing on the relationship between alpaca colostral quality measured by viscosity or sugar refractometry and cria post-suckle IgG levels. One earlier study examining the correlation between IgG concentration in colostrum and cria serum IgG in camelids was performed approximately 20 years ago.[7] However, the investigators used other assay methods, and the results were inconsistent, making practical inferences quite difficult. The report found the correlation between colostral IgG concentration and cria serum IgG to be low ($P < 0.04$) however, looking more closely at particular colostral IgG cut off points there was a strong relationship between low colostral levels and FPT. For instance, while 8 out of 9 crias (89%) with FPT had a colostral IgG level of less than 10 000 mg/dl, only 1 cria out of 59 (1.7%) with a colostral IgG level of more than 10 000 mg/dl had FPT. There was also a strong relationship between cria mortality and low colostral IgG levels since of 11 dams with colostral IgG < 7000 mg/dl, 4 (36%) had crias with FPT that died. In comparison, only 3 of the 38 (7.9%) dams with colostral IgG >13100 mg/dl had crias with FPT that died.

The aim of this study is therefore to evaluate the colostral quality by sugar refractometry and by visual assessment of viscosity and their use as rapid field tests in the prediction of poor passive transfer status in crias.

Materials and Methods

Samples were collected from 77 alpaca dams and their corresponding crias in Peru, divided between 54 samples at La Raya research station 4200 metres over sea level, and 23 samples at Macusaní, 4600 metre over sea level. All alpacas were kept on native pastures and apart from routine worming not supplemented with neither alimentation nor vaccinated. Both suri and huyacaya alpacas were sampled. Additional information as parity of dam and weight of cria was noted, but was impossible to obtain for all samples due to lack of farm records.

Determination of colostral total solids

A small colostral sample (~ 1 ml) was taken immediately postpartum and pre-suckle. The total solid content of the colostrum was measured with a temperature-compensated sugar refractometer (0-90 % Brix, E-line 90 Bellingham & Stanley). Refractometry is an indirect way of measuring total solids in a solution by the means of refractive light.[4,5] The Brix scale is based on the refractive index of pure sucrose in water concentration; thus the refractive index of sucrose is 1% Brix. However, despite its name the sugar refractometer measures total solids as it cannot differentiate between different elements.[a] As the refractometer could not be calibrated to 0 % Brix but was set at 2 % Brix, this sum was deducted from all colostral sample readings.

Viscosity

All colostral samples were evaluated optically by the author and the viscosity was judged on a scale from 1-5. A viscosity value of 1 was defined as very runny, milk-like colostrum with no or low stickyness. A viscosity value of 5 was defined as thick, slow moving and with a high degree of stickiness.

Cria serum collection

A small blood sample was taken aseptically into a serum tube by venipuncture of the jugular vein from the corresponding crias 36-48 hours post partum. The blood was centrifuged and the separated serum was stored at -20ºC for later analysis. Due to lack of electricity the 23 blood samples from Macusaní could not be centrifuged and stored for 5 days. The crias at La Raya research station were routinely given profylactic antibiotics orally for the first three days of life.

Laboratory Analysis

All of the 77 blood samples, and 26 of the colostral samples, were analysed with a radial immunodiffusion kit for camelid IgG (Triple J Farms) according to the manufacturers instructions. Briefly, 5 µg of serum was added to 1 well of a 24-well plate containing antiserum against camelid IgG in agarose together with 3 control serums provided by the manufacturer. The plate was incubated overnight in room temperature (20-24º C). The diameter of the precipitations was read after 24 hrs and the controls plotted against a standard curve.

Picture 1: The diameters of the samples were determined seperately by two people and a mean value was selected if discrepancy occurred. Controls from four of the plates coincidenced with the manufacturers references and the IgG values were therefore extrapolated from the provided reference table. All values over 9.4 mm/diameter were outside the reference table and therefore considered > 3215 mg/dl. These samples were however recorded as 3215 mg/dl.

The colostral samples were manually diluted 1/10 with saline buffer before analysed. The controls from one plate of colostrum did not correspond to the provided references and the IgG concentrations were therefore calculated from a standard curve. All samples with a diameter of ≥8.5mm were considered as having a IgG concentration of 32150 mg/dl or above, however they were recorded as 32150 mg/dl.

Picture 1. One plate of the radial immunodiffusion assay.

Statistics

The data was tested for normality and the results analysed using parametric correlation models and un-paired t-tests. Prism software was used for all statistical calculations. A P-value of <0.05 vas deemed as significant. Mean and standard deviation were also calcualated.

Results

Results are expressed as the minimum mean ± SD. Serum IgG levels of < 1000 mg/dl at 48 hours of age are considered diagnostic of FPT in crias. Out of 77 crias in this study, only one cria had less than 1000 mg/dl. 22 crias had >3215 mg/dl. This was recorded as 3215 mg/dl. The average serum IgG level of the crias was 2678.9 mg/dl (± 603.4 mg/dl).

The range of % Brix reading in 77 samples was between 28-50 %. The mean was 37. 3 (± 4.5) % Brix. Colostrum IgG concentrations in 26 samples ranged from 14390 ->32150 mg/dl with a mean of 28 337 mg/dl (± 5593 mg/dl). Values above 32 150 mg/dl could not be determined. The IgG concentration of the colostrum was well correlated to the % Brix scale (P=0.0007; r^2 = 0.38) **Fig.1** The % Brix scale was also strongly correlated to the viscosity of the colostrum. (P= <0.0001; r^2 = 0.69) **Fig. 2**

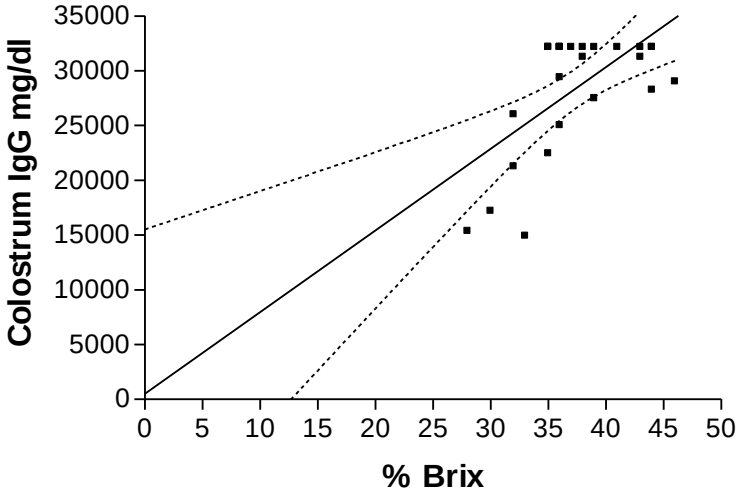

Fig.1 The relationship between colostral IgG concentration and % Brix readings.

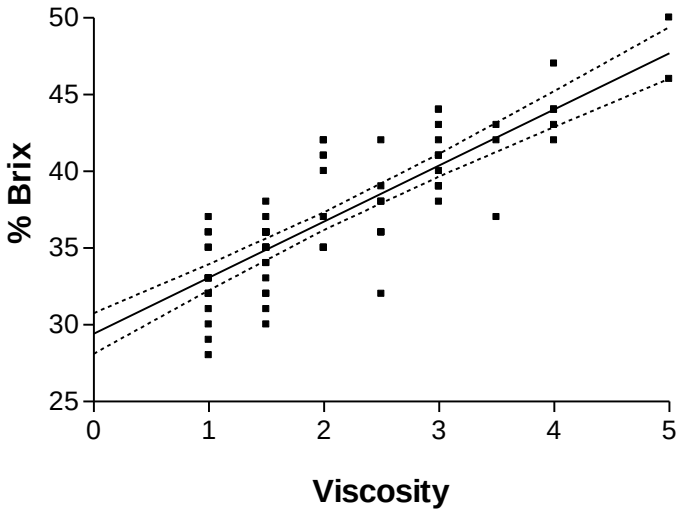

Fig 2. The relationship between viscosity and % Brix readings.

However, when comparing the viscosity with the IgG concentration the correlation was lower but still significant (P=0.044). **Fig. 3**

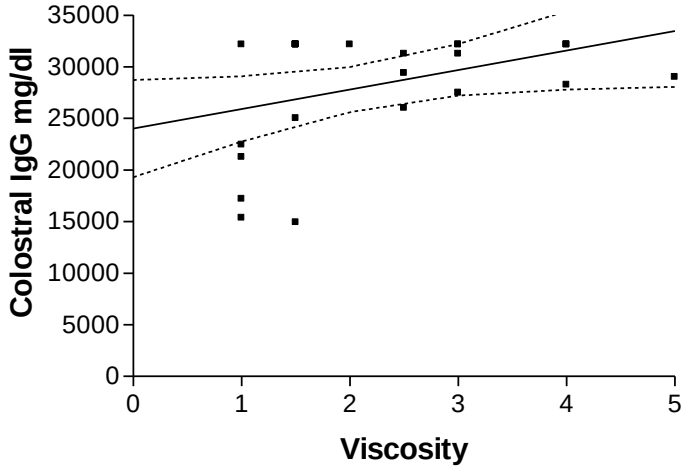

Fig.3. The relationship between viscosity and colostral IgG.

There was a visual linear relationship between the % Brix of the colostrum and cria serum IgG levels, however statistical analysis showed poor correlation between the values. (P=0.338) **Fig. 4** There was also poor correlation between the IgG concentration of the colostrum and the cria serum IgG levels. (P = 0.15) **Fig. 5.**

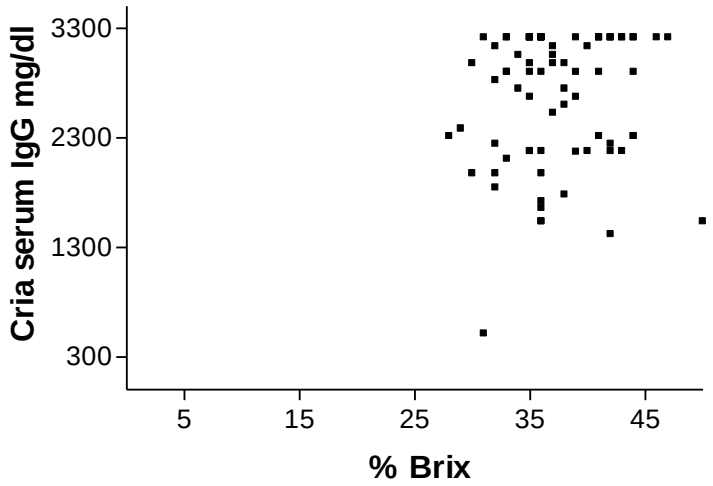

Fig.4 The relationship between cria serum IgG and % Brix of colostrum. All serum samples > 3215 mg/dl were recorded as 3215 mg/dl .

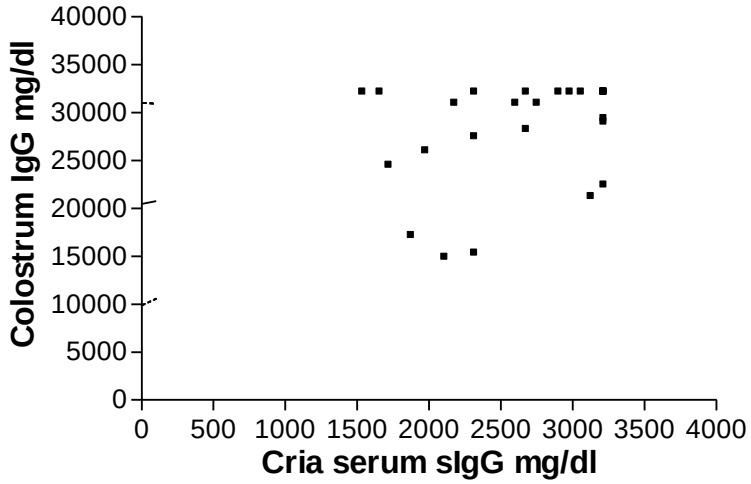

Fig. 5 The relationship between Cria serum IgG and the IgG concentration of colostrum. All values >3215 mg/dl (serum) or >32150 mg/dl (colostrum) were recorded as 3215 and 32150 mg/dl respectively.

There was no significant difference (P= 0.84) in neither cria serum IgG, nor % Brix (P=0.86) between animals located at La Raya or Macusani. There was also no significant difference (P=0.66) in serum IgG levels between female and male. (Female 2766 mg/dl, ± 531.9; male crias 2705 mg/dl, ± 494.4) **Fig 6.**

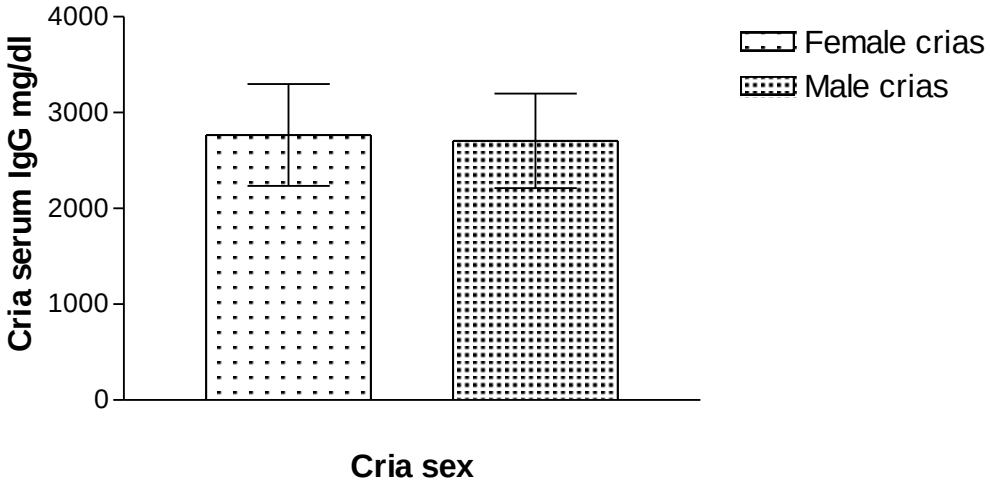

Fig 6. Mean + SD of female and male cria serum IgG.

There was no correlation between cria weight and serum IgG (P=0.9001). The % Brix readings showed no significant difference (P= 0.41) between maiden or multiparous dams. Nor was there any difference (P=0.99) in serum IgG of crias belonging to maiden (2667 mg/dl, ± 526.6) or multiparous dams (2668 mg/dl, ± 671.2) **Fig 7**

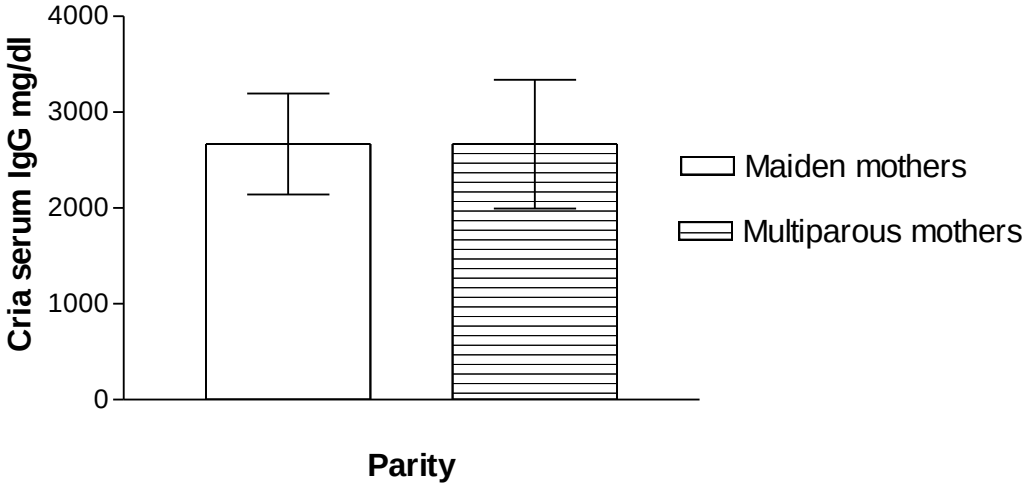

Fig 7. Serum IgG levels of crias belonging to maiden or multiparous mothers. Mean + SD.

Discussion

Some of the results from this study were different to the results of previous papers. The average cria serum IgG was 2678.9 mg/dl in this study, whilst two other studies reported a mean of 2370 mg/dl [2] and 1806 mg/dl [23] . No significant difference in serum IgG was found between female and male crias in this review, other papers show conflicting results.[2,7] The reported prevalence of FPT in crias has been between 9-20.5%.[7, 23] In this study only one cria had serum IgG values below 1000 mg/dl, making the prevalence of FPT 1.3% The mortality and morbidity rate of the crias in this study was not determined, it has however been demonstrated on several occasions that although FPT is associated with increased mortality and morbidity, the alpaca cria is capable of surviving despite low serum IgG levels if under right husbandry conditions. [3, 23]

Alpaca crias are born agammaglobulinemic and several studies have shown a rapid linear increase in serum IgG concentrations 24-48 hrs after the ingestion of colostrum, similar to other farm animals.[2, 3, 7, 8] However, in other species, the main determinant of offspring serum IgG levels is the IgG concentration of colostrum,[10, 11, 19, 20] and in foals the serum IgG level is directly correlated to the specific gravity of colostrum.[9, 10, 11, 15, 19] Studies in calves have shown that animals absorp more colostral IgG if 1 litre of colostrum containing 100 mg of Ig/ml is fed than if 2 litres of colostrum with 50 mg of Ig/ml is given.[20]

The results from this report demonstrate that a similar link between colostral IgG concentration and cria serum IgG does not appear to exist in alpacas. The low correlation between colostral IgG and cria serum IgG is consistent with the findings of Garmendia 20 years ago.[7] Unfortunately, due to the absence of both low serum and colostral IgG values in this present study, it was impossible to fully determine their association. The strong correlation between % Brix and colostral IgG shows that the sugar refractometer correctly measures antibody levels and is a good assessor of colostral quality. However, the poor correlation between % Brix and cria IgG limits the value of using a refractometer as a predictor of passive transfer status.

This lack of correlation between colostral IgG and cria serum IgG suggest that factors other than colostral concentration might be important in the absorption of immunoglobulins. As the intestines are only able to absorb antibodies for a limited period, the time of first ingestion of colostrum has also proven to be important in other domestic livestock species.[10, 17, 20] In calves, the amount IgG ingested affects the gut closure in a linear fashion.[17, 20, 21] Investigations in the horse have shown that the colostral antibodies bind to specialised Fc receptors on the intestinal epithelial cells called FcRn, and are actively pinocytosed and passed into the systemic circulation via the lacteals. These cells are replaced by mature epithelial cells and between 18 and 24 after birth the gut of the foal is no longer able to absorb immunoglobulins.[1, 9, 12, 16, 19]

In this study high cria serum IgG levels were interestingly achieved by colostral IgG concentrations on both ends of the scale and vice versa. This pattern may suggest a feedback mechanism yet to be documented. The exact physiology of immunoglobulin absorption in alpaca crias has so far not been investigated, nor has the precise time of intestinal closure been determined, but due to the linear increase in serum IgG concentration for the first 24 hrs followed by a decrease at 48 hours, one study suggests a similar absorption pattern to calves with optimal time of absorption at 0-12 hrs of age.[8] The time of first ingestion was not observed in this present study, but it is recommended in future studies.

The main immunoglobulin absorbed in the cria is IgG, which accounts for >85 % of the passively transferred proteins in serum of 24-hour-old crias. IgM is also transferred, but to a much lesser, (1:7) degree than IgG.[8] To date no isotypes have been determined. It is however very interesting to note that the immunoglobulins of camelids differ dramatically from other species in that over 75% of the serum proteins are IgG molecules lacking a light chain. It has been demonstrated that IgG_2 and IgG_3 consist of only heavy chains, making them smaller than conventional antibodies and thus allowing better tissue penetration and biodistribution.[25] These antibodies, which have a molecular weight of 90kDa compared to normal antibodies of 150 kDa, are also more efficient in neutralising enzymes than common antibodies. How this affects passive transfer has not been established. South american camelids further differ from other farm species in the way they produce colostrum. Investigations in the cow, sow, mare and ewe have demonstrated a passage of IgG molecules from the bloodsteam to the mammary gland just prior to parturition.[2, 9, 21] In alpacas and llamas the serum blood IgG levels remain constant throughout the pregnancy, whilst the mammary secretion concentrations are elevated prior to parturition, a ten-fold difference from the sera IgG levels, and drop dramatically after parturition.[2] This points to a local production of colostral IgG in the mammary gland. The isotypes of these IgG molecules have not been determined and it is therefore unclear whether alpaca colostrum contains IgG molecules lacking light chains.

Studies of the effect of dam age and parity in other farm species have shown variable results, [6, 10, 19] however, one study[18] in calves concluded that colostral quality improved with increased parity. The results from this review show no difference between multiparous and maiden dams neither in % Brix levels, nor the cria IgG levels. At the time of sampling the author noted that although both multiparous and maiden dams produced colostrum from the full range of viscosity, the multiparous dams seemed to more often produce a greater quantity of colostrum than the maiden dams. Volume of ingested colostrum was however not determined in this study. It has been calculated[25] that a 10 kg cria would need to consume approximately 100 ml of normal quality (22000 mg/dl) colostrum in order to obtain a IgG level greater than 1000 mg/dl, but so far this calculation has not been confirmed.

The strikingly high correlation ($P<0.0001$) between % Brix and viscosity is interesting, as this relationship is not so prominent in horses where stickiness of the colostrum is regarded as a more reliable means of visually assessing colostrum.[1, 9, 11, 13] The data in Figure 2 of this study clearly shows that a viscosity value of 1.5 was never above 40 % Brix, and a viscosity of 3 was never below a % Brix reading of 35. Figure 3 further demonstrates that there were no IgG concentrations below 25 000 mg/dl above a viscosity of 1.5. These results suggest that viscosity could be really useful at differentiating between colostral total solid concentrations. As there unfortunately were no remarkedly low colostral IgG values present in this study (<10 000 mg/dl), it was not possible to either define the threshold of poor colostrum on the Brix-scale, or fully evaluate viscosity as a method of detecting poor colostrum. However, the strong association between viscosity and % Brix in the range examined offers great promise of using visual inspection as a predictor of colostral quality. In future studies, the author recommends that the colostral samples should be saved and compared to each other instead of assessed immediately on collection as in this report. This is to increase further accuracy of correct classification.

In conclusion; results from this study show that the correlation between dam colostral IgG concentration and cria serum IgG concentration is low. The sugar refractometer is accurate at assessing colostral IgG concentration but fails to demonstrate a significant relationship between Brix % and cria serum IgG. Therefore the sugar refractometer has limited value in predicting passive transfer status in crias. Cria serum IgG levels were not evaluated by sugar refractometery, but could nevertheless be included in future studies. Visual assessment of colostral viscosity and % Brix showed to be significantly related in the range examined and warrants further studies in order to properly evaluate this relationhip.

One major flaw of this study was that the samples outside the reference range of the radial immunodiffusion assay were not diluted and rerun. This limited the top reference to either 3215 mg/dl or 32 150 mg/dl. The data in this study is therefore most likely skewed to the left. Other limitations of this study include using visual means to dilute the colostral samples, which presents the possiblity of the samples not being diluted exactly 1/10 and thereby affecting the

subsequent colostral IgG results. As this study was conducted under uncontrolled field conditions there is also the chance that some of the crias suckled dams others than their own. In future studies suckling behaviour should be monitored. Apart from investigating the association between viscosity and low colostral IgG values, further areas of study suggested are quantity and time of first ingestion, investigation of a possible feedback mechanism and the isotypes involved in passive transfer.

[a], technical literature for E-line-90 Brix refractometer, Bellingham & Stanley

References

1. Blanchard, Varner etc al. *Manual of equine reproduction*. 2nd ed, C.V Mosby
2. Bravo PW, Garnica J, Fowler ME. Immunoglobulin G concentrations in periparturient llamas, alpacas and their crias. *Small ruminant research* 1997; 26: 145-149
3. Drew M, Fowler ME. Comparison of methods for measuring serum immunoglobulin concentrations in neonatal llamas. *JAVMA* 1995; 206: 1374-1380
4. Cash R.S.G. Colostral quality determination by simple refractometry, a preliminary investigation. *Centaur* 1995; XI:56-59
5. Chavatte P. Field determination of colostrums quality by using a novel practical method. *AAEP Proceedings* 1998; 44: 206-209
6. Erhard MH, Luft C. Assessment of colostral transfer and systemic availability of immunoglobulin G in new-born foals using a newly developed enzyme- linked immunosorbent assay (ELISA) system. *J Anim Physiol Anim Nutr* 2001; 85: 164-173
7. Garmendia AE et al. Failure of passive immunoglobulin transfer: a major determinant of mortality in newborn alpacas. *Am J Vet Res* 1987;48:1472-1476
8. Garmendia AE et al. Mechanism and isotypes involved in passive immunoglobulin transfer to the newborn alpaca. *AJVR* 1987; 48: 1465-1471
9. Koterba et al. Immunological considerations. *Equine Clinical Neonatology.* 1st ed, Lea& Febiger 1990
10. LeBlanc MM. Factors that influence passive transfer of immunoglobulins in foals. *JAVMA* 1992; 200: 179-183
11. LeBlanc MM. Relationships among serum immunoglobulin concentration in foals, colostral specific gravity and colostral immunoglobulin concentration. *J Am Vet Med Assoc.* 1986; 189: 57-60
12. Lewis. *Veterinary clinical Immunology.* 1st ed, Lea & Febiger 1989
13. Mair, Love, Schumacher & Watson. *Equine medicine, surgery & reproduction* 1st ed, Saunders 1998
14. McKinnon A.O, Voss J.L et al. *Equine reproduction* 1st ed, Lippincott Williams & Wilkings 1993
15. McGuire TC et al. Failure of colostral immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. *JAVMA* 1977; 170: 1302-1304
16. McGuire TC, Banks KL. Neonatal immunology. *Veterinary clinical immunology*, 1st ed, Saunders 1989
17. Michanek P. Transfer of colostral immunoglobulins to newborn dairy calves. Dissertation SLU, Sveriges Lantbruks Universitet 1994
18. Mohammed HO. Factors associated with the levels of immunoglobulins in colostrum. *Acta Vet Scand. (Suppl)* 1988; 84: 119-121
19. Morris DD. Passive transfer failure in horses; incidence and causative factors on a breeding farm. *AJVR* 1985; 46 2294-2299
20. Stott GH, Fellah A. Colostral immunoglobulin absorption linearly related to concentration for calves. *J Dairy Science* 1983; 66: 1319-1328
21. Tizard. *Veterinary immunology*, 6th ed, Saunders 2000
22. Waelchi RO. Relationships of total protein, specific gravity, viscosity, refractive index and latex agglutination to immunoglobulin G concentration in mare colostrum. *Equine Vet J* 1990; 22: 29-42
23. Weaver D. Passive transfer of colostral immunoglobulin G in neonatal llamas and alpacas. *AJVR*, 2000, vol 61; 4
24. Weaver D. Evaluation of assays for determination of passive transfer status in neonatal llamas and alpacas. *JAVMA* 2000; 216: 559-563
25. Wernery U. Camelid immunoglobulins and their importance for the new born – a review. *J. Vet. Med* 2001; B 48: 561-568

Acknowledgements:
This study was financed by UFAW Small Projects and Travels awards, and by The Zebra Foundation. The author is hugely indebted to the support of Dr. Jane Wheeler and Dr Raul Rosadio, Dr Brian Aldridge, Juan Olazabal, Hugo Castillo Doloriert, Gustaf Rydevik and all involved staff at La Raya UNA research station and
Alianza, Macusani. Peru.