Sveriges lantbruksuniversitet
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Slutrapport

Projekttitel
Råmjölkens antikroppskvalitet och kalvars upptag av antikroppar från råmjölken hos mjölkkor: betydelse för hälsa och produktion, samt möjligheter till genetisk selektion

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Del 1: Utförlig sammanfattning

Syftet med detta projekt var att identifiera genetiska och skötselfaktorer som kan ligga bakom skillnaden i råmjölkskvalitet hos kor och upptag av råmjölksantikroppar hos kalv. Det slutliga målet är att kunna förbättra hälsa och produktion genom att inkludera råmjölkskvalitet och upptag av råmjölksantikroppar i framtida avelsprogram. Vi följde kor och kalvar vid tre försöksgårdar under en tvåårsperiod genom att samla råmjölks- och blodprover samt utförlig information om råmjölksrutiner, hälsa och produktion. Råmjölk och blodprover analyserades med avseende på antikroppar med hjälp av refraktometer och laboratorieanalyser. Genetiska data för alla djur samlades också in. Tack vare goda skötselrutiner och hög biosäkerhet såg vi inga signifikanta skillnader i kalvhälsan mellan kalvar med lågt respektive högt upptag av råmjölksantikroppar. Däremot såg vi att kalvar med lågt upptag av råmjölksantikroppar löpte mycket högre risk att drabbas av diarré och lunginflammation i samband med sjukdomsutbrott, medan kalvar med högt upptag av

råmjölksantikroppar var skyddade. Vi fann att råmjölkskvalitet och upptag av råmjölksantikroppar är ärftliga egenskaper på ungefär samma eller högre nivå än egenskaper som nu finns med i avelsprogrammet, till exempel mastit och klövhälsa. Våra resultat tyder på att det inte finns någon genetisk korrelation till mjölkproduktion, vilket betyder att kons mjölkproduktion inte påverkas vid urval för råmjölkskvalitet. Det enklaste och bästa sättet vore därför att inkludera råmjölkskvalitet utvärderat genom Brix-refraktometer i ett framtida avelsprogram. På detta sätt kan råmjölkskvaliteten förbättras och indirekt även kalvens upptag av råmjölksantikroppar, då detta är kopplat till kons råmjölkskvalitet.

Del 2: Rapporten (max 10 sidor)

Inledning / Introduction

The ruminant fetus is not capable of receiving maternal antibodies in the uterus because of the placental barrier. Thus, the newborn calf is dependent on the intake of colostrum, where maternal antibodies (especially immunoglobulin G; IgG) are concentrated during the final period before calving, to get a sufficient protection before developing its own immunity. The absorption of antibodies from colostrum is also called passive transfer. When this process is not leading to a sufficient serum concentration of IgG (<10 g/L or 5.5 g/dL of serum total protein according to the literature; Gooden, 2008), it is called failure of passive transfer (FPT). Previous studies have shown that FPT can lead to impaired calf health and welfare due to increased risk for infections. Most Swedish dairy herds have a very good calf health with a low mortality in calves at age 1-60 days (median 0.7%), but still there are differences with 10% of the herds having a calf mortality above 5.9% (Växa Sverige, 2018). These differences might be explained by colostrum management routines (e.g. volume and time from birth when colostrum is given) and FPT, as well as infection pressure and biosecurity measures. Previous studies have also indicated that FPT is common in Swedish dairy herds with 30-50% of calves having FPT (Silverlås et al., 2010; Torsein et al., 2011). There is also an observed variation in colostrum antibody content between cows (Gulliksen et al., 2008; Morrill et al., 2012), and several factors are known to cause this variation, e.g. number of lactations (Gulliksen et al., 2008; Morrill et al., 2012) and time from calving to first milking (Moore et al., 2005). There are thus a number of environmental and management factors affecting the colostrum antibody content and absorption of antibodies from colostrum, but these cannot solely explain the observed variation, neither in serum-IgG levels in calves nor in colostrum antibody content in cows. Several studies indicate that genetics is also accountable for this variation. For example, the apparent efficiency of absorption (AEA) of IgG from colostrum was highly variable between calves even when a standardized feeding regime was used (Halleran et al., 2017). Although there is obviously a genetic effect, there are so far only a limited number of studies where the role of genetics has been studied in more detail. These studies have suggested a genetic variation in the transfer of IgG to colostrum (Zhang et al., 2009) and in absorption of colostrum antibodies in calves (Laegreid et al., 2002; Clawson et al., 2004), linked to variations in the neonatal Fc receptor (FcRn) gene. This receptor is an important component in the specific transport of antibodies in the udder of lactating animals to concentrate them in colostrum, and in the gut epithelium of neonatal animals to transport antibodies to serum (Cervenak & Kacskovics, 2009). Certain variants of the FcRn gene have been associated with impaired uptake of antibodies in newborn beef calves in the USA (Laegreid et al., 2002; Clawson et al., 2004), while the only published study in dairy calves has not found this association (Maltecca et al., 2008).

The overall aim of this project was therefore focused on mapping the genetic factors explaining the variation in colostrum quality and passive transfer. We followed three research farms over a 2-year period and collected samples from cows and calves to establish colostrum quality and passive transfer in these animals. We carefully recorded data on colostrum management routines and health, and retrieved health and production data from the databases of Växa Sverige and at the herds.

Materiell och metoder / Material and methods

Study design

Three research farms were included in the study: 1) The Swedish Livestock Research Center Lövsta (Lövsta); 2) Röbäcksdalen Research Station SITES (Röbäcksdalen); and 3) Nötcenter Viken (Viken). The herds were selected partly because of animals at the herds already are being genotyped on a routine basis for genetic evaluation. Lövsta and Viken include both Swedish Holstein (SLB) and Swedish Red (SRB), while Röbäcksdalen only includes SRB. The farms were studied from January 2015 to April 2017. Basically, colostrum from all cows giving birth to a calf were sampled, and serum and blood samples from all calves born during the study period were collected. In addition, serum samples prior to calving and first test milk after calving for a subset of cows were collected.

Sampling of first milking colostrum was carried out by staff at the farms, representing the first meal of colostrum given to calves. Farm staff also recorded data on time between birth and colostrum sampling, volume of first meal and during the first 24 h. Data on treatments, birth weight, etc were also retrieved from the farms, and health and production data were retrieved from the Swedish Official Milk Recording Scheme, Växa Sverige.

Estimation of immunoglobulins in colostrum

Colostrum samples were analyzed using a digital refractometer (Atago 3810 PAL-1; Atago, Tokyo, Japan) to measure Brix percentage as an estimate for IgG content. All samples were analyzed in the lab, where each sample was measured at least three times to average a sample value. Most samples were also measured on farm.

Estimation of serum total protein

Optical refractometer was used to estimate serum total protein levels as a proxy for colostrum antibody uptake in the calves. A subset of calf serum samples was also analyzed using the Brix refractometer.

Measurement of total IgG in colostrum and serum

Colostrum IgG was measured only in samples with a matching calf serum sample using a commercial ELISA (Bovine IgG ELISA Kit E-10G; Immunology Consultants Laboratory Inc, OR, USA). In total, 686 colostrum samples were analyzed.

Serum IgG concentration was determined using another commercial ELISA (Bovine IgG ELISA Quantitation Set E10-118; Bethyl Laboratories Inc, TX, USA). In total, 810 samples were analyzed. For detailed description of the methodology, see Cordero Solórzano, 2020.

Measurement of natural antibodies (NAb) in colostrum, milk and serum

Titers for natural antibodies were measured in all sample types for cows (colostrum, first-test milk and pre-parturition serum) and calves (serum) using previously described inhouse ELISAs (Ploegaert et al., 2010). For detailed description of the methodology, see Cordero Solórzano, 2020.

Pedigree data and genotypes

Pedigree data for all animals were made available by Växa Sverige and contained 29048 records (20 generations). Most animals had already been genotyped as part of the Nordic Cattle Genetic Evaluation (NAV) routine, but for 445 animals not retained for breeding (bull calves and non-replacement heifers) were genotyped within the project. This was done by EuroFins GenoSkan (Aarhus, Denmark) using BovineLD BeadChip 7K chip assay (Illumina, San Diego, CA, USA), the same chip as used for the animals already genotyped. Imputed 50 K SNP genotypes were provided by NAV. Imputations were only performed on purebred SRB or SLB individuals. Finally, 706 calves and 829 cows from the project had imputed 50K genotypes.

Statistical analysis

We used ASReml 4.1 (Gilmour et al., 2015) for our models to estimate correlations (genotypic, phenotypic incl. production and health data) and to perform genome-wide association studies. For detailed description of models, see Cordero Solórzano, 2020.

Candidate genes

We searched for genes within the regions associated with a trait using NCBI (NCBI Resource Coordinators, 2018) and Ensembl (Zerbino et al., 2018) databases. The identified genes were analyzed by WebGestalt (Wang et al., 2017) to determine which biological pathways they were related to.

Resultat och discussion / Results and discussion

Descriptive statistics – number of cows and calves included in the project

In total, 1340 cows were sampled (SRB: n=682, SLB: n=460 and SLB/SRB crossbreds [CRB]: n=198). Parities ranged from 1 to 6 with around 90% of the animals between 1 and 3 parities. During the sampling period 504 cows calved twice and 29 calved three times. We collected 330 serum samples from cows 1-23 days before calving and 290 milk samples from the first-test milking after calving (6-24 days in mik). In addition, we retrieved milk production data from 1283 cows.

In total, 886 calves were sampled at Lövsta and Röbäcksdalen, however, 59 of them had an unknown pedigree and were thus excluded from the study. The remaining calves were 520 SRB, 211 SLB and 96 CRB.

Descriptive statistics – colostrum quality and passive transfer

For both colostrum quality (estimates of IgG content through Brix refractometer and IgG-ELISA) in cows and passive transfer (STP and serum [S]-IgG), there was a high individual variation. The colostrum quality ranged 14.5 – 28.7 Brix% (excluding outliers) with a mean of 21.9±4.2 (Figure 1), just at the cutoff for colostrum of sufficient quality according to literature (22 Brix%; Buczinski & Vandeweerd, 2016). When using IgG-ELISA, the colostrum IgG concentration range was 19.7 – 106.6 g/L (excluding outliers), with a mean of 56.8±26.9 g/L. Thus, the mean value is above the cutoff for good quality colostrum according to the literature (≥50 g/L; Buczinski & Vandeweerd, 2016).

The STP values in calves varied from 4.9 – 7.2 g/dL (excluding outliers) with a mean of 6.0±0.7 g/dL. Thus, the average STP was above the threshold for passive transfer (STP <5.5 g/dL when failure of passive transfer; Godden, 2008). Likewise, the mean value of S-IgG (22.8±12.8) was above the cutoff of 10 g/L (Godden, 2008), although with a high individual variation (range: 5.6 – 48.7 g/L; excluding outliers).

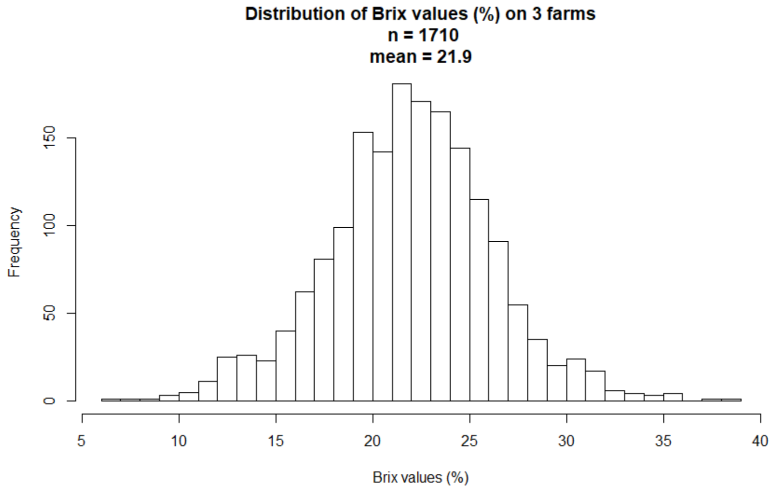

Figure 1. Distribution of Brix values of all colostrum samples.

Factors influencing colostrum quality

As expected, we also observed several previously described factors influencing the observed colostrum quality, and these factors were needed to take into account in our model. These factors include parity and time from calving to colostrum sampling. Increasing parity (from parity 3 and above) clearly leads to a higher concentration of colostrum IgG (Figure 2), which is consistent with previous literature (Conneely et al., 2013). In consistence with previous literature (Moore et al., 2005), we could also see a decrease in colostrum IgG concentration when the time from calving to colostrum sampling increased. It is therefore important to milk the cow short after calving to get as a high colostrum quality as possible.

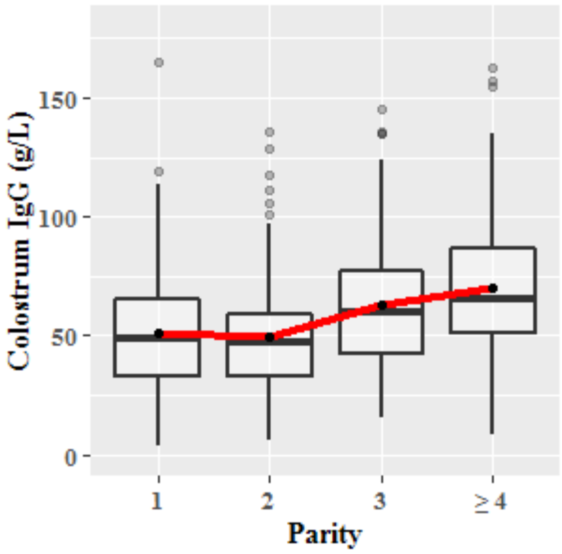

Figure 2. Plot of colostrum IgG for each parity (Cordero Solórzano, 2020).

Methodological considerations when evaluating passive transfer

STP by optical refractometer is a commonly used method to estimate passive transfer. It has previously been compared to radial immunodiffusion, which is considered to be the gold standard for IgG measurement (Godden, 2008). We measured all serum samples by optical refractometer and by a commercially available IgG-ELISA. In addition, a subset of samples (n=161) was also analyzed by Brix refractometer. The correlation between optical and Brix refractometer was very high (Figure 3; de Haan, 2018), and a STP of 5.5 g/dL corresponded to 8.5 Brix%, similar to what have been reported in other studies (Morrill et al., 2013; Deelen et al., 2014; Thornhill et al., 2015). Overall, the S-IgG values were unexpectedly high, thus we are not confident that the observed value reflects the true S-IgG value (Larsson, 2018).

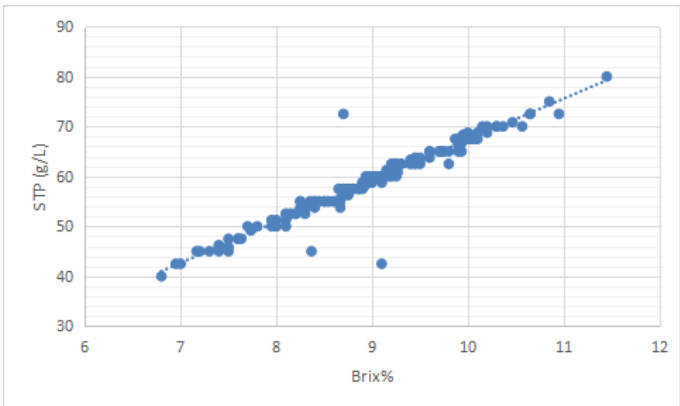

Figure 3. Comparison of optical and Brix refractometry in a subset of samples (n=161; de Haan, 2018).

Heritability

Based on the pedigree data, we were able to estimate the heritability of colostrum quality and passive transfer (Cordero Solórzano, 2020; Cordero Solórzano et al., in preparation). For passive transfer, the heritability was judged moderate based on S-IgG (0.25±0.13), but not significant for STP. The heritability for colostrum quality, was judged moderate for both Brix refractometry (0.31±0.06) and colostrum IgG (0.20±0.09) measured by ELISA. The heritability for these traits is similar or higher than traits already included in the breeding program. For example, milk yield has a heritability of 0.22-0.38 (Mäntysaari et al., 2006), clinical mastitis 0.07-0.08 (Urioste et al., 2012) and claw health 0.03-0.18 (Heringstad et al., 2018). Thus, there is a possibility to include passive transfer and colostrum quality in the breeding program to improve these traits. More practically, using Brix refractometry to evaluate colostrum quality would be a fairly easy tool to use for a breeding program.

There was a significant breed effect, where SLB and CRB on average had higher values for colostrum quality compared to SRB. For SLB, Brix and total IgG had a mean of 23.3 Brix% and 65.9 g/L respectively, as opposed to SRB with 21.2 Brix% and 53.1 g/L with the difference between breeds being statistically significant for both traits (*p-value* $< 1 \times 10^{-8}$). This means that if the same breeding goal is set for colostrum quality on both breeds, it might take more generations or stronger selection intensity for SRB to increase the colostrum quality compared to SLB.

We also estimated the heritability for various natural antibodies in colostrum and in calf serum as a result of passive transfer. Heritability for these traits were also moderate to high.

Genome-wide association study on colostrum quality in cows

Based on the genotypic data of the cows, we were able to perform a GWAS and identified two significant genome regions for colostrum IgG (Cordero Solórzano, 2020; Cordero Solórzano et al., in preparation). The strongest association was on bovine chromosome 20. We propose Myosin X as a candidate gene, which is involved in Fc gamma receptor mediated phagocytosis. Other candidate genes identified are involved in various immunological pathways, such as T cell receptor signaling, cytokine-cytokine receptor interaction and immunoglobulin secretion.

In a study on similar traits in a Dutch Holstein-Friesian cattle population, we could show that a higher density of the genetic markers (imputed 777 000 SNPs compared to 50 000 SNPs in the Swedish data) lead to increased likelihood of finding accurate candidate genes and at a higher resolution (Cordero-Solorzano et al., 2019). Thus, it would be important to re-genotype the Swedish study population (cows and calves) using a higher number of genetic markers to better assess the possibilities for genetic selection.

Genome-wide association study on passive transfer in calves

For passive transfer in calves, we were able to identify one significant and two suggestive genome regions for S-IgG (Cordero Solórzano, 2020; Cordero Solórzano et al., in preparation). In addition, several other genomic regions were identified for natural antibodies. For S-IgG, the strongest association was on bovine chromosome 6, and for this chromosome we found three candidate genes related to salivary secretion. Candidate genes for natural antibodies included genes involved in gastric acid secretion, tight junction, but also immunological pathways, similar to the ones found for colostrum quality in cows.

Colostrum quality, passive transfer and effects on health and production
We investigated whether genetic selection for colostrum quality would negatively affect milk production (milk yield, milk composition and lactation average somatic cell score). We could not find any such significant genetic correlations, suggesting that breeding for improved colostrum quality would not negatively impact milk production.
Regarding calf health, overall data for animals during the first three months of age showed no significant differences between FPT and normal ones. However, for animals born during summer 2015, sick calves (pneumonia and diarrhea) have significantly lower levels of STP (sick: 5.1 g/dL vs. healthy: 5.8 g/dL; $p = 0.006$) and S-IgG (sick: 7.7 g/L vs. healthy: 16.6 g/L; $p = 0.009$). These results suggest that in an event of increased infection pressure, such as an outbreak of infectious disease, calves with low passive transfer or FPT are at a significantly higher risk for developing clinical signs. Thus, these results are in line with previous literature, showing higher risk for FPT calves to contract various diseases (Raboisson et al., 2016).

Slutsatser / Conclusions

A sufficient passive transfer in calves is essential for the calf health, but good management and a high biosecurity can reduce the impact on calf health. In case of an outbreak situation, calves with low passive transfer or FPT are at significantly higher risk for developing clinical signs of pneumonia and diarrhea.
There is a moderate heritability for colostrum quality and passive transfer, similar or higher than traits already included in the current breeding program. Selective breeding could therefore be used to improve colostrum quality and reduce the frequency of FPT. For calves, this would require an extensive phenotyping using IgG-ELISA, since STP analysis by refractometry did not result in any significant heritability. Evaluation of colostrum quality by a Brix refractometer on farm could be implemented in the breeding program to improve the colostrum quality and indirectly reduce the frequency of FPT. We identified a few candidate genes, although the genomic regions were not strongly associated with the traits. It would therefore be important to re-genotype the Swedish study population (cows and calves) using a higher number of genetic markers to be able to pinpoint genetic markers at a much higher resolution.

Nytta för näringen och rekommendationer

The project results show that colostrum quality and passive transfer are heritable, and our results suggest no significant genetic correlation with production traits. Selective breeding could therefore be used to improve colostrum quality, but further studies would be needed to elucidate the calves’ genetic component. We recommend genetic studies using higher density of genetic markers to further understand whether colostrum quality and passive transfer would negatively or positively affect other production traits. Furthermore, we recommend that long-term effects of colostrum absorption are included in such studies.

Good management and a high biosecurity can reduce the impact of failure of passive transfer (FPT) on calf health. During outbreak events, calves with low passive transfer or FPT are at a high risk for contracting disease. Good colostrum feeding routines are therefore crucial. We recommend all farmers to evaluate the colostrum quality in all cows using a Brix refractometer. This tool is hand-held and very easy to use. Colostrum of sufficient quality (≥22 Brix%) can be used to feed calves and if the colostrum is of poorer quality (18-22 Brix%), the calf should be supplementary fed with high quality colostrum. Colostrum of high quality (>25 Brix%) should be archived in a colostrum bank.

If a recording system at farm level for colostrum quality could be put in place, we believe that this could increase our understanding for the variation of colostrum quality and indirectly failure of passive transfer.

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Del 3: Resultatförmedling

Ange resultatförmedling av projektet, inklusive titel, referens, datum, författare/talare, och länk till presentation eller publikation om tillämpligt. Planerade publiceringar (med preliminära titlar) ska ingå i tabellen. Ytterligare rader kan läggas till i tabellen.

Vetenskapliga publiceringar	J.M. Cordero-Solorzano, J.A.J. Arts, H.K. Parmentier, H. Bovenhuis (2018). Detection of candidate regions affecting bovine IgM natural antibodies in milk. Proceedings of the World Congress on Genetics Applied to Livestock Production, Volume Biology - Disease Resistance 1, 559, 2018. http://www.wcgalp.org/proceedings/2018/detection-candidate-regions-affecting-bovine-igm-natural-antibodies-milk
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	Juan Cordero Solórzano (2020). Genetics of colostrum, milk, and serum antibodies in dairy cattle. Implications for health and production. Doctoral thesis No. 2020:4. Swedish University of Agricultural Sciences and Wageningen University & Research. https://pub.epsilon.slu.se/16658/
	Cordero-Solorzano J, Wensman JJ, Parmentier HK, Arts JAJ, Tråvén M, Bovenhuis H, De Koning DJ. Genetic parameters of colostrum and calf serum antibodies in Swedish dairy cattle. Manuscript in preparation.
	Cordero-Solorzano J, Wensman JJ, Parmentier HK, Arts JAJ, Tråvén M, Bovenhuis H, De Koning DJ. Total IgG and natural antibodies in colostrum of Swedish dairy cows: a genome-wide association study. Manuscript in preparation.
	Cordero-Solorzano J, Wensman JJ, Parmentier HK, Arts JAJ, Tråvén M, Bovenhuis H, De Koning DJ. Genomic regions associated with antibody uptake in newborn calves. Manuscript in preparation.
	Cordero-Solorzano J, Jouffroy M, Larsson A, De Haan T, Tråvén M, Wensman JJ. Comparison of refractrometry and IgG-ELISA to evaluate colostrum quality in dairy cows and failure of passive transfer in dairy calves. Manuscript in preparation.
	Cordero-Solorzano J, Emanuelson U, Tråvén M, Wensman JJ. Evaluation of passive transfer and health in Swedish dairy calves. Manuscript in prepration.
Övriga publiceringar	Tidningen Husdjur deltog i och uppmärksammade den workshop om råmjölkens betydelse som vi hade i mars 2020 på bl.a. FaceBook och Instagram.

Muntlig kommunikation	Wensman JJ (2015). Betydelsen av råmjölkens antikroppskvalitet och kalvars upptag av antikroppar från råmjölk för kalvhälsa och -tillväxt. Presentation på Växa Sveriges vårmöte 2015, Uppsala.
	Wensman JJ, Cordero J, de Haan T, Alenius S, Emanuelson U, Hetta M, Holtenius K, de Koning DJ, Tråvén M (2017). Variation av råmjölksantikroppar hos mjölkkor och serumtotalprotein hos kalv: möjliga orsaker och betydelse för kalvhälsa. Veterinärkongressen 2017, Uppsala.
	Wensman JJ (2018). Råmjölkens och smittryckets betydelse för kalvhälsa. Presentation på Växa Sveriges vårmöte 2018, Uppsala.
	Avslutande workshop om råmjölkens betydelse vid SLU Lövsta 2020-03-09. 18 deltagare representerande SLU, SVA, Växa Sverige, Gård och Djurhälsan, Distriktsveterinärerna, Tidningen Husdjur, Interbull. Program: Wensman JJ – Introduction Cordero Solorzano J - Genetics of colostrum, milk, and serum antibodies in dairy cattle – Implications for health and production Hernandez C – Colostrum feeding routines - Passive transfer of immunity, behaviour and welfare in dairy calves Hurri E - Good enough colostrum for the calf – how do I know? Åkerlind M - How can we help the farmers to prioritize and follow our advice? Wensman JJ - Short update from ongoing research on calf diarrhea Wensman JJ – Final reflections of the day Österberg J - Ongoing research at Lövsta (tour at the show room of the stables)
Studentarbete	Therese de Haan (2018). Hur råmjölkskvalité och upptag av immunoglobulin påverkar kalvhälsa. Examensarbete veterinärprogrammet 2018:46. https://stud.epsilon.slu.se/13666/
	Andrea Larsson (2018). Evaluation of passive transfer, apparent efficiency of absorption and health in dairy calves. Examensarbete veterinärprogrammet 2018:73. https://stud.epsilon.slu.se/14397/
	Aline Myrthe (2018). Quality of colostrum and its absorption by the calf in two Swedish breeds. Internship report. Ecole Supérieure D'Agricultures Angers Loire, France.
	Mathilde Jouffroy (2019). Evaluation of failure of passive transfer by optic refractometry for the calf. Internship report. AgroSup Dijon, France.

Övrigt	Presentation av projektet för Viking Genetics (Sören Borchersen och Hans Stålhammar), 2017-06-01 (Juan Cordero Solorzano och Jonas Johansson Wensman)
	Presentation av projektet och preliminära resultat har gjorts regelbundet för personal vid Nötcenter Viken. Vid ett tillfälle närvarade även besättningsveterinär Cor van der Beek, Distriktsveterinärerna Falköping.
	Presentation av projektet (2014/15) och preliminära resultat (2018) för SLU Röbäcksdalen.
	Presentation av projektet (2014) och preliminära resultat (2018) för SLU Lövsta.