Authors

C.G. McAloon, P. Whyte, L. O' Grady, I. Lorenz, M.G. Green, I. Hogan, A. Johnson, M.L. Doherty

Title

Relationship between selected perinatal paratuberculosis management interventions and passive transfer of immunity in dairy calves

Abstract

The objective of this cohort study was to assess the relationship between perinatal calf management practices relevant to the control of paratuberculosis, and passive transfer of immunoglobulin in calves born in an endemically infected Irish dairy herd. Data from 176 calves were used to assess the effect of time spent in the calving area, individual versus non-designated calving and colostrum pasteurisation on serum total protein, zinc sulphate turbidity, globulin and gamma-glutamyltransferase. In addition, the effects of colostrum quality, volume of colostrum fed, method of colostrum administration and calving season on passive transfer were quantified. Serum samples were collected as part of routine herd health monitoring from calves aged between 1-7 days of age. Multivariate linear and logistic regression models were used to assess the effect of each variable on the test result and failure of passive transfer as determined using a cut-off point for each diagnostic test. Colostrum pasteurisation and calving area were not significantly associated with passive transfer whereas increased time spent in the calving pen was consistently associated with a detrimental effect. In addition, a strong seasonal effect was apparent which appeared to be unrelated to colostrum quality and calf management. The authors are unaware of published studies documenting such a significant seasonal effect on passive transfer.

Introduction

Bovine paratuberculosis is a disease characterised by chronic granulomatous enteritis which manifests clinically as a protein-losing enteropathy causing diarrhoea, hypoproteinaemia,

emaciation and, eventually death (Sweeney 2011). Calves are recognised as being most susceptible to infection (Windsor and Whittington 2010) and protective calf management is regularly advised as part of national control programmes (Geraghty and others 2014). Common “protective calf management” interventions advocated in order to reduce transmission of paratuberculosis in infected herds include the use of individual calving pens, prompt removal of the calf from the calving environment and feeding of low risk feeds with the aim of reducing exposure of calves to the aetiological agent, *Mycobacterium avium* subspecies *paratuberculosis* (MAP).

The use of an individual calving pen over a group calving pen has been associated with reduced transmission of paratuberculosis in endemically infected herds (Pithua and others 2013) or reduced number of seropositive animals in positive herds (Tiwari and others 2009). In addition, during the course of the calving season on a commercial farm, a small number of cows are often not moved to the calving pen in time and calves may be born in “non-designated” calving areas such as close-up or far-off dry pens. Whilst there is no direct evidence to suggest these animals are at a greater risk of infection, environmental samples from adult cow areas are often more likely to be positive than those collected from calving areas (Raizman and others, 2004), suggesting a greater risk of exposure to MAP for calves born in these areas.

Prompt separation of the calf from the dam and removal from the calving area is commonly advocated as part of a JD control programme. The probability of testing positive has been reported to be higher in herds where calving was not supervised (Cashman and others 2013), or in herds where there was late separation from the dam with possible suckling (Beaudeau and others 2005).

MAP has also been isolated from milk and colostrum of subclinical animals (Sweeney and others 1992; Streeter and others 1995) and the feeding of milk replacer has been advocated as a result. More recently the use of on-farm pasteurisers has gained popularity as an intervention for the control of paratuberculosis. Whilst a reduction in levels of MAP in milk (Stabel, 2001) and colostrum (Godden and others 2006) has been shown, a definitive effect on within herd transmission is yet to

be demonstrated (Godden and others 2015).Separation of the calf from the dam within 2 hours is also commonly advocated in order to promote passive transfer and calf health (McGuirk and Collins 2004). Bovine neonates are born virtually agammaglobulinaemic (Klaus and others 1969) and successful passive transfer of maternal immunoglobulin depends on efficient absorption of an adequate volume of colostrum of sufficient quality. Immunoglobulin absorption by enterocytes in the neonatal calf is greatest for the first 4 hours of life and declines rapidly from 12 hours of age (Weaver and others 2000). Similarly, in the dam, colostrum immunoglobulin is highest immediately after calving and progressively declines from 6 hours post calving, the mechanism for this decline is unclear (Moore and others 2005).

Recently, colostral bacterial count has also been shown to negatively impact the efficiency of immunoglobulin absorption (Gelsinger and others 2015). Consequently, colostrum pasteurisation has been developed as a method of reducing bacterial counts with limited effect on immunoglobulin content (Donahue and others 2012). Heat treatment of colostrum has also been studied as a method of significantly reducing the level of MAP in colostrum (Godden and others 2006).

Therefore, considerable crossover exists between recommendations for control of paratuberculosis in infected dairy herds and practices advocated in order to promote calf health. However, whilst there is some anecdotal evidence to suggest that the implementation of a paratuberculosis control programme is likely to have positive implications for calf health (Garry 2011), there is little empirical evidence to support this.

The aim of this study was to investigate the association between the degree to which calf management practices, introduced with the aim of reducing the spread of paratuberculosis, affected passive transfer as measured by serum total protein (STP), zinc sulphate turbidity (ZST), globulin and gamma glutamyl transferase (GGT).

Materials and Methods

One hundred and seventy-six (176) calves born between September 2014 and June 2015, were monitored in a 350-cow, split (autumn and spring) seasonal calving dairy herd in southwest Ireland. The average 305 day yield of lactating cows in the herd was approximately 5900 litres. Considerable data regarding the perinatal management of individual calves in the herd were available due to the herd's involvement in a HACCP-based paratuberculosis control programme (McAloon and others 2015). This system involved intensive monitoring of control measures at critical control points relating to peri-parturient area management, calving, new-born calving management and colostrum management. As part of the implementation of this programme, a tailored on-farm written recording system was developed with farm management and workers, capturing data on traceability and management of cows and calves in the periparturient period including area calved in and time spent in the calving area for each calf.

For the purpose of this study, the recording system was further developed to investigate the effects of the control measures relevant to paratuberculosis, whilst also quantifying and controlling for the effects of possible confounding factors such as volume, method and timing of colostrum administration. The final dataset consisted of date of birth, calving area, calving difficulty, time of calving, time of calf removal from calving area, time of colostrum administration, volume of colostrum administered and method of colostrum administration for each calf born on the farm. Calving difficulty was initially recorded on a 4-point scale ranging from a normal calving to considerable difficulty requiring veterinary assistance, however, given the small number of difficult calvings recorded on the farm, this was subsequently simplified to a 2-point scale; non-assisted and assisted calvings.

A total of 8 individual calving pens were present on the farm to which cows were moved immediately prior to calving. Each pen was cleaned out and bedded with straw following every calving. Calvings occurring accidentally in areas other than these calving pens, such as the far-off or close-up dry cow pen were recorded as non-designated calvings. Calves were removed from the

calving pen or non-designated calving area as soon as possible after calving to a pre-weaning calving shed where they were grouped in batches of 8 until weaning. Heifer and bull calves were reared in separate pens within the same shed. From day 3 of life, heifers were fed a commercial milk replacer (Triple A Golden Maverick, Volac Ireland, Co. Cavan, Ireland) until weaning, whereas bulls were reared on waste milk.

All calves in the herd were fed low-risk donor colostrum. Each calf was fed one feed of the first milk from a donor cow, followed by 2 feeds from the second milking. Risk status of animals in the herd was assigned based on ongoing paratuberculosis testing and the ID of the donor was recorded for each calf.

Colostrum quality was measured before and after pasteurisation using an on-farm portable brix refractometer with a range of 0-32% Brix. All colostrum and transition milk intended for heifer calves was pasteurised using a commercial colostrum pasteuriser (MilkWorks GOLD, DairyTech Inc., Greely, Colorado, USA), frozen for storage and thawed when required. All colostrum and transition milk used for bull calves was frozen on collection and thawed before use. Calves were fed from a milk bottle fitted with a teat and those not ingesting sufficient quantities were tube fed via oesophageal tube feeder.

Routine evaluation of passive transfer on the farm was conducted by the farm's private veterinary practitioner as a part of routine herd health diagnostics. Blood samples were collected from all calves from 1-7 days of age at the time of the practitioner's visit. Samples were transported to the local regional veterinary laboratory. Samples were centrifuged on arrival and the tests for FPT performed without delay. The ZST test used the standard operating procedure in place at Limerick Regional Veterinary Laboratory, as described by McEwan (1970) with the modification that the concentration of the zinc sulphate solution used was 250 mg/L rather than 208 mg/L (Hudgens and others 1996). Testing for GGT, STP and albumin was carried out using an Rx Daytona autoanalyser; globulin levels were then determined by subtracting albumin levels from STP. GGT levels were

124 evaluated by a colourimetric method where the L-γ-glutamyl-3-carboxy-4-nitroanilide is converted in
125 the presence of glycylglycine by GGT to 5-amino-2-nitro-benzoate which absorbs at 405nm (Szasz
126 1974). Total protein levels were determined by formation of a coloured complex between protein
127 and cupric ions in an alkaline medium (Weichselbaum 1946). Albumin levels were determined by
128 quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonphthalein (bromocresol
129 green) (Doumas and others 1971).

130 Statistical Analysis

131 Paper records were collected from the farm at the end of the calving season and transferred to an
132 Excel spreadsheet (Microsoft Corporation, USA), statistical analysis was conducted using MLwiN
133 (version 2.29, Centre for Multilevel Modelling, University of Bristol 2013) and Stata (version 13.1,
134 StataCorp, College Station, Texas, USA).

135 Four outcomes of interest were evaluated; serum total protein, zinc sulphate turbidity (ZST), globulin
136 and GGT. Each outcome was investigated as a continuous outcome and as a binary outcome (success
137 or failure of passive transfer).

138 Univariate linear regression was first used to evaluate the effect of each explanatory variable on
139 each continuous outcome. Serum GGT was not normally distributed and was natural log
140 transformed to meet the assumptions of the linear model.

141 All explanatory variables with a P value <0.2 were carried forward to a multivariate linear regression
142 and a backwards stepwise elimination was conducted to fit the final model. Variables remained in
143 the final model when $P<0.05$.

144 Logistic regression was used to evaluate the association between the measured variables and the
145 outcome 'failure of passive transfer' as determined by the cut-off points selected for each outcome.
146 Cut-off points of 52mg/mL (Calloway and others 2002), 20 units (McEwan and others 1970),

20mg/ml (Garry and others 1993) and 100IU/L (Parish and others 1997) were selected for serum total protein, ZST, globulin and GGT respectively.

Results

Data were available for 176 calves including 102 females and 74 males. Mean serum total protein, ZST, globulin and GGT were 57.1, 22.8, 28.8, and 360.6 respectively. Using the cut-off points identified in the literature, the prevalence of FPT in the herd was 32.4%, 42.0%, 8.5% and 22.5% when using STP, ZST, globulin and GGT respectively.

Linear Regression Model

Univariate analysis

Results of the univariate analysis are shown in Table 1. Season, time spent in calving pen, time until administration of colostrum, volume of colostrum administered were identified as $P<0.2$ across 3 of the methods of assessing passive transfer. Method of colostrum administration yielded P-values of 0.162, 0.157, 0.118, when passive transfer was assessed by serum total protein, ZST and globulin respectively. Colostrum quality was associated with elevated STP and globulin, pasteurisation was associated with an elevated globulin and volume of colostrum administered was associated with elevations across all 4 measures of passive transfer.

Multivariate analysis

The results of the multivariate analysis are displayed in Table 2. Season was identified as a significant factor across 3 of the outcomes assessed. Markers of passive transfer were consistently higher in autumn born calves even when volume and quality of colostrum administered were corrected for. The amount of time spent in the calving pen was also significant across 3 of the outcomes assessed, markers of passive transfer declined with increasing time spent in the calving pen. Colostrum quality as measured on farm by means of a Brix refractometer was associated with improved passive transfer across 2 of the outcomes assessed. Volume of colostrum administered was associated with

improved passive transfer across all 4 outcomes. Calf serum markers of passive transfer increased from less than 3 litres to 3 litres and from 3 litres to 3.5, however PT declined when the volume of colostrum administered increased from 3.5 greater than 4 litres. Tube feeding of colostrum was significantly associated with poorer passive transfer compared with bottle and tube feeding.

Logistic regression model

Univariate analysis

Results of the univariate analysis with failure of passive transfer as a binary response variable are displayed in Table 3. Season, time spent in calving area and method of colostrum administration were significantly associated with failure of passive transfer as determined by serum total protein, globulin and GGT. Volume of colostrum administered was significantly associated with FPT as identified by ZST and GGT. Calving area and level of calving assistance and time spent in calving pen were considered significant when FPT was evaluated by globulin. Colostrum quality and feeding of pasteurised colostrum were significantly associated with ZST assessment of FPT. In addition, calving area, time spent in calving pen and time until colostrum administration were considered significant when FPT was evaluated by GGT.

Multivariate analysis

Results of logistic regression are shown in Table 4. Season had a significant effect on the OR for FPT. The odds ratio (95% CI) ranged from 3.00 (1.40, 6.41) for serum total protein, to 5.3 (1.86, 15.22) for GGT ($P<0.05$). The seasonal effect size for FPT when determined by globulin was also large (OR=4.23, 95% CI = 0.90, 19.83), however this association was not significant (p=0.067). Increased time spent in the calving area was associated with increased odds of FPT as evaluated by STP, globulin or GGT. Tube feeding of colostrum rather than feeding from bottle and teat was significantly associated with FPT when assessed by GGT, this observation was also observed with STP but the effect did not quite reach significance (p=0.053). Volume of colostrum administered was significantly associated with

FPT risk as assessed by ZST; odds ratios were lowest for 3.5 litres, followed by 3 litres and greater than 4 litres, all of these categories were significantly better than feeding less than 3 litres.

Discussion

Estimation of the prevalence of FPT in this herd varied considerably from 8.5% as identified by serum globulin, to 42% as measured by ZST. However, 17% of all samples tested were classified as having a FPT on ZST, despite being negative on all 3 of the remaining tests. Zinc Sulphate Turbidity is commonly associated with comparably poor specificity for the detection of FPT (Hogan and others 2015). It therefore seems likely that the true prevalence of FPT in the herd was lower than that as estimated by ZST. There are limited published data regarding the prevalence of FPT in commercial Irish dairy herds, however a recent UK study found that this prevalence varied from 5-51% across 7 commercial dairies which was in agreement with similar estimates from North America (MacFarlane and others 2015).

The present study found that there was no difference in passive transfer between calves born in individual calving pens compared to those born in non-designated calving areas. This contrasts with a Swedish study which found that calf plasma IgG was greatest in calves born in individual calving pens when compared to group pens (Michanek and Ventorp 1993). However, a US study found no difference in calf health between calves born in individual calving pens and those born in group calving pens (Pithua and others 2009).

Calving difficulty was not significantly associated with FPT in any of the models. However there was a tendency for those calves which required some degree of farmer intervention during parturition to have a greater chance of FPT as determined by serum globulin ($p=0.051$). Dystocia is often commonly cited as a reason for FPT, however the biological mechanism behind this finding is somewhat unclear, and may be related to the fact that calves suffering from combined respiratory and metabolic acidosis as a result of dystocia, are less likely get up and suckle, rather than any inability to absorb immunoglobulin *per se* (Weaver and others 2000).

Increased time spent in the calving pen was consistently associated with a lower assessment of passive transfer and an increased risk of FPT when assessed by methods other than ZST. This finding is somewhat unsurprising as time spent within the calving pen is likely to be related to time from birth to colostrum administration which has a well-defined role in the efficiency of immunoglobulin absorption. In this dataset, time spent in the calving pen and time until administration of colostrum were moderately correlated (r=0.476). However, it is interesting to note that the majority of calves in this study were removed from the dam within 1 hour of birth and that a significant effect of time spent in calving pen was observed even within the relatively small spread of removal times. Feeding of colostrum shortly after birth may result in earlier intestinal closure in the neonate than if feeding is delayed (Stott and others 1979). Calves that have opportunity to suckle in the calving pen may therefore experience earlier cessation of absorption and a poorer efficiency of absorption of subsequent colostrum feeds.

Colostrum quality as determined by Brix refractometer was observed to have a significant effect on passive transfer as measured by STP and globulin in the linear regression and when measured by ZST in the logistic regression. Optical brix refractometry is known to correlate quite well with the gold standard, radial immunodiffusion method for quantifying colostral immunoglobulin concentration (Bielmann and others 2010).

Volume of colostrum administered was significantly associated with passive transfer in the linear analysis for all 4 evaluation methods, however logistic regression identified this factor as being only significant when evaluated by ZST. Improvement in passive transfer increased when volume of colostrum increased from less than 3 litres to 3.5 litres. However, the coefficient for feeding greater than 4 litres was consistently less than 3.5 litres across all models, although the difference between these two groups was not statistically significant. Interestingly, a recent Irish study found that feeding colostrum at 8.5% body weight resulted in better passive transfer than 10% bodyweight

(Conneely and others 2014) although the biological mechanism behind this observation is somewhat unclear.

Colostrum pasteurisation produced contradictory results from the 2 statistical analyses conducted. In the linear models there was no significant effect of pasteurisation though the model coefficients suggested a non-significant positive effect of pasteurisation. However in the logistic regression, pasteurisation was significantly associated with an increased risk of FPT as determined by ZST this may be the result of poor specificity associated with ZST for the diagnosis of STP.

Feeding of colostrum by tube was generally associated with poorer passive transfer when assessed by STP and globulin and an increased risk of FPT as determined by STP. This observation has been made before (Adams and others 1985), and has been attributed to closure of the oesophageal grove with earlier delivery of colostrum to the abomasum and small intestine. However the magnitude of this effect is generally considered to be small and not clinically significant (Godden 2008). A recent randomised control trial found that this effect was only significant when small (1.5 litre) volumes of colostrum were administered (Godden and others 2009). In the present study calves were preferentially fed colostrum by bottle and teat and only tube fed when they did not drink a sufficient quantity of colostrum. Therefore, it is possible that these calves may have been suffering from acidosis as a result of unobserved or undocumented dystocia which may have affected efficiency of absorption.

An interesting outcome of this study was the strong seasonal effect on passive transfer. Spring born calves had significantly poorer passive transfer and a greater risk of FPT when compared to autumn born herd mates. Poorer calf health is often observed in the spring born population of calves in Irish dairy herds, when compared with autumn born herdmates. The most likely explanation for this is that the proportion of cows calving in autumn in a split calving season dairy herd is usually smaller than the proportion calving in the spring. Higher numbers of calves born in spring may result in strains on farm labour, and a delay in the average time from calving to colostrum administration may

occur as a result. In addition, an increase in stocking density may also result in poorer hygiene and increased pathogen exposure. The dry period diet of spring calving cows is likely to differ substantially from those calving in the autumn, body condition scores were also not available for autumn and spring calving cows. However the role of the dry cow diet in determining colostrum quality is somewhat unclear and studies have generally shown that colostrum quality is relatively insensitive to manipulation of the dry cow diet (Godden 2008). Similarly, the relationship between BCS and colostrum quality and passive transfer is also unclear with studies often finding a lack of association between BCS at calving and passive transfer (Lake and others 2002), others have found increases in colostrum quality only when BCS increased from dry-off to calving, but not the BCS at calving *per se* (Shearer and others 1992). However, the dry-cow diet and BCS could only be expected to affect passive transfer by affecting colostrum quality whereas in the present study, both the quality of colostrum as assessed by refractometer and the time from calving to colostrum administration were recorded and included in the final models when appropriate. Therefore it was expected that both the quality of colostrum and the length of time until colostrum was administered would have had a limited effect on this particular finding in the final model. Indeed, the average refractometer reading for colostrum was higher in spring (27.2%) than in autumn (26.1%), whereas poorer passive transfer was observed in the spring born calves. These findings would suggest that on this particular farm there was an unidentified factor affecting apparent efficiency of absorption resulting in increased passive transfer in autumn born calves as opposed to spring born calves. The authors are unaware of similar studies investigating the seasonality associated with passive transfer having accounted for quality of colostrum and time until administration. This finding warrants further investigation.

This study utilised a range of non-gold standard methods for assessing passive transfer in calves, each method has varying sensitivities and specificities when compared with the gold standard method (Hogan and others 2015). In addition, GGT levels can vary significantly according to the age of the calf with levels declining after 24 hours of age. The most significant decline occurs between 24

and 48 hours with the recommended cut-off dropping from 200 to 135 IU/L. A moderate decline is expected between 2 and 7 days of age with the recommended cut-off reduced from 135 to 75 IU/L (Parish and others 1997). In this study the age of the calf at sampling was not available, therefore given that all calves were known to be less than 7 days of age, the cut-off point for a 4 day old calf, i.e. 100IU/L was used.

Caution must be taken when extrapolating the results of studies conducted on a single herd to the wider population of dairy herds, particularly those operating widely different management systems to study herds. However, due to the level of data available on individual calves, many of the known confounders for passive transfer could be accounted for in the statistical analysis. Similarly, assigning colostrum treatments according to male and female calves facilitates possible confounding as a result of the sex of the calf. Whilst the authors are unaware of biological reasons why apparent efficiency of absorption may vary between sexes, management of heifer and bull calves often differs on farms with the health of heifers often prioritised on dairy farms. However in the present study, management issues known to affect passive transfer in neonatal calves such as volume, timing and method of colostrum administration, were quantified and controlled for in the statistical analysis.

In conclusion, this study utilised detailed data on the perinatal management of calves born in a herd endemically infected with paratuberculosis. The effect of management practices on calf passive transfer were assessed. Increased time spent in the calving pen was consistently associated with a detrimental effect on passive transfer. In addition, a strong seasonal effect was apparent which was unrelated to colostrum quality and calf management.

Acknowledgements

This work was carried out as part of the ICONMAP multidisciplinary research programme funded by the Irish Department of Agriculture, Food and the Marine. The authors also wish to thank the herdowner, farm manager and farm workers of the study herd for their assistance and co-operation over the course of the study.

320 **References**

321 ADAMS, G. D., BUSH, L. J., HORNER, J. L. & STALEY, T. E. (1985) Two Methods for Administering
322 Colostrum to Newborn Calves1. *Journal of Dairy Science* **68**, 773-775

323 BEAUDEAU, F., LEDOUX, D., POUMEROL,N. & JOLY SEEGERS, A.H., (2005) Risk factors for
324 Mycobacterium avium subspecies paratuberculosis infection in dairy herds in Brittany (Western
325 France). Proceedings of the 8th International Colloquiumon Paratuberculosis, International
326 Association for Paratuberculosis. Madison, Wisconsin, p.255.

327 BIELMANN, V., GILLAN, J., PERKINS, N. R., SKIDMORE, A. L., GODDEN, S. & LESLIE, K. E. (2010) An
328 evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle.
329 *Journal of Dairy Science* **93**, 3713-3721

330 CALLOWAY, C. D., TYLER, J. W., TESSMAN, R. K., HOSTETLER, D. & HOLLE, J. (2002) Comparison of
331 refractometers and test endpoints in the measurement of serum protein concentration to assess
332 passive transfer status in calves. *Journal of the American Veterinary Medical Association* **221**, 1605-
333 1608

334 CASHMAN, W., J. BUCKLEY, T. QUIGLEY, S. FANNING, S. MORE, J. EGAN, D. BERRY, I. GRANT, and
335 O'FARRELL, K. (2008) Risk factors for the introduction and within-herd transmission of
336 *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection on 59 Irish dairy herds *Irish*
337 *Veterinary Journal* **61**, 1.

338 CONNEELY, M., BERRY, D. P., MURPHY, J. P., LORENZ, I., DOHERTY, M. L. & KENNEDY, E. (2014) Effect
339 of feeding colostrum at different volumes and subsequent number of transition milk feeds on the
340 serum immunoglobulin G concentration and health status of dairy calves. *Journal of Dairy Science*
341 **97**, 6991-7000

342 DONAHUE, M., GODDEN, S., BEY, R., WELLS, S., OAKES, J., SREEVATSAN, S., STABEL, J. & FETROW, J.
343 (2012) Heat treatment of colostrum on commercial dairy farms decreases colostrum microbial

344 counts while maintaining colostrum immunoglobulin G concentrations. *Journal of Dairy Science* **95**,
345 2697-2702

346 DOUMAS, B. T., WATSON, W. A. & BIGGS, H. G. (1971) Albumin standards and the measurement of
347 serum albumin with bromcresol green. *Clinica Chimica Acta* **31**, 87-96

348 GARRY, F. (2011) Control of Paratuberculosis in Dairy Herds. *Veterinary Clinics of North America:*
349 *Food Animal Practice* **27**, 599-607

350 GARRY, F., ADAMS, R. & ALDRIDGE, B. (1993) Role of colostral transfer in neonatal calf
351 management: current concepts in diagnosis. *The Compendium on continuing education for the*
352 *practicing veterinarian* (USA) **15**, 335-342

353 GELSINGER, S. L., JONES, C. M. & HEINRICHS, A. J. (2015) Effect of colostrum heat treatment and
354 bacterial population on immunoglobulin G absorption and health of neonatal calves. Journal of Dairy
355 Science **98**, 4640-4645

356 GERAGHTY, T., GRAHAM, D. A., MULLOWNEY, P. & MORE, S. J. (2014) A review of bovine Johne's
357 disease control activities in 6 endemically infected countries. *Preventive Veterinary Medicine* **116**, 1-
358 11

359 GODDEN, S. (2008) Colostrum management for dairy calves. *Veterinary Clinics of North America:*
360 *Food Animal Practice* **24**, 19-39

361 GODDEN, S., MCMARTIN, S., FEIRTAG, J., STABEL, J., BEY, R., GOYAL, S., METZGER, L., FETROW, J.,
362 WELLS, S. & CHESTER-JONES, H. (2006) Heat-treatment of bovine colostrum. II: effects of heating
363 duration on pathogen viability and immunoglobulin G. *Journal of Dairy Science* **89**, 3476-3483

364 GODDEN, S. M., HAINES, D. M., KONKOL, K. & PETERSON, J. (2009) Improving passive transfer of
365 immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed.
366 *Journal of Dairy Science* **92**, 1758-1764

GODDEN, S. M., WELLS, S., DONAHUE, M., STABEL, J., OAKES, J. M., SREEVATSAN, S., & FETROW, J. (2015). Effect of feeding heat-treated colostrum on risk for infection with *Mycobacterium avium* ssp. *paratuberculosis*, milk production, and longevity in Holstein dairy cows. *Journal of Dairy Science* **98**, 5630-5641.

HOGAN, I., DOHERTY, M., FAGAN, J., KENNEDY, E., CONNEELY, M., BRADY, P., RYAN, C. & LORENZ, I. (2015) Comparison of rapid laboratory tests for failure of passive transfer in the bovine. *Irish Veterinary Journal* **68**, 1-10

HUDGENS, K., TYLER, J., BESSER, T. & KRYTENBERG, D. (1996) Optimizing performance of a qualitative zinc sulfate turbidity test for passive transfer of immunoglobulin G in calves. *American Journal of Veterinary Research* **57**, 1711-1713

KLAUS, G., BENNETT, A. & JONES, E. (1969) A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. *Immunology* **16**, 293

LAKE, S. L., SCHOLLJEGERDES, E. J., SMALL, W. T., BELDEN, E. L., PAISLEY, S. I., RULE, D. C., & HESS, B. W. (2006). Immune response and serum immunoglobulin G concentrations in beef calves suckling cows of differing body condition score at parturition and supplemented with high-linoleate or high-oleate safflower seeds. *Journal of Animal Science* **84**, 997-1003.

MACFARLANE, J. A., GROVE-WHITE, D. H., ROYAL, M. D. & SMITH, R. F. (2015) Identification and quantification of factors affecting neonatal immunological transfer in dairy calves in the UK. Veterinary Record **176**, 625

MCALOON, C. G., WHYTE, P., MORE, S. J., O'GRADY, L. & DOHERTY, M. L. (2015) Development of a HACCP-based approach to control paratuberculosis in infected Irish dairy herds. *Preventive Veterinary Medicine* **120**, 152-161

MCEWAN, A., FISHER, E., SELMAN, I. & PENHALE, W. (1970) A turbidity test for the estimation of immune globulin levels in neonatal calf serum. *Clinica Chimica Acta* **27**, 155-163

391 MCGUIRK, S. M. & COLLINS, M. (2004) Managing the production, storage, and delivery of colostrum.
392 *Veterinary Clinics of North America: Food Animal Practice* **20**, 593-603

393 MICHANEK, P. & VENTORP, M. (1993) Passive immunization of new-born dairy calves on three farms
394 with different housing systems. *Swedish Journal of Agricultural Research* **23**, 37-43

395 MOORE, M., TYLER, J. W., CHIGERWE, M., DAWES, M. E., & MIDDLETON, J. R. (2005). Effect of
396 delayed colostrum collection on colostral IgG concentration in dairy cows. *Journal of the American*
397 *Veterinary Medical Association* **226**, 1375-1377.

398 PARISH, S. M., TYLER, J. W., BESSER, T. E., GAY, C. C. & KRYTENBERG, D. (1997) Prediction of serum
399 IgG1 concentration in Holstein calves using serum gamma glutamyltransferase activity. *Journal of*
400 *Veterinary Internal Medicine* **11**, 344-347

401 PITHUA, P., WELLS, S. J., GODDEN, S. M. & RAIZMAN, E. A. (2009) Clinical trial on type of calving pen
402 and the risk of disease in Holstein calves during the first 90 d of life. *Preventive Veterinary Medicine*
403 **89**, 8-15

404 PITHUA, P., ESPEJO, L. A., GODDEN, S. M., & WELLS, S. J. (2013). Is an individual calving pen better
405 than a group calving pen for preventing transmission of *Mycobacterium avium* subsp
406 *paratuberculosis* in calves? Results from a field trial. *Research in Veterinary Science* **95**, 398-404.

407 RAIZMAN, E. A., WELLS, S. J., GODDEN, S. M., BEY, R. F., OAKES, M. J., BENTLEY, D. C., & OLSEN, K. E.
408 (2004). The distribution of Mycobacterium avium ssp. paratuberculosis in the environment
409 surrounding Minnesota dairy farms. *Journal of Dairy science* **87**, 2959-2966.

410 SHEARER, J., MOHAMMED, H. O., BRENNEMAN, J. S., & TRAN, T. Q. (1992). Factors associated with
411 concentrations of immunoglobulins in colostrum at the first milking post-calving. *Preventive*
412 *Veterinary Medicine* **14**, 143-154.

413 STABEL, J. R. (2001). On-farm batch pasteurization destroys Mycobacterium paratuberculosis in
414 waste milk. *Journal of Dairy Science* **84**, 524-527.

415 STREETER, R. N., HOFFSIS, G. F., BECH-NIELSEN, S., SHULAW, W. P., & RINGS, D. M. (1995). Isolation
416 of Mycobacterium paratuberculosis from colostrum and milk of subclinically infected cows.
417 *American Journal of Veterinary Research* **56**, 1322-1324.

418 STOTT, G. H., MARX, D. B., MENEFEE, B. E. & NIGHTENGALE, G. T. (1979) Colostral Immunoglobulin
419 Transfer in Calves I. Period of Absorption1. Journal of Dairy Science **62**, 1632-1638

420 SWEENEY, R. W., WHITLOCK, R. H., & ROSENBERGER, A. E. (1992). *Mycobacterium paratuberculosis*
421 cultured from milk and supramammary lymph nodes of infected asymptomatic cows. *Journal of*
422 *Clinical Microbiology* **30**, 166-171.

423 SWEENEY, R. W. (2011) Pathogenesis of paratuberculosis. *Veterinary Clinics of North America: Food*
424 *Animal Practice* **27**, 537-546

425 SZASZ, G. (1974) New substrates for measuring gamma-glutamyl transpeptidase activity. *Zeitschrift*
426 *für klinische Chemie und klinische Biochemie* **12**, 228

427 TIWARI, A., VANLEEUWEN, J. A., DOHOO, I. R., KEEFE, G. P., HADDAD, J. P., SCOTT, H. M., &
428 WHITING, T. (2009). Risk factors associated with Mycobacterium avium subspecies paratuberculosis
429 seropositivity in Canadian dairy cows and herds. *Preventive Veterinary Medicine* **88**, 32-41

430 WEAVER, D. M., TYLER, J. W., VANMETRE, D. C., HOSTETLER, D. E. & BARRINGTON, G. M. (2000)
431 Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine* **14**,
432 569-577

433 WEICHSELBAUM, T. (1946) Calorimetric methods for the determination of total serum protein.
434 *American Journal of Clinical Pathology* **16**, 40

435 WINDSOR, P. A. & WHITTINGTON, R. J. (2010) Evidence for age susceptibility of cattle to Johne's
436 disease. *The Veterinary Journal* **184**, 37-44

437

438

439

440

441 **TABLE 1**: Univariate linear regression of the effect of measured variables on Serum Total Protein
442 (STP), Zinc Sulphate Turbidity (ZST), globulin and loge transformed Gamma-glutamyltransferase
443 (lnGGT)

Variable		**STP**			**ZST**			**Globulin**			**lnGGT**		
		β	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***
Season	Autumn	REF			REF			REF			REF		
	Spring	-5.37	1.32	<0.001	-1.75	1.64	0.286	-6.20	1.37	0.000	-0.87	0.17	0.000
Calving Area	Individual	REF			REF			REF			REF		
	NonDes	-0.66	1.39	0.632	-1.38	1.65	0.400	-1.02	1.45	0.482	-0.17	0.18	0.354
Assisted Calving	No	REF			REF			REF			REF		
	Yes	-0.82	1.98	0.679	-0.42	2.35	0.859	-0.61	2.07	0.770	-0.15	0.26	0.562
Time in calving pen (minutes)	Continuous	-0.07	0.03	0.017	-0.05	0.03	0.186	-0.08	0.03	0.009	-0.01	0.00	0.024
	0	2.68	2.06	0.193	1.44	2.47	0.560	2.98	2.15	0.166	0.29	0.27	0.273
	0-10	3.62	1.70	0.033	1.80	2.04	0.378	4.13	1.78	0.020	0.45	0.22	0.040
	10-60	4.88	2.26	0.030	4.34	2.71	0.109	5.32	2.36	0.024	0.66	0.29	0.025
	60+	REF			REF			REF			REF		
Colostrum Quality (%)		0.44	0.26	0.088	0.31	0.31	0.308	0.38	0.27	0.162	-0.03	0.03	0.362
Time until colostrum admin	Continuous	-0.01	0.01	0.253	0.01	0.01	0.382	-0.01	0.01	0.199	0.00	0.00	1.000
	0-30	REF			REF			REF			REF		
	31-60	-0.86	1.87	0.647	0.00	2.25	0.999	-2.00	1.95	0.305	-0.22	0.25	0.367

	61-120	-2.05	1.75	0.242	-2.07	2.10	0.324	-2.78	1.83	0.128	-0.40	0.23	0.084
	121+	-4.09	1.80	0.023	-0.42	2.16	0.847	-4.84	1.88	0.010	-0.32	0.24	0.171
Volume of Colostrum (litres)	<3.0	REF			REF			REF			REF		
	3	4.63	2.85	0.103	6.75	3.38	0.046	5.16	2.98	0.084	0.68	0.37	0.068
	3.5	7.12	2.72	0.009	9.77	3.22	0.002	7.62	2.85	0.007	0.87	0.36	0.015
	4+	5.19	2.77	0.061	7.07	3.28	0.031	6.39	2.90	0.027	0.82	0.36	0.024
Method	Teat	REF			REF			REF			REF		
	Tube	-2.66	1.90	0.162	-3.19	2.26	0.157	-3.11	1.99	0.118	-0.24	0.25	0.331
Pasteurised	No	REF			REF			REF			REF		
	Yes	1.60	1.35	0.235	-0.14	1.61	0.930	2.04	1.41	0.147	0.18	0.18	0.293

444

445 β=coefficient of variable, SE = Standard Error, REF = referent category, NonDes = non-designated

446 calving area

447 **TABLE 2:** Multivariate linear regression models of the effect of measured variables on Serum Total

448 Protein (STP), Zinc Sulphate Turbidity (ZST), globulin and loge transformed Gamma-

449 glutamyltransferase (lnGGT)

Variable		**STP**			**ZST**			**Globulin**			**lnGGT**		
		β	**SE**	**p**	**β**	**SE**	**p**	**β**	**SE**	**p**	**β**	**SE**	**p**
Season	Autumn	REF											
	Spring	-5.82	1.34	<0.001				-6.55	1.39	<0.001	-0.83	0.17	0.000
Time spent in calving pen (mins)	continuous	-0.05	0.03	0.046				-0.06	0.03	0.024	-0.01	0.003	0.020
Colostrum quality (%)		0.75	0.25	0.003				0.73	0.26	0.005			
	<3.0	REF			REF			REF					
	3	4.00	3.01	0.184	6.75	3.38	0.046	4.26	3.13	0.174	0.47	0.38	0.222

Volume of colostrum (litres)	3.5	6.43	2.92	0.028	9.77	3.22	0.002	6.47	3.04	0.033	0.66	0.37	0.073
	4+	4.19	3.00	0.161	7.07	3.28	0.031	4.92	3.12	0.114	0.52	0.37	0.166
Method	Teat	REF						REF					
	Tube	-3.43	1.96	0.080				-3.70	2.04	0.069			

450

451 β=coefficient of variable, SE = Standard Error, REF = referent category

452 **TABLE 3:** Univariate logistic regression of the effect of measured variables on failure of passive
453 transfer as determined by Serum Total Protein (STP), Zinc Sulphate Turbidity (ZST), globulin and
454 gamma-glutamyltransferase (GGT)

Variable		**STP**			**ZST**			**Globulin**			**GGT**		
		β	SE	p	β	SE	p	β	SE	p	β	SE	p
Season	Autumn	REF			REF	SE		REF			REF		
	Spring	1.08	0.37	0.004	0.21	0.32	0.502	1.44	0.77	0.062	1.68	0.51	0.001
Calving area	Individual	REF			REF			REF			REF		
	NonDes	0.14	0.33	0.670	0.11	0.32	0.729	0.76	0.54	0.161	0.47	0.37	0.199
Assisted calving	No	REF			REF			REF			REF		
	Yes	0.34	0.46	0.459	-0.35	0.47	0.451	1.00	0.63	0.114	0.46	0.49	0.348
Time spent in calving pen (mins)	continuous	0.01	0.01	0.086	0.00	0.01	0.462	0.02	0.01	0.024	0.02	0.01	0.004
	0	REF			REF			REF			REF		
	0-10	-0.08	0.45	0.868	0.41	0.43	0.337	1.12	1.09	0.304	-0.07	0.51	0.884
	10-60	-0.05	0.59	0.927	-0.33	0.58	0.570	0.00	0.90	1.000	-1.09	0.85	0.200
	60+	0.58	0.50	0.248	0.41	0.48	0.403	1.95	1.10	0.076	0.84	0.54	0.118
Colostrum quality (%)		-0.02	0.06	0.747	-0.11	0.06	0.072	-0.06	0.10	0.586	0.06	0.07	0.394
Time until colostrum admin (mins)	continuous	0.00	0.00	0.968	0.00	0.00	0.699	0.00	0.00	0.757	0.00	0.00	0.183
	0-30	REF			REF			REF			REF		
	31-60	0.29	0.46	0.530	0.01	0.45	0.977	-0.94	1.14	0.409	0.60	0.55	0.282

	61-120	0.28	0.43	0.516	0.54	0.41	0.186	0.53	0.70	0.451	0.59	0.52	0.261
	121+	0.32	0.44	0.468	0.34	0.42	0.421	0.64	0.71	0.365	1.19	0.51	0.018
VolCol	<3.0	REF			REF			REF			REF		
	3	-0.11	0.67	0.869	-2.06	0.84	0.014	0.63	1.13	0.575	-0.32	0.67	0.634
	3.5	-0.74	0.65	0.253	-2.26	0.82	0.006	-0.36	1.16	0.757	-1.09	0.66	0.100
	4+	-0.27	0.65	0.674	-1.83	0.82	0.026	-0.13	1.17	0.913	-1.41	0.70	0.044
Method	Teat	REF			REF			REF			REF		
	Tube	0.78	0.44	0.076	0.66	0.44	0.131	-0.08	0.79	0.920	0.78	0.46	0.093
Pasteurised	No	REF			REF			REF			REF		
	Yes	0.21	0.33	0.521	0.50	0.31	0.115	0.41	0.57	0.477	-0.29	0.36	0.428

β=coefficient of variable, SE = Standard Error, REF = referent category, NonDes = non-designated calving area

TABLE 4: Multivariate logistic regression of the effect of measured variables on failure of passive transfer as determined by Serum Total Protein (STP), Zinc Sulphate Turbidity (ZST), globulin and gamma-glutamyltransferase (GGT)

Variable		**STP**			**ZST**			**Globulin**			**GGT**		
		OR	**95% C.I.**	***p***	**OR**	**95% C.I.**	***p***	**OR**	**95% C.I.**	***p***	**OR**	**95% C.I.**	***p***
Season	Autumn	1.00			1.0			1.0			1.0		
	Spring	3.00	1.40, 6.41	0.005				4.23	0.90, 19.83	0.067	5.33	1.86, 15.22	0.002
Assisted Calving	No							1.00					
	Yes							4.08	0.99,	0.051			

									16.76				
Time spent in calving pen (mins)	Continuous	1.01	1.00, 1.03	0.049				1.03	1.00, 1.05	0.018	1.03	1.01, 1.04	0.002
Colostrum Quality (%)					0.86	0.76, 0.98	0.022						
Volume of colostrum (litres)	<3.0				1.00								
	3				0.10	0.02, 0.56	0.009						
	3.5				0.08	0.02, 0.44	0.003						
	4+				0.15	0.03, 0.81	0.027						
Method of colostrum admin	Teat	1.00									1.0		
	Tube	2.61	0.99, 6.91	0.053							3.2	1.08, 9.50	0.04
Pasteurised	No				1.00						1.0		
	Yes				2.22	1.09, 4.53	0.029				3.2	1.08, 9.50	0.04

461

462 OR = odds ratio, 95% CI = 95% confidence intervals