

# Salivary IgG levels in neonatal calves and its association to serum IgG: an observational pilot study

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**ABSTRACT:** The diagnosis of inadequate transfer of colostrum immunoglobulin G (IgG) to calf serum, often known as failure of passive transfer (<10 g/L IgG1 at 24 to 48 h), necessitates blood sampling from the calf and in some instances the presence of a veterinarian. Sampling saliva is both less invasive and easy for the producer. Previous research has shown that quantification of saliva IgG is possible in juvenile and adult cattle. The objectives of this observational pilot study were to investigate whether IgG can be quantified in neonatal calf saliva, if it is correlated to serum IgG concentrations, and if the indirect quantification of saliva IgG is achievable by use of a digital refractometer. Paired blood and saliva samples were collected from 20 healthy dairy calves aged 1 to 3 d. In these samples, IgG was quantified directly with single radial immunodiffusion and indirectly by use of a digital refractometer indicating Brix % (a subsample of  $n = 12$  saliva samples). A strong positive correlation

( $r = 0.7$ ,  $P < 0.001$ ) between saliva IgG (mean  $\pm$  SD;  $0.2 \pm 0.11$  g/L) and serum IgG ( $32.1 \pm 11.94$  g/L) was found. Saliva IgG ranged from the lowest detectable value, 0.1 g/L ( $n = 6$  samples) to 0.6 g/L. Saliva Brix ( $1.2 \pm 0.69\%$ ) was not significantly correlated to serum IgG ( $n = 12$ ,  $r = 0.43$ ,  $P = 0.155$ ); however, it was significantly correlated to saliva IgG ( $n = 12$ ,  $r = 0.7$ ,  $P = 0.018$ ) and Brix in serum ( $n = 12$ ,  $r = 0.7$ ,  $P = 0.013$ ). We conclude that IgG was quantifiable in most of the saliva samples. For saliva IgG to be of any value with regards to detecting failure of passive transfer, future studies should investigate methods that can detect IgG <0.1 g/L. The results indicate that saliva IgG can be used to predict serum IgG at levels above 10 g/L, which may warrant further exploration of the use of saliva in the surveillance of failure of passive transfer. The results of the current pilot study did not support the potential usage of a Brix % refractometer to quantify saliva IgG.

**Key words:** dairy, health, immunity, welfare

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## INTRODUCTION

A successful colostrum management program requires that calves receive a sufficient

volume of clean, high-quality colostrum within the first few hours of life (Godden, 2008). Inadequate transfer of immunoglobulines (Igs; isotypes IgG1 through IgG4; IgG1 being the most predominant) is commonly defined by serum IgG1 levels of <10 g/L at 24 to 48 h of age, a condition called failure of passive transfer (FPT; Besser et al., 1991). Because FPT is associated with increased mortality, and decreased weight gain (Robison et al., 1988; Wells et al., 1996; Weaver et al., 2000), it represents a risk for poor calf welfare, i.e., increased risk of injury, disease, negative feelings, or failure to cope (EFSA, 2012). To investigate FPT, serum has been used; however, blood sampling is invasive. The presence of a veterinarian is also required in some instances (as in Norway and Italy; Ministry of Agriculture and Food, 2001 (Norway), and National Association of Veterinarians Italy, 2019). If absorbed maternal IgG1 transfers into the calf's saliva and its concentration predicts that of serum, saliva may represent a noninvasive medium from which FPT status of calves could be evaluated. In young (4 mo) and adult cattle, IgG has been determined in saliva (e.g., Butler et al., 1972; Duncan et al., 1972). However, due to the intestinal transmission of Igs to the blood circulation occurring until "gut closure" (Deutsch and Smith, 1957), the saliva IgG profile of neonatal calves may differ from that of juvenile or adult cattle. To the authors' knowledge no study has investigated salivary IgG levels in neonatal calves and its correlation to serum IgG.

Recently, methods to determine the adequacy of both colostrum quality, i.e., colostrum IgG >50 g/L; McGuirk and Collins (2004), and calf serum IgG have been developed for use on dairy operations. Digital refractometers indirectly assess IgG levels through relative density (Brix %) and have proven valuable for use by producers (Chigerwe and Hagey, 2014; Elsohaby et al., 2015; Morrill et al., 2015) for colostrum as well as serum. If IgG in saliva reliably predicts that of serum, its indirect quantification by use of a digital refractometer could represent an easy way for producers to classify each calf's passive transfer status without blood sampling.

The purpose of this observational pilot study was to investigate whether it is possible to detect and quantify IgG1 in saliva of neonatal calves using both single radial immunodiffusion (SRID) and digital refractometry and to investigate associations to both serum IgG and serum Brix.

## MATERIALS AND METHODS

### *Animals and Management*

All procedures were in accordance with the regulations controlling experiments and procedures in live animals in Norway (Ministry of Agriculture and Food, 2015). We used 20 Norwegian Red dairy calves (10 bull calves and 10 heifer calves; the experimental units) kept at the research facility of the Norwegian University of Life Sciences. Calves were separated from the dam immediately after birth and housed in single pens with straw bedding. Only calves without clinical signs of disease were included. Within 4 h of birth, calves were bottle-fed 4 L of colostrum from their own dam. Colostrum quality was measured with a digital refractometer (Digital Brix refractometer MA871; Milwaukee Instruments, Inc., Rocky Mount, NC, USA). Colostrum with Brix % >24 was considered high quality whereas colostrum with Brix 18% to 24% was considered of medium quality. Calves that did not drink the entire amount, or received colostrum of medium quality were again offered colostrum 2 h later. During the first 3 d after birth, the calves were bottle-fed colostrum or transition milk (7 L divided on 5 meals per d) from their own dam and had free access to hay, concentrate, and water from the first day.

### *Sampling and Analyses of Saliva and Serum*

Between d 1 and 3, matched samples of saliva and blood from the calves were obtained. To minimize risk of contamination from previous milk meals, milk was retained for at least 2 h prior to sampling. Saliva samples were taken with a clamped cotton swab (IVF Schaufhauser Dental Rolls No. 2; Paul Hartmann AG, Neuheusen, Switzerland), which was held into the calves' mouth for 1 min until it was soaked with saliva (Geburt et al., 2015). Thereafter, the cotton was compressed using a 20 mL syringe, following which saliva was collected in Eppendorf tubes (ref. 72.706.400, Sarstedt AG & Co, Nümbrecht, Germany). Subsequent to saliva collection, blood was collected via jugular vein puncture into 10 mL vacutainer tubes (VACUETTE, Greiner Bio-One, Kremsmünster, Austria). Then, the blood sample was centrifuged immediately in order to extract serum. With a digital refractometer (same as earlier the Brix values of both serum and subsample of ( $n = 12$ ) saliva samples were obtained within 60 min after sampling. Serum and saliva samples were thereafter stored at  $-18^{\circ}\text{C}$ . Once all samples were collected, they were submitted in cool, insulated boxes to TINE

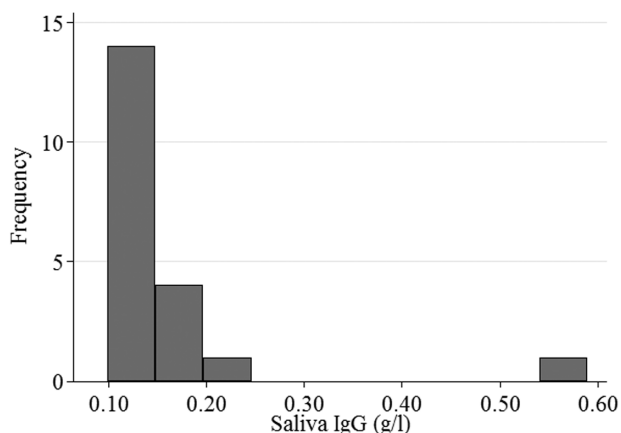
Mastittlaboratoriet i Molde, Norway, for analyses. To determine IgG in saliva and serum, SRID Ultra Low Level and SRID (Triple J Farms, Bellingham, WA, USA) respectively were used according to the manufacture instructions. Lower detection limits of the two tests are 0.1 and 1.9 g/L, and the detection range of the digital refractometer was 0% to 85% according to manufacturers (Triple J Farms).

### Statistical Analysis

A normal distribution of serum IgG was confirmed by visual inspection (Stata, version 14, StataCorp LLC, College Station, TX, USA). Saliva IgG was right skewed and Spearman rank correlation coefficients were calculated to investigate associations between saliva IgG and serum IgG as well as saliva Brix, and also serum IgG and serum Brix. A linear regression was used to determine how well saliva IgG explains variation in serum IgG. Inspection of the residuals in the residuals-vs.-fitted values plot (rvfplot command) revealed two outlying cases that were far removed in value from the others. The model was rerun without these outliers to investigate their effect of on the model output. Results from both models are presented. Significance was declared at  $P < 0.05$ .

## RESULTS AND DISCUSSION

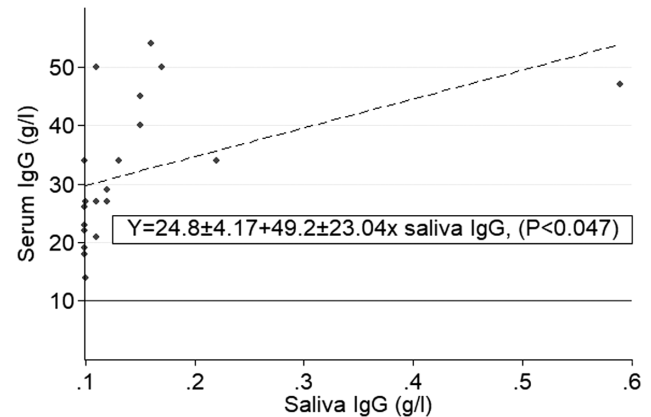
Mean ( $\pm$ SD) serum IgG was 32.1 ( $\pm$ 11.94) g/L ranging from 14 to 54 g/L and mean serum Brix % was  $9.9 \pm 0.82$  ranging from 8.2% to 11.2%. Mean saliva IgG was 0.2 ( $\pm$ 0.11) g/L ranging from the lowest detectable value, 0.1 g/L ( $n = 6$  samples) to 0.6 g/L. A strong significant positive correlation ( $r = 0.7$ ,  $P < 0.001$ ) between saliva IgG and serum IgG was found. Except for one sample (0.6 g/L), all samples had values around 0.1 to 0.2 g/L (Figure 1).



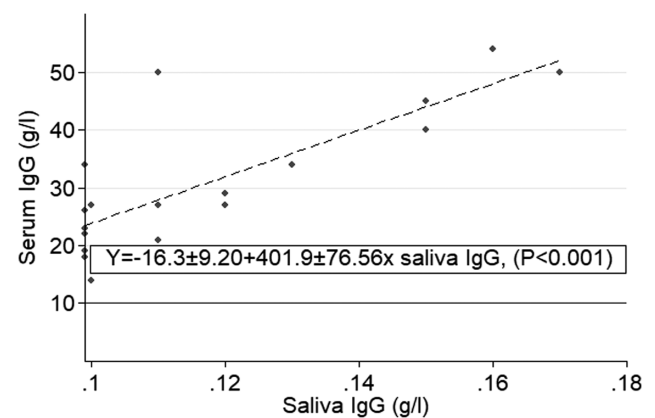
**Figure 1.** The frequency distribution of calf saliva IgG in 20 samples taken from 1- to 3-d-old dairy calves.

Linear regression showed that saliva IgG significantly explained variation in serum IgG, model fit was  $R^2 = 0.20$ ,  $P = 0.047$  (Figure 2). Two of the outlying saliva IgG values (0.2 and 0.6 g/L IgG, respectively) had a strong influence on the regression equation, and removal of the outliers resulted in an increase in  $R^2 = 0.63$ ,  $P < 0.001$  (Figure 3).

Mean Brix in saliva were 1.2 ( $\pm$ 0.69)% with ranges from 0.0% (one sample) to 2.5%. Saliva IgG also correlated significantly with saliva Brix values ( $n = 12$ ,  $r = 0.7$ ,  $P = 0.018$ ). Although a significant correlation between the Brix values of saliva and serum IgG measured by SRID was not confirmed ( $n = 12$ ,  $r = 0.43$ ,  $P = 0.155$ ), Brix values of saliva did correlate with Brix in serum ( $n = 12$ ,  $r = 0.7$ ,  $P = 0.013$ ). As expected, serum IgG correlated with serum Brix ( $n = 20$ ,  $r = 0.8$ ,  $P < 0.001$ ).



**Figure 2.** Relationship between serum IgG and saliva IgG measured with single radial immunodiffusion in 20 one- to three-d-old dairy calves. The solid horizontal reference line indicates serum IgG levels below which calves are diagnosed with failure of passive transfer. The dotted line indicates the line of best fit.



**Figure 3.** Relationship between serum IgG and saliva IgG measured with single radial immunodiffusion after removal of two outlying saliva IgG values ( $n = 18$  dairy calves). The solid horizontal reference line indicates serum IgG levels below which calves are diagnosed with failure of passive transfer. The dotted line indicates the line of best fit.

IgG was quantifiable in 14 of 20 of the saliva samples and the results indicate that saliva IgG can predict that of serum IgG. Saliva IgG of neonatal calves were higher than that of adult cattle: 0.034 g/L, range 0.017 to 0.050 (Butler, 1983). There was variability in the saliva IgG values, which should be evaluated using larger study samples. It is unknown why IgG in one of the saliva samples was threefold that of average IgG. An inadvertent laceration of the mucous membranes by the metal clamp used to hold the cottons swab could potentially cause a contamination of the sample, although no apparent signs of blood were seen on any of the cotton swabs. This calf was very lively at sampling, which possibly could influence the measurement of IgG. Residual colostrum in the calves' mouth may also have caused a false inflation in the saliva IgG levels. Measures were taken to prevent this, since milk was retained for at least 2 h prior to saliva sampling. However, optimal sampling routines with respect to time of the day, time since feeding etc. can be investigated in future research. Further studies are also needed to investigate saliva IgG with more sensitive methods. For comparison, Duncan et al. (1972) found small amounts of IgG1 have been found in (juvenile and adult) ruminant tears ( $0.15 \pm 0.09$  g/L) and nasal secretions ( $0.16 \pm 0.12$  g/L). IgA and not IgG is the most abundant Ig in saliva, which affects the total protein content (Butler et al., 1972). The latter may also affect the indirect quantification of IgG by use of Brix. Owing to low IgG concentrations in saliva relative to IgA, other fluids may be more suitable for the assessment of passive immunity. The studies of Duncan et al. (1972) and Butler et al. (1972) revealed higher IgG levels in e.g., nasal secretions, tears, and vaginal secretions.

Limitations to the dataset exist which should be taken into consideration while evaluating the results. Of the saliva samples, 30% had IgG levels below the detection limit of the SRID test ( $<0.1$  g/L). More sensitive methods to quantify IgG (e.g., enzyme-linked immunosorbent assay or turbidimetric immunoassay) should be explored in future studies (McVicker et al., 2002; Dunn et al., 2018). The correlation of saliva IgG to other indirect measures of IgG, as serum total protein, is of interest in future research. None of the sampled calves had serum IgG levels  $<10$  g/L. Therefore, it is unknown how the lower serum IgG levels (and thus calves with FPT) can be predicted by saliva IgG given that IgG values  $<0.1$  g/L can be quantified using other methods.

Most of the IgG in human saliva originates from the blood circulation, and thus represents

systemic immunity by passive diffusion from serum (Brandtzaeg, 2013). The design of our study cannot explain the origin of the IgG1 found in the calf saliva. However, it is likely to be derived from local synthesis, selective transport from the blood, or both (Pedersen, 1973). The study of Husband and Lascelles (1975) indicates that the calves' self-synthesized IgG appears first at 32 d postpartum, which suggests that the IgG measured in the calves' saliva originated from colostrum. On the other hand, self-synthesis of IgG1 has been proven already during the first week of life by others (Sasaki et al., 1977; Devery et al., 1979). The selective transport of IgG1 from plasma to tears has been proven in 4- to 10-mo-old cattle (Pedersen, 1973). A decreased permeability of the calves' intestinal mucosa to large molecules, "gut closure," occurs already subsequent to the first colostrum-feeding (Michanek et al., 1989) and a similar mechanism may occur in the oral mucosa (Brandtzaeg, 2013). However, colostrum IgG may similarly pass into calf saliva as Smith et al. (1976) showed that colostrum IgG passes into nasal mucus of lambs. Unfortunately, this study did not investigate IgG1 levels in pre-colostrum saliva that could yield more knowledge on the origin of the saliva IgG. We encourage future studies to include this measure to improve the understanding of the saliva IgG kinetics.

Although only 12 of the calves' saliva were investigated using the digital refractometer, saliva Brix was associated with saliva IgG. However, this study could not confirm that saliva Brix % is a valid indirect measure of serum IgG. Possible reasons for the lack of correlation between serum IgG and saliva Brix can be related to different fractions and concentrations of Igs in saliva vs. that of serum (Butler, 1983). A recent review revealed that the diagnostic accuracy of Brix refractometry to diagnose FPT in calves is varying (Buczinski et al., 2018). The results of this study could not support that saliva Brix can predict serum IgG.

Our study was based on a low number of calves and as such results can only provide the basis for future studies on the relationship between calf serum and saliva IgG. However, the usefulness of saliva as a medium to assess calf passive transfer of immunity from colostrum may deserve further attention.

*Conflict of interest statement.* None declared.

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