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PAPER

Reduction in morbidity and mortality of dairy calves from an injectable trace mineral supplement

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Abstract

The effect of a multimineral preparation on the health and growth of spring born, dairy calves was investigated on four New Zealand pastoral farms. Calves were randomly allocated injections within 24 hours of birth, 35 days and 70 days after birth. Injections contained 40 mg zinc, 10 mg manganese, 5 mg selenium, 15 mg copper and 5 mg chromium per ml (Multimin+Se+ Cu+Cr Cattle, Virbac South Africa) at 1 ml/50 kg body weight. Morbidity, mortality from natural challenge and growth rates were recorded for 140 days. There were no differences in morbidity and mortality within 48 hours of birth for treated calves compared with controls, P=0.192. Morbidity and mortality were highest at 3–35 days (7.5 per cent [95 per cent CI 5.00 to 9.99] treated calves sick and 15.6 per cent [95 per cent CI 12.48 to 18.73] controls sick, P<0.001). For this period, mortality was lower at 4.4 per cent (95 per cent CI 2.49 to 6.41) treated calves and 10.4 per cent (95 per cent CI 7.78 to 13.03) controls, P<0.001. Allowing for potential confounders, the adjusted OR of treated calves scouring between 3 and 35 days was 0.44 (95 per cent CI 0.24 to 0.82, P=0.009). Allowing for potential confounders, from 0 to 140 days a second model predicted treatment approximately halved the probability of morbidity and mortality (P<0.001). There was no difference in the daily rate of gain (0.67 kg/day [95 per cent CI 0.66 to 0.67] for treated calves).

Introduction

There is increasing interest in the role morbidity and mortality play in the efficiency and waste of dairy farms.[1] In calf rearing systems, the definition and incidence of calf disease and mortality vary between farms and the inclusion criteria in studies.[2] Generally, perinatal morbidity and mortality refers to calves born full term and in the first 48 hours after birth.[3] Postnatal morbidity and mortality refers to the period after the first 48 hours, up to a defined time point such as three months[2] or weaning.[4]

In their review, Compton and others[1] reported that internationally perinatal mortality incidence risks ranged from 3 per cent to 9 per cent of full term births, and postnatal mortality from 5 per cent to 11 per cent.

A prospective observational study on New Zealand (NZ) pasture-based dairy farms,[4] reported a perinatal mortality risk of 5.7 per cent, similar to the 4.3 per cent recorded by Mee and others[3] on Irish pasture-based dairy farms and consistent with levels of perinatal mortality reported by Compton and others[1] from housed systems.

Levels of postnatal mortality in NZ systems (eg, 4.1 per cent[4]) seem to be at the low end of international reports. There are also differences in the causes of calf morbidity. In housed calves, diarrhoea and pneumonia account for most calf morbidity; Windeyer and others[2] reported that in their first three months calf diarrhoea and pneumonia affected 23 per cent and 22 per cent of calves, respectively. In contrast, Cuttance and others[5] reported that before weaning diarrhoea and pneumonia were recorded as occurring in 5 per cent and 0.1 per cent of calves, respectively, with 1 per cent of calves being recorded as having omphalophlebitis.

In pastoral systems, several factors have been identified increasing mortality incidence including sex, week of birth, rain on the day of calving, poor colostrum quality and management,[4] dystocia and dam parity.[3] To the authors' knowledge, the impact of trace mineral supplementation (TMS) on morbidity and mortality in these systems has not been reported.

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The neonatal calf is born immunocompetent, but with a naïve immune system that needs time to develop specific antibody responses. Improvements in colostrum management and feeding practices were identified by Cuttance and others[6] as a key driver of reducing calf mortality and failure of passive transfer (FPT) under NZ pastoral systems has recently been quantified.[6] However, from birth, the calf does have a functioning innate cellular immune system in the form of peripheral leucocytes.[7] Functionality can be heavily influenced by the availability of essential trace minerals that are important for multiple biochemical processes, including immune response, cell replication and skeletal development.[8] These are particularly relevant for the neonate.[9] Understanding the internal and external factors contributing to the immunological responses of calves to stressors has been identified as a key driver reducing calf morbidity and mortality.[10]

Palomares and others[11] showed injection of calves 3.5 months of age with a TMS containing zinc (Zn), copper (Cu), selenium (Se) and manganese (Mn) increased the humoral and cellular immune response, the latter detectable within 7 days of supplementation, while Arthington and Havenga[12] showed an increase in antibody production as a response to vaccination in yearling calves that had concurrently been given a TMS supplement containing Zn, Cu, Se and Mn.

Teixeira and others[8] found postnatal injections of a TMS containing Zn, Mn, Cu and Se at 3 and 30 days after birth increased neutrophil function and glutathione peroxidase activity at 14 days and reduced morbidity in the first 50 days of life. Together, these results suggest injection of TMS at anticipated periods of stress may have beneficial implications for the animals' immune system and lead to a reduction in morbidity and mortality.

The objective of the present study was to record the incidence of farmer diagnosed morbidity and mortality from birth to 140 days on four commercial farms and to evaluate the effect of supplementation of an injectable multimineral supplement containing Zn, Mn, Cu, Se and chromium (Cr) at and after birth on morbidity, mortality and growth of Friesian-Jersey cross heifer calves during the preweaning period under a pastoral rearing system. The authors' null hypothesis was that supplementation would make no difference to the recorded morbidity and mortality between 0 and 140 days.

Materials and methods

Farms and animals

A total of 971 calves were enrolled from four seasonally calving pastoral dairy farms in Canterbury, NZ. Farms were selected as a convenience sample of commercial dairy farms serviced by Vetlife NZ. Calves were recruited over a three-week period from the planned start of calving (PSC) for each farm (October 24–27, 2016). All female calves born alive and individually tagged at collection from the calving paddock were recruited for the study. The authors excluded calves born before these dates, born alive but identified for culling within 24 hours of birth and male calves so that the study cohort was representative of full term, heifer replacement calves.

A precalving farm visit was carried out on each farm to determine dry period and preweaning and postweaning practices for replacement heifers. Milking cows were fed a pasture dominant feed with supplementary milled wheat at 1–2 kg/cow/day depending on stocking rate and pasture supply. Throughout lactation, all cows were on a mineralised water supply using customised blend (AgVance Nutrition, Howick, NZ) providing an estimated average daily dose per cow of 10 mg cobalt as cobalt sulphate, 80 mg copper as copper glycinate, 137.5 mg copper as copper sulphate, 16.0 mg iodine as ethylenediamine dihydroiodide, 5.8 mg selenium as selenium selenite and 450 mg zinc as zinc sulphate.

All dry cows were wintered on fodder beet *(Beta vulgaris)* without further mineral supplementation, transitioning to a grass-based diet, 2–3 weeks before calving and returning to the mineralised water supply with an additional 60 g magnesium chloride, 60 g magnesium oxide and 50 g dicalcium phosphate per cow per day mixed into perennial ryegrass (*Lolium perenne*) silage. On all farms, calves were born at pasture and collected once a day in the morning. Calves were housed in covered, open fronted bay sheds in groups (5–10 per shed) of up to 20 and bedded on wood chips. Colostrum and feeding management was similar on all farms. Pooled colostrum from the first two milkings after calving was fed to calves at collection and then again within six hours on the same day. All calves were tube fed with four litres of colostrum at the first feed if farm personnel judged the calf had not suckled.

Thereafter, calves were fed a total of four to six litres divided into two equal feeds (07.00 and 15.00 hours) using pooled colostrum mixed with non-saleable milk. All calves had access to a commercially produced, 20 per cent crude protein, 13 MJ/kg ME calf starter feed (NRM GrowUp 20 per cent, NRM, Canterbury, NZ) and ryegrass hay from three days of age. Calves were turned out in batches of 20–60 onto designated calf paddocks of perennial ryegrass at four to five weeks, when the group were consuming on average 12 litres of milk per day. Ad libitum access to the calf starter meal continued until weaning when calves weighed between 85 and 95 kg and were eating an average of 1 kg of meal per day.

After weaning calves were moved to a separate farm and grazed pastures of perennial ryegrass and white clover (*Trifolium repens*) with access to a commercially produced, 16 per cent CP, 13 MJ/kg ME (NRM GrowUp 16 per cent) calf meal maintained for four to eight weeks. All calf meals contained lasalocid sodium at 180 mg/kg (Bovatec, Zoetis, NZ) to control coccidiosis.

Table 1 Allocation of injections of a trace mineral supplementation (TMS) containing 40 mg zinc, 10 mg manganese, 5 mg selenium, 15 mg copper, 5 mg chromium per ml and control treatment to experimental groups in 971 calves from 4 seasonally calving dairy farms in New Zealand

Birth	35 days	70 days
Control	Control	Control
Control	Control	TMS
TMS	Control	Control
TMS	Control	TMS
TMS	TMS	Control
TMS	TMS	TMS

No calves received any additional mineral supplementation until after the end of data recording at 140 days of age.
TMS, calves given TMS; control, calves received no treatment.

As part of standard animal health practice, on each farm serum samples were taken from a farmer-convenience selection of 10 multiparous milking cows 40–60 days before the end of the previous lactation. Liver biopsy samples were also obtained on each farm from a convenience sample of five of these cows. After calving, a separate farmer convenience selection of 10 multiparous milking cows were blood sampled at 40–60 days in milk in the same manner. Samples were tested at a commercial laboratory (Gribbles Veterinary Pathology, Invermay Research Centre, NZ) for serum copper, selenium and inorganic iodine concentrations and liver fresh weight copper concentrations.

Study design and treatment

At birth, calves were allocated into one of two treatment groups: TMS or control. Each farm had a randomised list (produced using the random number function within Microsoft Excel[Microsoft, 2013]) of 'supplement days' and 'non-supplement days'. On the former, all calves born that day were given an injection at birth of TMS. With the latter, no calves born that day were given an injection. An equal number of treatment and control days were allocated to each farm in a random order, unique for each farm so that each farm's list of days was different. This reduced the chance that weather would influence the results. At collection, TMS calves received a 0.75 ml subcutaneous injection by farm staff containing 40 mg Zn, 10 mg Mn, 5 mg Se, 15 mg Cu, 5 mg Cr per ml (Multimin+Se+Cu+Cr Cattle, Virbac South Africa, imported to NZ under Special Import Licence) administered within 24 hours of birth. Each week, total volume of product used was reconciled against number of calves recorded as injected to quantify that as many doses had been dispensed as recorded. Control calves were not injected.

Subsequently, each calf was randomly allocated to receive up to two more treatments, 5 and 10 weeks after birth. On each occasion, restricted randomisation was used to allocate calves to treatment schedules to ensure a minimum of 100 calves in all treatment groups. Supplemented calves were injected by veterinary staff with TMS at 1 ml/50 kg body weight; control calves received no mineral supplementation. The allocation of TMS and control to the experimental groups is indicated in table 1. The authors anticipated that morbidity and mortality would be highest in the first 30 days with a smaller peak at weaning.[4] Consequently, the allocation of injections was chosen to maximise the opportunity to detect differences at these times.

Sample size was calculated for the three dependent variables (morbidity, mortality and weight gain using GLIMMPSE—http://glimmpse.samplesizeshop.org/#/[13] allowing for clustering of calves within farm and repeat measurements within calf to detect a difference in incidence risk of 10 per cent in control calves and 5 per cent in TMS calves, with a 95 per cent CI and 80 per cent power.

Morbidity, mortality and growth

At the precalving farm visit, calf rearing staff were briefed on disease definitions. Perinatal mortality was defined as death within 24 hours of collection (so within 48 hours of birth) but no attempt was made to define the cause. Daily records of disease events and mortality were maintained by farm personnel using standardised forms and robust case definitions (table 2). Data were collected weekly for the first 5 weeks of rearing by one study team member to maintain engagement and standardisation. Thereafter, morbidity and mortality data were collated by veterinary staff at the monthly weighing sessions. All farm and veterinary staff involved in daily calf care and weighing were blinded to treatment as injected calves were not marked or identified.

All calves were weighed as soon after birth as possible (median age 5 days, range 1–18 days) and

Table 2 Standardised case definitions provided to farm personnel on four commercial seasonally calving dairy farms in New Zealand for recording morbidity in 971 calves from birth to 140 days

Disease	Case definition
Perinatal mortality	Heifer calf born alive, identified with a unique identification tag that died within 24 hours of entry to the calf rearing sheds.
Respiratory disease	Increased resting respiratory rate, sound or effort AND fever (›39.5°C or ›103°F) with one or more additional signs such as coughing, nasal discharge, depression, decreased appetite or rough hair coat.
Diarrhoea/scours	Liquid manure which would puddle, flow or drip with one or more additional signs such as depression, decreased appetite or rough hair coat.
Omphalitis/Navel ill	Warm, enlargement of or foul smelling discharge from the umbilical structures due to infection.
Joint ill	Swelling of one or more joints resulting in lameness±fever, dullness, depression±decreased appetite±fever of unknown origin.
Bloat	Distension of the abdomen resulting in discomfort±respiratory distress and persisting for more than 20 minutes.
Otitis media	Drooping of one or both ears±head tilt±dullness.
Other	Traumatic injury, birth defect, ringworm, warts, other (specify).

Table 3 Characteristics of farms recruited for an analysis of the effect of trace mineral supplementation (TMS) on farmer diagnosed morbidity and mortality for 971 New Zealand dairy calves in the first 140 days of life

	Farm A	Farm B	Farm C	Farm D	P value and statistical test
Herd size (predicted number calving)	2000	1600	800	850	
Calves recruited (number, per cent and 95 per cent CI)	432 21.6 per cent (19.8 per cent to 23.4 per cent)	190 11.9 (10.3 to 13.5)	174 21.7 (18.9 to 24.6)	175 20.6 (17.9 to 23.3)	X^2 test
Estimated median birth weight of calves with range (kg)	32.3 (24.4–49.3)	30.8 (24.1–40.9)	30.7 (20.6–43.0)	31.3 (21.4–45.4)	0.785 K-W test
Median age at first weighing with range (days)	5 (1–16)	5 (2–10)	5 (1–18)	5 (2–18)	0.896 K-W test
Adult median liver copper concentration 40–60 days predry off (µmol/kg) with range*	2320 (1870–2600)	2230 (1560–2690)	2180 (1400–2610)	1200 (940–2560)	0.272 K-W test
Adult median serum selenium concentration 40–60 days predry off (nmol/l) with range*	860 (750–1010)	545 (230–670)	630 (360–860)	380 (160–680)	<0.001 K-W test
Adult median inorganic iodine concentration 40–60 days predry off (µg/l) with range*	35 (32–44)	26 (16–47)	32 (29–42)	25 (24–31)	0.029 K-W test
Adult median serum copper concentration 40–60 days after calving (µmol/l) with range†	12 (10–14)	12 (10–15)	13 (8–15)	14 (11–15)	0.286 K-W test
Adult median serum selenium concentration 40–60 days after calving (nmol/l) with range†	625 (530–690)	535 (460–770)	680 (535–690)	960 (620–1050)	<0.001 K-W test
Adult median inorganic iodine concentration 40–60 days after calving (µg/l) with range†	65 (48–71)	128 (80–170)	78 (63–104)	46 (42–68)	0.006 K-W test
	Minimum	First quartile	Median	Third quartile	Maximum
Daily rainfall (mm) from PSC to PSC+140 days‡	0.00	0.00	0.00	0.20	20.40
Soil water deficit (mm) from PSC to PSC+140 days‡	0.00	7.9	15.7	40.3	52.0

*Measured from a coccygeal blood sample taken from 10 multiparous cows on each farm sampled 40–60 days predry off. Liver biopsy samples were also obtained on each farm from a convenience sample of five of these cows.
†Measured from a coccygeal blood sample taken from 10 multiparous cows on each farm sampled 40–60 days after calving.
‡Data obtained from Timaru Airport weather station.
K-W test, Kruskal-Wallis rank sum test; PSC, planned start of calving.

approximately 35, 70, 105 and 140 days after birth using an XR 3000 weighing system (Tru-Test, Hamilton, NZ). All weights were recorded electronically and manually before entry into a Microsoft Excel spread sheet, along with date of birth, breed, age of dam and farm, extracted from Minda database (LIC, Hamilton, NZ). Discrepancies and missing data were resolved after following up with farm personnel.

Climatic and soil data

Daily weather records of rain (mm) and soil water deficit (mm) were collected from the National Institute Water and Atmospheric Research (www.niwa.co.nz) website for the nearest weather station (Timaru Airport) with a similar soil type—Fluvial Recent—to the study farms. Soil type data were accessed from Landcare Research, NZ (www.landcareresearch.co.nz) and no farm was more than 30 km from Timaru Airport. In pastoral dairy systems with calves born outside, climatic factors such as the level of rainfall for the calving period and soil type influence the level of contamination and challenge for new born calves. Soil water deficit is a measure of the water loading in the soil and can be thought of as the amount of water that can be added to a soil before visible signs of ponding and excess water content are visible. A high deficit indicates a dry soil. It is a more temporally useful measure of the 'wetness' of a soil than daily rainfall, where one very wet day can have effects on hygiene for several subsequent days but is only recorded as one temporal event.

Statistical analysis

The aim of the experimental design was to investigate the effect of using TMS at time periods that coincided with calf handling under NZ conditions: in the first few days when calves are housed, at disbudding (approximately 21–28 days) and close to/at weaning (75–90 days). The number of likely disease and death events after the first 35 days was anticipated to decrease greatly, but the authors suspected they might see a small rise around weaning.[4] In this situation, a standard 3×2 factorial design would have been underpowered for the periods where disease incidence was low. Consequently, the schedule of injections was designed to maximise statistical power into the first period of 35 days but to allow us to also investigate the effect of injections at 35 and 70 days by using a mixed model.

In looking at the entire observation period of 0–140 days, the authors have repeated observations on the same calf and they have changes in a calf's TMS status over the period of observation. A mixed model with time varying variables allows the authors to analyse this type of data.[14] However, it generates a degree of complexity in the analysis and so the authors also used simple univariate analysis of the risk of morbidity and mortality unadjusted for the effect of confounders or covariates for the period where TMS status was constant for individual calves (perinatal and 3–35 days). Then the authors repeated the analysis of this time-period with a multivariate model so that they could allow for the effect of confounders and covariates.

In this way, the authors have presented a three-layer analysis which they hope will subject the data to an appropriate degree of statistical rigour but also be transparent and accessible to the reader.

Mortality data were aggregated for the first 48 hours of life and then separate morbidity and mortality data were aggregated every 5 weeks up to 20 weeks of age. The cumulative incidence risk of morbidity and mortality for each period was calculated as the number

Table 4 Distribution of the potential calf level predictor variables by administration of trace mineral supplementation at birth (TMS [n=435]) vs no administration of TMS (control [n=536]) in an analysis of farmer diagnosed morbidity and mortality from four farms in the Canterbury region of New Zealand

	Control	TMS treated	P value and statistical test
Number of calves	536	435	
Categorical variables			
Variable	Per cent and 95 per cent CI	Per cent and 95 per cent CI	
Calves born to primiparous cows (per cent). Dichotomous (0=multiparous dam, n=806; 1=primiparous dam, n=165)	21 (16.9 to 24.6)	14 (11.2 to 17.0)	0.006 x^2 test
Calves born to Friesian cows (per cent) Dichotomous (0≤75 per cent Friesian, n=282; 1>75 per cent Friesian, n=689)	72 (68.0 to 76.6)	70 (66.1 to 73.8)	0.659 x^2 test
Calves born on farm A (per cent adult cows) n=432	44 (39.4 to 48.9)	45 (40.6 to 48.9)	0.296 x^2 test
Calves born on farm B (per cent of adult cows) n=190	18 (14.0 to 21.3)	21 (17.6 to 24.4)	
Calves born on farm C (per cent of adult cows) n=174	20 (16.4 to 24.3)	16 (13.1 to 19.2)	
Calves born on farm D (per cent of adult cows) n=175	18 (14.2 to 21.6)	18 (14.9 to 21.3)	
Calves born in the presence of BVD virus (per cent) Dichotomous (0=no viraemia detected in calf cohort, n=174; 1=viraemia detected in calf cohort, n=797)*	20 (16.4 to 24.1)	16 (13.1 to 19.2)	0.100 x^2 test
Calves born in herd size<1000 Dichotomous (0≤1000 milking cows, n=349; 1>1000 milking cows, n=622)	38 (33.5 to 42.7)	34 (30.3 to 38.3)	0.222 x^2 test
Continuous variables			
Variable	Median and range	Median and range	P value and statistical test
Age at first weighing (days)	5 (1–18)	5 (1–19)	0.5125 Wilcoxon rank-sum statistical test
Calf breeding value	152 (0–224)	150 (0–228)	0.646 Wilcoxon rank-sum statistical test
Number of calves born per farm per week	51 (23–109)	50 (24–109)	0.143 Wilcoxon rank-sum statistical test
Variable	Mean and 95 per cent CI	Mean and 95 per cent CI	
Weight at first weigh (kg)	35.3 (34.81 to 35.88)	35.3 (34.84 to 35.71)	0.850 Two sample t-test
Estimated birth weight (kg)	31.4 (31.09 to 31.64)	31.8 (31.61 to 32.06)	0.187 Two sample t-test

*Persistent BVD viraemia was diagnosed as part of routine veterinary involvement in farm C in five calves born in the second and third week. All calves identified as antigen positive for BVD virus either died (n=2) or were culled within 35 days of birth (n=3).

of calves diagnosed sick for the first time or died during each period divided by the number of calves present at the start of each period.[15] For each disease, case fatality risk was defined as the proportion of calves with the disease that died over each time period.[15]

Estimated birth weight was corrected for the age at first weighing by using the formula:

Estimated birth weight=weight at first weigh date–(rate of gain in the first 35 days×age at first weigh date)

Separate general estimating equations (GEE) were used to model the probability of the perinatal mortality in the first 48 hours of life and of morbidity and mortality from 3 to 35 days. A binomial distribution was assumed and calf identity was nested within farm with an exchangeable or independent correlation structure selected. The proportion of the total variance for calves within farm was calculated as the intraclass correlation coefficient (ICC). Given that the number of clusters was small an F distribution was used to calculate the P values for group variables and a t distribution for single variables.[16] Parameter estimates from the GEE models were also compared with those from fixed (not shown) and were within 10 per cent of those estimated by the GEE model.

To assess the appropriateness of the chosen correlation structure, the quasi-likelihood under the independence model criterion (QAIC) statistic[17 18] was calculated.

For the 140 days from birth, repeated observations on the same calf were available. TMS status of individual calves changed between periods for some calves. The logit of the probability of the morbidity and mortality from birth to 140 days was modelled using two separate GEE with a binomial distribution and with one data row for each observation period (n=4) for each calf. Average marginal probability of morbidity and mortality by TMS status were calculated with all categorical predictor variables set to zero and continuous variables set to their mean value. For this same period, a GEE with a normal distribution was used to model daily gain (kg/day) for each calf. In these models, farm was included as a fixed effect and observation was included as a repeated effect within calf identity. The proportion of the total variance for repeat measures on the same calf was calculated as the ICC. To account for repeated measures within an individual, various covariance structures (autoregressive, exchangeable and independent) were added. The QAIC statistic was again used to assess the model with the best fit to the data. F and t distributions were again used to calculate the P values.[16] Parameter estimates from the GEE models were also compared with those from fixed (not shown) and were within 10 per cent of those estimated by the GEE model.

Independent variables were classified at the farm and calf level and are described in tables 3 and 4. Testing for collinearity, linearity of association and model construction methods followed standard statistical practice and are outlined in the supplementary

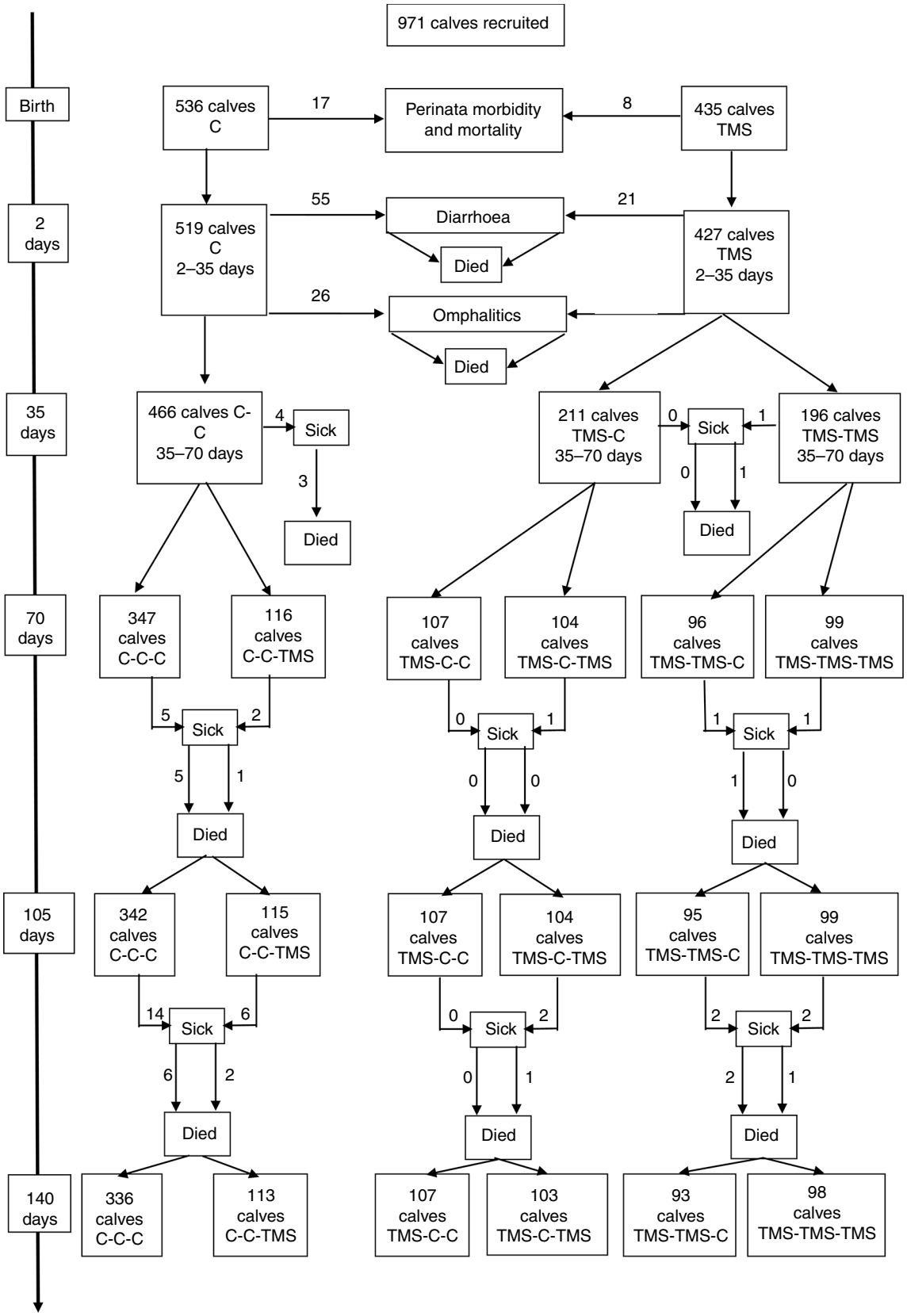

Figure 1 Allocation to treatment groups and morbidity and mortality from birth to 140 days of age for 971 dairy calves from four farms in the Canterbury region of New Zealand. Treatment consisted of up to three injections of a trace mineral supplementation (TMS) at 1 mg/50 kg containing 40 mg Zn, 10 mg Mn, 5 mg Se, 15 mg Cu, 5 mg Cr per ml. Control calves received no supplementation.

material. All analyses were performed using R Core Team (2017), with GEE models constructed using Zelig.[19 20]

Results

There were no adverse events recorded during the trial period.

Enrolment

Farm characteristics: the characteristics considered as potential independent variables for the four farms are given in table 3.

Mineral status: the copper, selenium and iodine status for all individual animals and for the group mean

Table 5 Final general estimating equation predicting the effect of trace mineral supplementation (TMS) on the logit of the probability of farmer diagnosed mortality in the first two days of life for 971 dairy calves from four herds in the Canterbury region of New Zealand

Input variable	Estimate	sem	P value*	OR (95 per cent CI)	LSM† Incidence risk per cent (95 per cent CI)*
Intercept	−5.48	0.73	<0.001		
Treatment group					
Control	Ref				3.60 (2.01 to 6.45)
TMS	−0.39	0.27	0.153	0.68 (0.39 to 1.16)	2.43 (1.73 to 3.41)
Presence of BVD viraemia in calves					
Absent	Ref				1.56 (1.19 to 2.05)
Present	1.28	0.21	<0.001	3.40 (2.38 to 5.45)	5.61 (3.17 to 9.90)
Breed of calf‡					
Cross-bred	Ref				2.19 (1.20 to 3.99)
Friesian	0.60	0.25	0.015	1.82 (1.12 to 2.95)	3.99 (3.07 to 5.17)
Birth weight§					
16.4–28.2 kg	Ref				1.45 (0.66 to 3.22)
28.3–35.3 kg	0.87	0.23	<0.001	2.40 (1.52 to 3.79)	3.48 (2.31 to 5.25)
35.4–49.3 kg	1.25	1.25	0.315	3.51 (0.30 to 4.54)	5.10 (0.87 to 13.04)
Herd size†					
<1000 cows	Ref				1.97 (1.33 to 2.92)
>1000 cows	0.81	0.02	<0.001	2.25 (2.15 to 2.35)	4.43 (2.98 to 6.59)
Dam age					
Multiparous	Ref				1.01 (0.62 to 1.64)
Primiparous	2.15	0.36	<0.001	8.60 (4.23 to 17.45)	8.67 (4.89 to 15.36)
Soil water deficit					
<30 mm	Ref				7.89 (6.59 to 9.35)
≥30 mm	−1.95	0.49	<0.001	0.14 (0.05 to 0.37)	1.11 (0.47 to 2.64)

ICC for calves within herds=0.0035 (95 per cent CI 0.001 to 0.096).
*Significance of coefficient in the model.
†Lease-square mean prediction from the regression model.
‡F≥75 per cent Friesian; cross-bred≤75 per cent Friesian. Overall significance of breed, P=0.015.
§Overall significance of birth weight, P<0.001.
ICC, intraclass correlation coefficient; Ref, reference category; RR, Relative risk.

were compared with reference ranges of >8 µmol/l for serum copper,[21] more than 300 µmol/kg fresh weight for liver copper,[22] more than 140 nmol/l for serum selenium[23] and more than 45 µg/l for plasma inorganic iodine.[24] All samples were above the threshold value in all herds except for plasma inorganic iodine at the end of the previous lactation.

Calf characteristics: over the period July 27, 2016 to August 25, 2016, 971 calves were recruited into the study from four farms and weighed for the first time. The allocation and fate of these calves to the different treatment groups is detailed in figure 1.

The calf level variables considered for inclusion in the model were evaluated to ensure similar distribution of potential confounder variables between calves treated with TMS at birth and control calves are listed in table 4. The restricted randomisation used to ensure 100 calves in each final treatment group (figure 1) led to the greater number of control calves at birth. None of the variables were highly correlated.

Perinatal mortality

The cumulative perinatal mortality in the first 48 hours of life for TMS-treated calves was 8/435=1.8 per cent (95 per cent CI 0.60 to 3.10) and for control calves 17/536=3.2 per cent (95 per cent CI 1.69 to 4.66), RR=0.58 (95 per cent CI 0.25 to 1.33). Morbidity not leading to mortality during the first 48 hours of life was not recorded.

The results for the GEE predicting the odds of mortality in the first two days of life are presented in table 5; there was a significant effect of presence of BVD virus, calf breed, birth weight, herd size, dam age and soil water deficit, but no effect of treatment. No confounding or interactions were detected.

Morbidity from 3 to 35 days

From 3 to 35 days the cumulative perinatal morbidity for TMS calves was 32/427 (7.5 per cent) and for control calves, 81/519 (15.6 per cent), RR=0.48 (95 per cent CI 0.33to 0.71). Only two categories of morbidity were recorded: diarrhoea (76/113; 67.3 per cent) and omphalitis (37/113; 32.7 per cent). In the TMS group, 21/427 (4.9 per cent) calves were recorded as having diarrhoea compared with 55/519 (10.6 per cent) in the control group, RR=0.46 (95 per cent CI 0.29 to 0.75). The median age for TMS and control calves with diarrhoea was 12 days (range 3–35) and 9 days (range 2–35), P=0.174. The median age for TMS and control calves diagnosed with omphalitis was 7 days (range 1–21) and 9 days (range 1–14), P=0.654. The results for the GEE predicting the odds of diarrhoea from 3 to 35 days of life are presented in table 6. There was a significant effect of treatment, presence of BVD virus,

Table 6 Final general estimating equation predicting the effect of trace mineral supplementation (TMS) on the logit of the probability of farmer diagnosed diarrhoea from 3 to 35 days of age for 971 dairy calves from four herds in the Canterbury region of New Zealand

Input variable	Estimate	sem	P value*	OR (95 per cent CI)	LSM Incidence risk per cent (95 per cent CI)†
Intercept	−3.42	0.08	<0.01		
Treatment group					
Control	Ref				17.57 (13.21 to 23.40)
TMS	−0.81	0.32	0.009	0.44 (0.24 to 0.82)	7.78 (5.17 to 11.7)
Presence of BVD viraemia in calves					
Absent	Ref				4.63 (3.92 to 5.48)
Present	1.85	0.10	<0.001	6.37 (5.24 to 7.74)	29.49 (23.72 to 36.67)
Herd size					
<1000 cows	Ref				6.32 (5.58 to 7.15)
>1000 cows	1.23	0.10	<0.001	3.40 (2.86 to 4.03)	21.63 (16.86 to 27.75)
Dam age					
Multiparous	Ref				10.10 (8.51 to 12.10)
Primiparous	0.28	0.16	0.078	1.33 (0.97 to 1.83)	13.50 (10.23 to 17.80)
Centred weight‡	−0.04	0.01	0.005	0.96 (0.94 to 0.99)	11.7 (9.88 to 13.80)

ICC for calves within herds=0.021 (95 per cent CI 0.004 to 0.261).
*Significance of coefficient in the model.
†Lease-square mean prediction from the regression model.
‡Weight centred around the mean at first weigh of 35.3 kg.
§F≥75 per cent Friesian; cross-bred≤75 per cent Friesian.
ICC, intraclass correlation coefficient; Ref, reference category.

calf weight, herd size and dam age. No confounding or interactions were detected.

Of the 37 calves with omphalitis, 11/427 (2.6 per cent) were in the TMS group and 26/519 (5.0 per cent) in the control group, RR=0.51 (95 per cent CI 0.26 to 1.03, P=0.051).

The median ages for TMS and control calves with omphalitis was 9 days (range 1–14) and 7 days (range 1–21), respectively, P=0.639. The results for the GEE predicting the odds of omphalitis from 3 to 35 days of life can be found in online supplementary information, online supplementary table 1s. The estimated OR for the effect of TMS was 0.55 (95 per cent CI 0.39 to 0.77).

Mortality from 3 to 35 days

Cumulative mortality over this period was 19/427 (4.4 per cent) for TMS calves and for control calves, 54/519 (10.4 per cent), RR=0.43 (95 per cent CI 0.26 to 0.71).

There was no difference in the case fatality risk for TMS-treated calves with diarrhoea (7/21; 33 per cent) compared with 13/55 (23.6 per cent) for control calves, RR=0.87 (95 per cent CI 0.62 to 1.22). The median age at death for TMS calves with diarrhoea was 9 days (range 3–31) and 14 days (range 4–25) for control calves, P=0.163.

Similarly, there was no difference in the case fatality risk for TMS-treated calves with omphalitis (5/11; 45.5 per cent) compared with 12/26 (46.2 per cent), RR=0.98 (95 per cent CI 0.46 to 2.13). The median age at death for TMS calves with omphalitis was 7 days (range 5–22) and 10 days (range 6–11) for control calves, P=0.552.

The results for the GEE with an independence correlation structure predicting the logit of mortality from 3 to 35 days of life are presented in table 7. There was a significant effect of treatment and herd size. No confounding or interactions were detected.

Table 7 Final general estimating equation predicting the effect of trace mineral supplementation (TMS) on the logit of the probability of mortality from 3 to 35 days of life for 971 dairy calves from four herds in the Canterbury region of New Zealand

Input variable	Estimate	sem	P value*	OR (95 per cent CI)	LSM Incidence risk per cent (95 per cent CI)†
Intercept	−2.04	0.08			
Treatment group					
Control					11.83 (10.07 to 13.90)
TMS	−0.90	0.32	0.004	0.41 (0.23 to 0.73)	4.79 (3.02 to 7.60)
Herd size					
<1000 cows					8.27 (7.07 to 9.67)
>1000 cows	−0.19	0.03	<0.001	0.85 (0.72 to 0.99)	6.85 (5.84 to 8.04)

*Significance of coefficient in the model.
†Lease-square mean prediction from the regression model.
Ref, reference category.

Table 8 Final general estimating equation predicting the effect of trace mineral supplementation (TMS) on the logit of the probability of morbidity from birth to 140 days of life for 971 dairy calves from four herds in the Canterbury region of New Zealand

Input variable	Estimate	sem	P value*	OR (95 per cent CI)	LSM Incidence risk per cent (95 per cent CI)†
Intercept	−1.24	0.40	0.002		
Treatment group					
Control					3.74 (2.80 to 5.00)
TMS	−0.55	0.19	0.004	0.58 (0.40 to 0.84)	2.04 (1.31 to 3.18)
Herd of origin‡					
A	Ref				1.98 (1.35 to 2.89)
B	−0.52	0.30	0.084	0.59 (0.33 to 1.07)	1.68 (1.02 to 2.73)
C	0.81	0.27	0.003	2.26 (1.33 to 3.83)	6.51 (4.30 to 9.86)
D	−0.11	0.28	0.683	0.89 (0.52 to 1.53)	2.69 (1.69 to 4.30)
Days from birth§					
0–35	Ref				19.34 (15.31 to 24.41)
36–70	−3.18	0.41	<0.001	0.04 (0.02 to 0.09)	0.67 (0.29 to 1.56)
71–105	−2.10	0.26	<0.001	0.12 (0.07 to 0.20)	2.45 (1.45 to 4.14)
105–140	−2.32	0.27	<0.001	0.10 (0.06 to 0.17)	1.84 (1.08 to 3.14)
Dam age					
Multiparous	Ref				1.95 (4.42 to 2.67)
Primiparous	0.61	0.21	0.004	1.84 (1.22 to 2.77)	3.92 (2.53 to 6.09)
Calves born on farm in birth week	−0.01	0.005	0.029	0.99 (0.98 to 0.99)	

ICC for repeat measurements within calf 0.53 (95 per cent CI 0.50 to 0.56).
*Significance of coefficient in the model.
†Lease-square mean prediction from the regression model.
‡Overall significance of herd P<0.001.
§Overall significance of days P<0.001.
ICC, intraclass correlation coefficient; Ref, reference category.

Morbidity and mortality from birth to 140 days

Cumulative morbidity over this period was 121/536 (22.6 per cent) for control calves, while cumulative mortality was 85/536 (15.9 per cent). Treatment status of TMS calves changed throughout this period (figure 1) and so the analysis of the effects of treatment was conducted using a GEE.

The results for the GEE predicting the odds of morbidity and mortality from birth to 140 days of life are presented in tables 8 and 9. There were significant

Table 9 Final general estimating equation predicting the effect of trace mineral supplementation (TMS) on the logit of the probability of mortality from birth to 140 days of life for 971 dairy calves from four herds in the Canterbury region of New Zealand

Input variable	Estimate	sem	P value*	OR (95 per cent CI)	LSM Incidence risk per cent (95 per cent CI)†
Intercept	−0.57	0.44	0.188		
Treatment group					
Control	Ref				1.22 (0.73 to 2.02)
TMS	−0.68	0.23	0.004	0.50 (0.32 to 0.80)	0.61 (0.32 to 1.17)
Herd of origin‡					
1	Ref				1.50 (0.89 to 2.52)
2	−0.97	0.33	0.003	0.38 (0.20 to 0.72)	0.57 (0.28 to 1.14)
3	−0.21	0.31	0.504	0.81 (0.44 to 1.50)	1.21 (0.59 to 2.51)
4	−1.02	0.33	0.002	0.36 (0.19 to 0.69)	0.54 (0.26 to 1.10)
Days from birth§					
0–35	Ref				8.53 (6.23 to 11.68)
36–70	−4.02	0.72	<0.001	0.02 (0.004 to 0.07)	0.15 (0.04 to 0.65)
71–105	−3.00	0.46	<0.001	0.05 (0.02 to 0.12)	0.43 (0.17 to 1.05)
105–140	−2.15	0.30	<0.001	0.12 (0.07 to 0.21)	0.99 (0.52 to 1.91)
Dam age					
Multiparous	Ref				0.79 (0.47 to 1.32)
Primiparous	0.18	0.26	0.488	1.20 (0.72 to 2.00)	0.94 (0.49 to 1.82)
Calves born on farm in birth week	−0.02	0.005	<0.001	0.98 (0.97 to 0.99)	0.86 (0.51 to 1.47)

ICC for repeat measurements within calf 0.82 (95 per cent CI 0.81 to 0.84).
*Significance of coefficient in the model.
†Lease-square mean prediction from the regression model.
‡Overall significance of herd P<0.001.
§Overall significance of days P<0.001.
ICC, intraclass correlation coefficient; Ref, reference category.

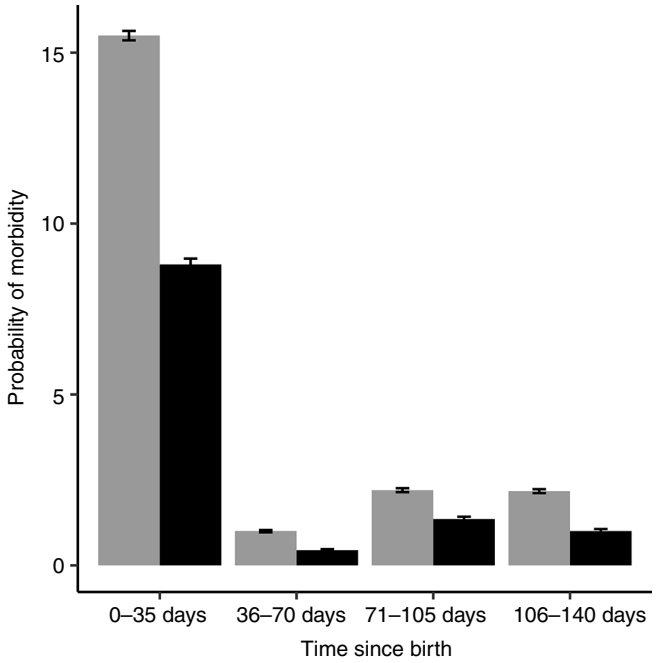

Figure 2 Model predicted average (±sem) marginal probability of farmer diagnosed morbidity from birth to 140 days for 971 calves on four herds in the Canterbury region of New Zealand. All categorical variables excluding trace mineral supplementation (TMS) status were set to zero and the continuous variables were set to their mean value. Black bars represent calves treated with TMS, grey bars control calves.

effects of treatment, herd, days since birth and dam age for morbidity and mortality plus for morbidity alone the number of calves born in the week of birth. No confounding or interactions were detected.

The predicted average marginal probabilities for morbidity and mortality from birth to 140 days by TMS status are shown in figures 2 and 3. There was a significant difference in the predicted probability of

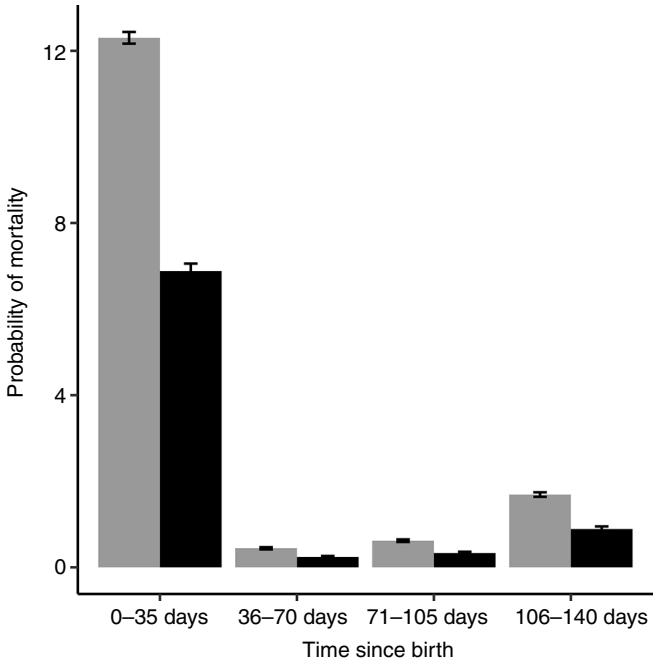

Figure 3 Model predicted average (±sem) marginal probability of farmer diagnosed mortality from birth to 140 days for 971 calves on four herds in the Canterbury region of New Zealand. All categorical variables excluding trace mineral supplementation (TMS) status were set to zero and the continuous variables were set to their mean value. Black bars represent calves treated with TMS, grey bars control calves.

Table 10 Final general estimating equation predicting the effect of trace mineral supplementation (TMS) on the daily gain in kg/day from birth to 140 days of life for 971 dairy calves from four herds in the Canterbury region of New Zealand

Input variable	Estimate	sem	P value*
Intercept	0.56	0.01	‹0.001
Treatment group			
Control	Ref		
TMS	−0.004	0.007	0.605
Herd of origin†			
1	Ref		
2	−0.10	0.01	‹0.001
3	0.04	0.01	‹0.001
4	0.16	0.01	‹0.001
Days from birth‡			
0–35	Ref		
36–70	0.13	0.01	‹0.001
71–105	0.28	0.01	‹0.001
105–140	0.11	0.01	‹0.001
Dam age			
Multiparous	Ref		
Primiparous	−0.04	0.01	‹0.001
Calves born on farm in birth week	−0.0003	0.0001	0.047

ICC for repeat measurements within calf 0.07 (95 per cent CI 0.04 to 0.11).
*Significance of coefficient in the model.
†Overall significance of herd P‹0.001.
‡Overall significance of days P‹0.001.
ICC, intraclass correlation coefficient; Ref, reference category.

morbidity and mortality in each period (0–35, 36–70, 71–140 days) for calves receiving TMS in that period.

Weight gain from birth to 140 days

Between birth and 140 days, the average rate of gain was 0.67 kg/day (95 per cent CI 0.66 to 0.67). The results for the GEE with an autoregressive correlation structure predicting the daily gain in kg/day from birth to 140 days of life are presented in table 10. The relationship between time from birth and growth rate was non-linear so the variable for time since birth (0–35, 36–70, 71–105, 106–140 days) was expressed as a factor. Inclusion of farm as a fixed factor meant that the farm level variable for BVD status could not be included in the model. There was no association between treatment with TMS and gain (P=0.605). No confounding or interactions were detected.

The average marginal growth rate in kg/day from birth to 140 days by TMS status is shown in online supplementary figure 2s as online supplementary information.

Discussion

This is the first known report on the effect of TMS supplementation on perinatal and postnatal morbidity and mortality. The results indicate that on these farms injection of TMS reduced the risk of mortality and morbidity in the postnatal period. The odds of morbidity and mortality changed with the time but the reduction in the OR as the result of TMS injection was the same for each time period. There was no effect of TMS on the rate

of weight gain for calves in agreement with the results from the study by Teixeira and others.[8] The purpose of the present study was to investigate the effect of TMS rather than the effect of morbidity on gain and there was no evidence for confounding by sickness on the relationship between TMS and gain and so morbidity was excluded from the GEE models.

Farmer reporting of morbidity and mortality was used in the present study and there was no confirmatory diagnostic or postmortem work carried out. Thus, underestimation of morbidity could have occurred[25] and the authors acknowledge that this may have led to inaccuracies in the estimation of morbidity. Consequently, the incidence of morbidity and mortality needs to be compared with that reported elsewhere. Calves born dead, male calves and female calves born after the first three weeks of calving were excluded but the levels of perinatal mortality described are comparable to the 5.7 per cent (95 per cent CI 5.4 per cent to 6.1 per cent) recorded by Cuttance and others.[4] However, postnatal mortality risk higher than the 4.1 per cent (95 per cent CI 3.6 per cent to 4.6 per cent) from 24 hours to weaning at an average of 13 weeks observed by Cuttance and others[4] and higher than the top end of the range reported by Compton and others.[1] Although the period of follow up was greater in the present study than in the study by Cuttance and others[4] and most of the studies assessed by Compton and others,[1] this does not completely explain the greater mortality observed. It is likely that this increased mortality is related to the increased morbidity seen in the present study compared with the study by Cuttance and others.[5] The latter study reported an overall postnatal morbidity rate until weaning (~13 weeks) of 8 per cent, whereas in the present study in the control cattle the morbidity for the shorter period from 2 to 35 days after birth was 16 per cent. This was reflected in considerable increases in calves affected with diarrhoea and omphalophlebitis. In the present study, between 2 and 35 days of age, 10.6 per cent and 5.0 per cent of calves were affected with diarrhoea and omphalophlebitis, respectively. The equivalent preweaning figures for the study by Cuttance and others[4] were 5 per cent and 1 per cent, respectively.

The mortality data were likely to be more robust as physical absence of a calf at one of the follow-up visits was always accompanied by back tracing of the animal to determine its fate. However, some animals that were identified as non-replacements after tagging may have been sold but incorrectly recorded as dead.

The reduction in the incidence of morbidity in TMS calves in the present study is similar to that reported elsewhere. The authors found the OR for diarrhoea in the period 3–35 days was 0.44 (95 per cent CI 0.24 to 0.82) for TMS calves compared with control calves. In a study looking at the effects of two injections each containing 60 mg of Zn, 10 mg of Mn, 5 mg of Se and 15 mg of Cu at 3 and 30 days after birth, Teixeira and others[8] found the OR for diarrhoea in the first 50 days of life was 0.72 (95 per cent CI 0.54 to 0.98) for TMS-treated calves compared with control calves. Morbidity was much higher in that study (49.7 per cent of control calves were diagnosed with diarrhoea and 49.1 per cent with pneumonia, otitis or both).

In the analysis of morbidity and mortality from 2 to 35 days, the use of a GEE model allowed us to include farm level factors such as herd size and presence of BVD on farm, which would not have been possible with farm included as a fixed effect with a generalised linear model[26] and is similar to the approach adopted by Mee and others.[3] However, the small number of clusters in the present study may have led to an increase in type 1 errors[27] despite using the F distribution to calculate the P values.

The authors have not been able to find reports of the impact for pen mates of coexisting with BVD viraemic calves under NZ conditions but believe the increase in morbidity and mortality observed is consistent with the immunosuppression associated with BVD virus seen in persistently and transiently viraemic calves.[28] The increase in the risk of morbidity in the first 35 days of life associated with the presence of BVD seen in this study, is in line with the finding from a farm study looking at transference of respiratory disease in beef calves.[29] However, that was a single farm study and respiratory disease was not diagnosed within the dairy calves from the present study.

Costa and others[30] concluded that factors such as colostrum transfer, group structure, pattern of feeding and hygiene were potentially more significant predictors of calf morbidity than housing system despite the differences in disease challenge and management. In NZ, Cuttance and others[4] reported that in addition to colostrum intake, region, farm and method of colostrum supplementation all affected calf morbidity, while mortality was affected by region, farm and date of calving.

The greater proportion of calves born to primiparous dams in the control group could have been a confounder in the present study and so it was forced into all models. Many of the identified risk factors for calf mortality may be influenced by dam parity including the risk of dystocia, quality of colostrum and mothering ability. Mee and others[3] reported an increase in perinatal mortality for primiparous dams and an interaction with dystocia such that the calves from primiparous dams with dystocia were more likely to die than the offspring of multiparous dams with dystocia. However, in other studies the increase in mortality associated with primiparous dams was not seen.[4 31] Several studies indicate that older cows tend to produce higher quality and quantities of colostrum.[32 33] However Pritchett and others[34] found an inverse relationship between colostrum concentration and volume and this may explain the observation

from a NZ study that the odds of a diagnosis of FPT increased with dam age.[6]

The association between morbidity-mortality and number of calves born in the week of birth could have been confounded by unrecorded factors related to FPT such as farm time pressure. Cuttance and others[4] found an increase in perinatal calf mortality in the first week of calving but in the authors' data set there was no correlation between the number of calves born and time from PSC (Spearman's correlation coefficient=−0.066). This may reflect a lack of power as data was only extracted from the first three weeks of calving where the range of calves born per day would be smaller than for the whole season but the authors recruited only calves born at full term, excluding those born before the PSC.

A study such as this one is not designed to establish the mechanism by which TMS may have reduced morbidity and mortality. Previous studies have suggested mechanisms such as improved functioning of antioxidant enzymes leading to an increased phagocytotic response,[8] and the role of Zn in cell proliferation and replication.[35] Data on FPT and on blood levels of minerals in the study calves were absent in the present study. Local gut immunity begins in the first week of life with the production of immunoglobulin M and by two weeks of age calves reach cell-mediated response levels similar to adults.[36] Given that the median time to morbidity for TMS calves in the first 42 days was 12 days for diarrhoea and 9 days for omphalitis, this is consistent with an effect of TMS via the cell-mediated innate immune system.

Data have been presented elsewhere[8 11 12 37] reporting the effect of TMS on blood and liver mineral profiles in calves. In the present study, and as with other published studies,[38] the authors are not attempting to link the reduction in morbidity and mortality to any particular change in mineral blood or liver level, rather to report the observed association. The authors plan a follow-up study looking in more detail at the effect of mineral supplementation on the immune response of calves and report these preliminary findings in that light.

Mineral concentrations in all cows tested were within the normal range and inclusion of mineral level as a farm level variable did not result in significant predictors or improve the quasi-likelihood of the model. However, quantification of herd mineral status by sampling individual cows is prone to error both in terms of selection of a representative sample, the choice of testing methodology and the availability of tests that reliably quantify an animal's mineral status.[22] The sample size in the present study was sufficient for assessment of serum selenium but those workers recommended a minimum of 12 liver biopsy assays for determination of liver copper status. Others have pointed out that a variable amount of copper is sequestered into the clot formed within a serum sample so that the agreement between serum copper status and other measures (plasma copper and plasma caeruloplasmin) is poor.[39] However, the intention in presenting the data on the mineral dosing levels used for these herds and their blood mineral levels was to confirm that the background mineral status on all farms was equivalent.

Conclusions

TMS improved the morbidity and mortality of calves from birth to 140 days, but had no effect on the rate of weight gain over this period. Injection of TMS close to birth appears to be an effective means of improving calf welfare and health outcomes on these pastoral NZ dairy farms.

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Competing interests The authors wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work. AB and MS are employees of Dairy Excellence Centre, owned by Vetlife Ltd; MW is an employee of Virbac NZ. RAL is employed by Massey University, NZ.

Ethics approval The study was approved by the Animal Ethics Committee of Massey University, New Zealand (study number 16/46).

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