

Production and performance of commercial beef breeding females in northern Australia. 4. Factors influencing the occurrence of lactating cows becoming pregnant within 4 months of calving

K. D. McCosker^{A,F,*} , N. R. Perkins^{B,G}, G. Fordyce^D , P. K. O'Rourke^E and M. R. McGowan^C

For full list of author affiliations and declarations see end of paper

***Correspondence to:**

K. D. McCosker
The University of Queensland, Queensland
Alliance for Agriculture and Food
Innovation, Centre for Animal Science,
Warrego Highway, Gatton, Qld 4343,
Australia
Email: kieren.mccosker@bigpond.com

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ABSTRACT

Context. Sound reproductive efficiency is a key determinant for the overall productivity of a beef breeding business. For beef breeding herds to obtain high levels of reproductive productivity, breeding females need to efficiently become pregnant while lactating. **Aims.** This study aimed to determine and quantify the major factors associated with lactating cows becoming pregnant within 4 months of calving (P4M) in commercial beef breeding herds of northern Australia.

Methods. A prospective epidemiological study was conducted using 78 commercial northern Australian beef breeding herds and involved 78 000 cattle that were monitored for 3–4 years. A multivariable model-building process was employed to scrutinise the resulting dataset to identify what herd-management practices, and nutritional, environmental and individual cow factors were major determinants of lactating cows becoming pregnant within 4 months of calving (P4M) and to estimate their magnitudes of effect. **Key results.** Overall, 41.6% of cows per production year were successful for P4M. Country type was strongly associated with 65.4%, 57.5%, 61.8% and 16.4% P4M for the Southern Forest, Central Forest, Northern Downs, and Northern Forest respectively. Between-year variability ranged between 3.3 and 11.7 percentage points. Cows calving in December–January (61%) had a substantially higher occurrence of P4M than did cows calving between July and September (15%). The difference in P4M when comparing availability of wet-season pasture protein and phosphorus was 12.7 and 20.3 percentage points respectively. Modelling of the impact of group seroprevalence and management group prevalence of recent infection with several infectious diseases was estimated, with a large negative association between group bovine viral diarrhoea seroprevalence and P4M suggested.

Conclusions. This study further demonstrated the substantial impact that environment, herd management practices, nutrition and disease factors can have on the reproductive performance of females. **Implications.** To optimise the performance of females (through increasing the occurrence of cows contributing calves in consecutive years) under commercial conditions in northern Australia, herd managers should focus on maximising the proportion of cows within a herd calving at the desired time of the year, ensuring that any nutritional deficiencies and herd health issues are managed, and that cows are managed such that they are of good body condition score at the time of calving.

Keywords: conception, beef cattle, fertility, northern Australia, pregnancy, reproduction, tropics.

Introduction

Approximately half of Australia's beef breeding herds are in northern Australia, which includes the state of Queensland, the Northern Territory and the northern part of the state of Western Australia. The subtropical–tropical region is characterised by distinctive dry and wet seasons, with a summer-dominated rainfall pattern. Soil fertility is highly variable, with most areas north of the Tropic of Capricorn being considered at least

marginally phosphorus (P) deficient. Many areas in central and southern Queensland have moderate- to high-fertility soils. Beef cattle graze either improved tropical pastures or native pastures that vary considerably in dry-matter digestibility and crude protein content according to season.

A diverse range of beef production systems exist in northern Australia, varying by marketing options, mating systems and environmental conditions. Characteristics, including multiple market options, level of development, proximity to infrastructure and access to high-energy by-products of other industries differ in availability among regions (Bortolussi *et al.* 2005a). The live-export market is the major outlet for cattle in northern Australia. Herds within northern, north-western and western Queensland, the Northern Territory and northern Western Australia are typically either partially or completely reliant on the market. Approximately two-thirds of cow herds in the dry tropical rangelands of northern Australia are continuously mated, whereas in areas with higher soil fertility and more intensive management, herds are control-mated, typically for periods of 3–7 months (McCosker *et al.* 2020). By contrast, herds within southern and central Queensland are primarily control-mated and turnoff is typically ≤ 2 -years of age and commonly to domestic markets.

The vast majority of beef cattle in northern Australia are of *Bos indicus* or *Bos indicus*-derived content (Holroyd *et al.* 2000), to enable them to better cope with high environmental temperatures, low-quality pastures and internal and external parasitism, in particular, cattle tick (*Rhipicephalus microplus*) and buffalo fly (*Haematobia irritans exigua*) infestations. Cattle are typically mustered (brought together from the paddock into a cattle-handling facility) twice a year for branding, weaning and other husbandry such as pregnancy diagnosis, usually in the late wet–early dry season and then again in the mid-dry season (Cowley *et al.* 2014). Helicopter mustering is now commonly used on most extensively managed properties.

The beef industry in northern Australia has been reported to be in an unprofitable and unsustainable state, with many beef enterprises tending to spend more than earnings in 6 of the previous 7 years (McLean *et al.* 2013). Productivity of the beef breeding component of a beef herd (reproductive productivity) is a function of the annual percentage of cows mated weaning calves and the liveweight of calves at weaning (Arthur *et al.* 1999). Since the efficiency with which lactating cows become pregnant influences both the annual percentage of calves weaned and the liveweight of calves at weaning, the average interval between calving and the establishment of their next pregnancy is a significant determinant of the productivity and profitability of northern Australian beef herds (Braithwaite and de Witte 1999; McLean *et al.* 2013).

For northern Australian beef herds, the percentage of cows annually contributing a calf is often low, ranging between 50% and 70% (Burns *et al.* 2010), which is largely attributed

to prolonged postpartum anoestrus interval (Entwistle 1983; Fordyce *et al.* 1997). Studies have reported the impacts of biological, breeding management and individual cow risk factors on the postpartum anoestrus interval of beef cattle, including the impact of parity, body condition score at calving, plane of nutrition, season of calving and genotype (Baker 1969; Short *et al.* 1990; Yavas and Walton 2000; Montiel and Ahuja 2005; Blanc and Agabriel 2008; Hawken *et al.* 2012). However, there have been few epidemiological studies that have determined and quantified the factors affecting the occurrence of commercially managed beef cows becoming pregnant while lactating.

The primary objective of this study was to identify the major associations among herd management, nutritional, environmental, disease and individual cow factors, with several animal-level outcomes summarising reproductive performance. The results are reported as a series of eight papers, with the current paper being the fourth. This research has provided the opportunity to quantify the probability of lactating cows being pregnant within 4 months of calving. It outlines which group of factors explains the largest amount of variation in performance on commercial beef cattle breeding properties in northern Australia.

Materials and methods

Overview of study design

A prospective population-based epidemiological study was conducted with commercial beef breeding herds in northern Australia. Seventy-eight properties (farms) located across each of the major beef breeding regions of Queensland, the Northern Territory and northern Western Australia participated (Fig. 1). Potential collaborating herds were identified by project regional coordinators or collaborating veterinarians as meeting the following criteria:

- (1) only herd managers who were keen to participate and support the project and thought to be likely to maintain accurate records were included;
- (2) properties were selected that were considered to be typical in their region for their property size and herd management;
- (3) herd managers were prepared to maintain the enrolled management groups on their property, with the exception of females culled as per normal property breeding herd management policy, for the duration of the study;
- (4) all enrolled females were individually electronically identified for the duration of the study;
- (5) The herd manager was prepared to attend a 1-day training workshop in assessing standing pasture biomass and land condition;
- (6) properties had access to reasonable working-condition cattle-handling facilities and herd managers were

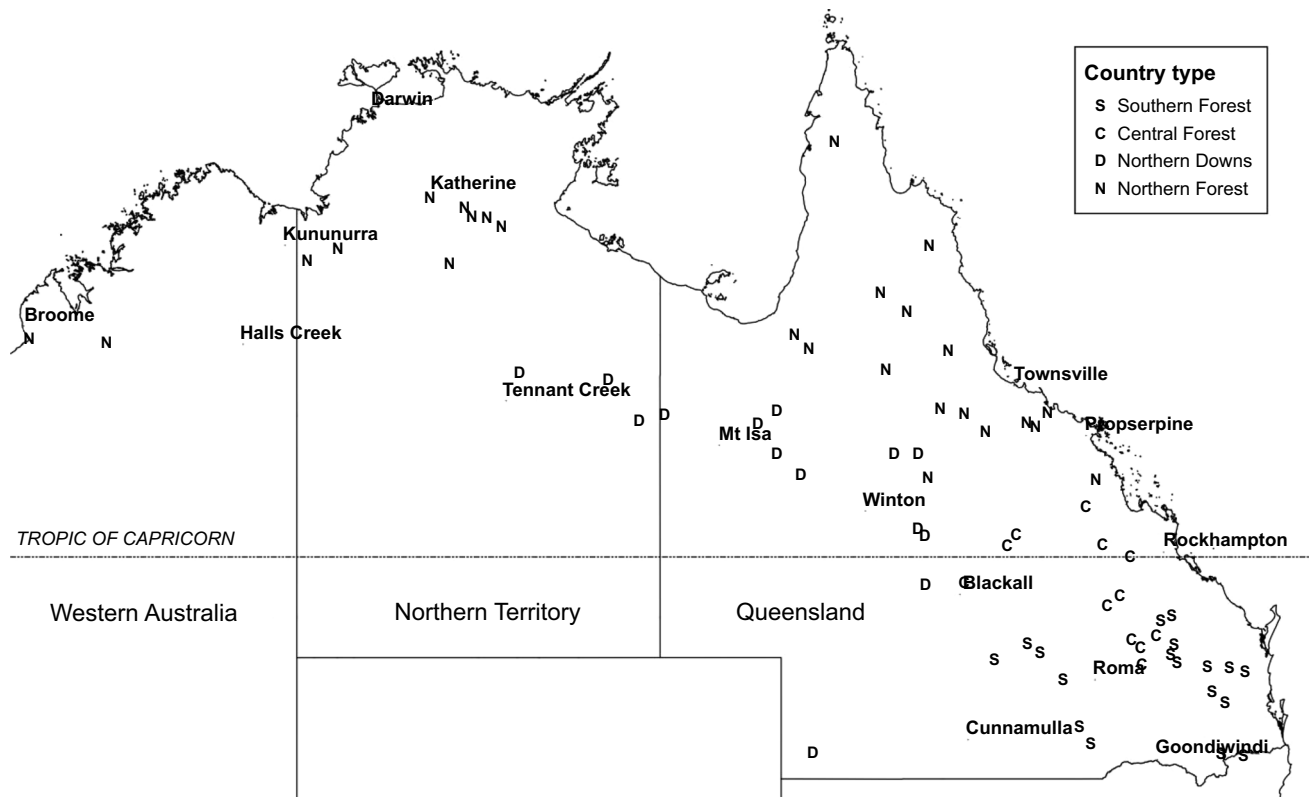


Fig. 1. Location of cooperating properties by country type.

prepared to ensure that all enrolled management groups were mustered a minimum of twice a year. In controlled-mated herds, musters were completed to brand calves in the first few months of the year and again for pregnancy diagnosis and to wean calves, which typically corresponded to at least 6 weeks after the end of mating or when the bulls were withdrawn; in continuously mated herds, two annual weaning musters (in April–June and August–October) were typically conducted, with pregnancy diagnosis of all females being completed at the second annual muster;

- (7) nearly all pregnancy diagnosis and fetal ageing of enrolled management groups was conducted by accredited cattle veterinarians (National Pregnancy Diagnosis Scheme, Australian Cattle Veterinarians); in the very few situations where an accredited cattle veterinarian could not attend, experienced beef production research officers who had been internally assessed on their ability to accurately estimate fetal age, were used;
- (8) properties had access to weighing facilities and, at a minimum, were prepared to record the individual liveweight and associated information for weaners from each enrolled herd at each weaning muster.

Cooperating properties were progressively enrolled over 2 years. Initially, a pilot study involving 13 properties was undertaken, with each property enrolling a management

group of recently mated heifers during 2007–2008 to inform the management and design the larger observational study that was conducted during 2008–2011. Each cooperating property typically enrolled two cohorts of females, a management group of heifers that had been exposed to bulls for the first time and a management group of mature cows. All females were enrolled in groups of between 100 and 500 females. However, in management groups that were larger than 500 females, a cross-sectional subset of 300 females was enrolled.

Animal performance monitoring

Commercially available individual animal data collection systems (e.g. BeefLink™, AgInfoLink) were utilised to systematically capture and store data against an individual's electronic animal identification number read from their National Livestock Identification System (NLIS; www.nlis.com.au) ear tag at the time of mustering. In most situations, a visual management tag displaying a unique five-digit identification number was also attached to the animal to identify that the animal was enrolled into the study and would establish data linkages to historical performance data in the event of the NLIS tag requiring replacing.

Animals were described at their first muster with information on estimated *Bos indicus* content, year of weaning (year brand) and hip height (Fordyce et al. 2013a)

being recorded. Performance and explanatory data were recorded twice a year for each cow enrolled into the project, at the main branding or weaning muster and at the pregnancy diagnosis muster, which, on average, occurred 3.8 months later. An exception to this was cohorts of heifers during their first year enrolled in the study, where a single pregnancy diagnosis muster was completed. At each muster, body condition score (Gaden *et al.* 2005) and lactation status were visually assessed and recorded. Liveweight of cattle was captured wherever possible and was for approximately 77% of mobs. Pregnancy status was recorded for all cows at the pregnancy diagnosis muster (or near the last annual weaning muster in June–October). Fetal age was estimated by rectal palpation for all pregnant cows; the mean interval from pregnancy diagnosis to predicted calving was 4.4 months. The animal's status within the herd was recorded as either Kept or Culled at each muster.

Potential risk factors assessed

Management and resource factors were derived from survey responses provided by the cooperating herd manager at the commencement of the study. A uniform interpretation of questions and responses was ensured by using a face-to-face survey method. The questionnaire contained 148 items, including descriptors of the property (e.g. property area, herd size, average rainfall), and grazing and herd management practices and policies (e.g. bull to female mating ratio, duration of mating, culling and selection policies, provision of supplements, weaning and vaccination policies).

The nutritive value of the diet selected by mobs of grazing cattle was evaluated by collecting fresh faecal samples from enrolled mobs and analysing them by near-infrared reflectance spectroscopy for dry-matter digestibility (DMD) and crude protein (CP; Dixon 2007; Dixon and Coates 2009) and wet-chemistry techniques for faecal P (FecP; Zarcinas *et al.* 1987). A composite faecal sample was assembled by herd managers in January, March, May, August and November by combining samples collected from approximately 10–15 different random cows observed to defecate from multiple locations within a paddock grazed by study mobs. If, for logistical reasons, a sample was not able to be collected in the scheduled collection month, such as because of inability to access the paddock due to weather, a sample was collected in the following month. In the few situations where the collection coincided with a mustering event, samples were sometimes collected per rectum. The composite faecal sample was dried by thinly (<~10 mm) spreading the faeces out on a clean flat piece of non-absorbent material that was exposed to direct sunlight for approximately 4 h. Once dry, samples were broken up and sent *via* post for analysis.

Predictions of diet attributes (DMD, CP, FecP) for collection months were summarised to generate several contextual variables representing the nutritional quality of diets consumed by study mobs during the wet and dry

seasons. The ratio DMD:CP was used to assess the risk of performance being restricted by insufficient available protein relative to energy (Dixon 2007). Established threshold values (8 and 10) to predict when responses to rumen-degradable N were applied to create risk factors representing wet and dry season conditions (Dixon and Coates 2005). The ratio of FecP to dietary ME (FecP:ME) was calculated to characterise the availability of dietary P and risk of insufficient P adversely affecting performance. Dietary ME content was estimated from faecal near infrared reflectance spectroscopy (F.NIRS) prediction of DMD by using equation 1.12A in Freer *et al.* (2007; $ME = 0.172 \times DMD - 1.707$). Threshold indicator values of 390, 420 and 460 for FecP:ME were applied to create risk factors representing the proposed requirements of lactating 400 kg breeders producing 5 L milk/day (Jackson *et al.* 2012).

Annual property rainfall and weather interpolations were obtained from the Australian Bureau of Meteorology (<https://www.longpaddock.qld.gov.au/silo/point-data>, accessed 17 November 2020). Cattle movements were documented by the cooperating herd manager. Paddock factors (paddock area, distances to water) were generated within a geographic information system (ArcGIS, Esri Inc.).

Infectious disease factors were derived from cross-sectional blood and matched vaginal mucus sampling of enrolled cows and heifers. Approximately 15–30 randomly selected heifers or cows per management group were sampled at the pregnancy diagnosis muster in 2009 and 2011. Blood was aspirated from the tail vein, allowed to clot at ambient temperature and chilled overnight prior to decanting off serum, which was stored frozen for subsequent assays. Vaginal mucus was collected using a ribbed hard-plastic device and stored in frozen saline for subsequent assay. Serological testing was conducted for serum antibodies against bovine viral diarrhoea virus (BVDV), bovine ephemeral fever (BEF) virus, *Leptospira borgpetersenii* serovar *hardo* type Hardjobovis (*L. hardjo*), *Leptospira interrogans* serovar *pomona* (*L. pomona*) and *Neopspora caninum*, and for vaginal mucus antibodies against *Campylobacter fetus* subsp. *venerealis* infection (McGowan *et al.* 2014).

Regionalisation of properties – country type

Data from each property was regionalised according to four country types assigned following a subjective assessment of the production potential of the grazing land and cross-referencing with pasture and vegetation descriptions reported by the herd managers. Herd managers were asked to provide an estimate of the annual growth of yearling steers (AGYS) for the country where the cattle enrolled in the study were grazed. Properties with forested land types and fertile soils in the central and south-eastern regions of Queensland were distinguished by being outside (Southern Forest; median AGYS 200 kg) and within (Central Forest; median AGYS 180 kg) the northern Brigalow Forest. In the northern areas

of Queensland, Northern Territory and Western Australia, properties that were predominantly large treeless black soil plains (Northern Downs; median AGYS 170 kg) were distinguished from those that were forested with low-fertility soils (Northern Forest; median AGYS 100 kg).

Deriving the outcome of pregnant within 4 months of calving (P4M) in lactating cows

P4M was defined as whether cows became pregnant or not within 4 months of calving, derived as a binomial measure for each annual production cycle. The period from the end of one pregnancy diagnosis muster to the end of the pregnancy diagnosis muster in the following year approximately 12 months later was an annual production cycle. Only those cows that successfully reared their first confirmed pregnancy after enrolment were eligible for this outcome variable to be generated.

Pregnancy within 4 months of calving derived as a binomial rather than a shorter period was selected as it represented cows that could possibly wean a calf in each year of two consecutive years. P4M was considered a practical discriminatory measure because, when measures based on shorter periods were applied, there was sufficient evidence to suggest that variability for observed mob-level performance was constrained, with several individual properties in the Northern Forest having no animals with positive outcomes in some age classes. As the purpose of modelling was to identify the factors explaining the greatest amount of variation, the reduced statistical dispersion was considered problematic for modelling.

Fetal ageing by manual rectal palpation of the reproductive tract was nearly all conducted by veterinarians accredited by the Australian Cattle Veterinarian's National Cattle Pregnancy Diagnosis Scheme. In the very few situations where an accredited cattle veterinarian could not be on site, experienced beef production research officers that had been internally assessed to be competent in the skill by accredited veterinarians were used. Estimated fetal age (months multiplied by 30.4), date of the diagnosis muster and an assumed gestation length of 287 days were used to calculate predicted month of conception and calving in each year. Females that had conceived in less than 4 months (i.e. expected to have a ≤ 13 m inter-calving interval) were defined as being positive for P4M.

Animals were not eligible for classification under P4M if they were recorded as having been non-pregnant in the previous annual reproductive cycle, or if they failed to lactate after being previously diagnosed pregnant, i.e. experienced fetal or calf loss. Females were recorded as successfully rearing a calf if they were diagnosed as being pregnant and were then recorded as lactating after the expected calving date. Females were recorded as having failed to rear their pregnancy if they were recorded as not lactating at the first muster after the expected calving date,

provided this muster occurred greater than 1 month after the expected month of calving, and they were not subsequently recorded as lactating.

Data management and statistical analyses

Data were managed using a relational database (Microsoft Access 2010 for Windows; Microsoft Corporation, Redmond, WA, USA) and a spreadsheet system (Microsoft Excel 2010 for Windows; Microsoft Corporation). All statistical analyses were performed using StataIC[®] (versions 13 for windows; Stata Corporation, TX, USA), with one animal production year for an animal as the unit of analysis.

Screening of candidate risk factors (Table 1) for inclusion in the multivariable model-building process was based on associations between potential risk factors and P4M, by using a random-effects logistic regression model with Stata's `xtlogit` command, fitting herd as a random effect. The overall significance of risk factors was assessed using Wald-test *P*-values. Risk factors were retained for consideration in the multivariable model-building process if their association with the outcome was significant at $P \leq 0.20$ (Dohoo *et al.* 2009).

The assumptions of linearity of continuous variables in the logit were evaluated by inspecting partial residual graphs following herd-adjusted logistic regression models fitting the continuous variables as the main effect of non-pregnancy, by using Stata's `lpartr` command (Hilbe 2009). Continuous variables that appeared to fail the assumption of linearity were categorised into two or more categories. Wherever possible, continuous variables were categorised using established threshold values, such as for the risk factor 'average ratio of DMD:CP during wet season' (Dixon and Coates 2005). However, in some cases, where these were not found to be discriminatory, cut points were determined by changes in the slope of cubic splines fitted to partial residual plots, such as the risk factor 'average ratio of FecP:ME during wet season'.

Examination of pairwise Spearman correlations were used to identify pairs of risk factors that were highly correlated ($r \geq 0.90$; Dohoo *et al.* 2009). Where pairs of risk factors were highly correlated, one risk factor was selected for inclusion in the multivariable model-building process on the basis of biological plausibility, fewer missing values and Akaike's and Schwarz's Bayesian information criteria estimates. Putative risk factors that had an excessive number ($\geq 40\%$) of missing values were also considered ineligible for consideration in the multivariable model-building process.

A multivariable model was built using a backwards elimination process, commencing with all significant ($P \leq 0.20$) risk factors derived from candidate variable screening being added to a starting model; non-significant variables with the highest *P*-value were dropped one at a time. This process was continued until only significant ($P \leq 0.05$) variables remained in an interim model. With the exception of those variables with a high degree of missing values, all risk factors

Table 1. List of candidate herd management, environment, nutrition and animal risk factors for the occurrence of lactating cows being pregnant within 4 months of calving (P4M) that were considered during univariate screening and potentially considered in the multivariable model building process.

Risk factor	
Herd management	
Percentage <i>Bos indicus</i> of heifers and cows	Culling rate of breeding females
Property management experience of manager	Culling age of breeding females
Reported size of the herd	Mating management
Size of management group at pregnancy diagnosis	Botulism vaccination policy
Bull selection policy	Leptospirosis vaccination policy
Annual bull management policy	Bulls vaccinated for bovine ephemeral fever
Environment	
Year observed	Cumulative number of days temperature humidity index exceeded 71 during month of calving
Timing of wet-season onset	Cumulative number of days temperature humidity index exceeded 79 during month of calving
Wet-season duration	Average temperature–humidity index during month of calving
Cumulative number of days maximum temperature exceeded 32°C during month of calving	
Cumulative number of days maximum temperature exceeded 39°C during month of calving	
Nutrition	
Minimum dry-season biomass	Average wet-season CP
Average dry-season crude protein (CP)	Average wet-season DMD
Average dry-season dry-matter digestibility (DMD)	Average wet-season DMD:CP ratio
Average dry-season DMD:CP	Average ratio faecal phosphorus to metabolisable energy during wet season
Provision of supplemental nitrogen	
Provision of supplemental phosphorus	
Proportion of the paddock grazed that was ≤ 2.5 km from permanent water around time of calving	
Animal	
Cow-age class	BCS at the branding or weaning muster
Estimated period of calving	BCS change between pregnancy diagnosis and branding or weaning musters
Liveweight at the pregnancy diagnosis muster	Hip height
Body condition score (BCS) at the pregnancy diagnosis muster	

previously eliminated during the model-building process were again reconsidered, one at a time, for inclusion into the interim model. The predictor country type was forced into all interim models due to specific interest in the effects of region that were being represented by country type. All potential interactions between pairs of risk factors remaining in the interim model were considered one at a time and were retained in the final model if their association was significant ($P \leq 0.05$) and their effects were biologically plausible. An appraisal of effects of potential confounding variables was completed by individually including each variable into the candidate model and assessing changes in the measure of association for statistically significant variables. Confounding

was considered important when odds ratios for statistically significant variables changed by >20 – 30% (Dohoo *et al.* 2009) and the variable was included in the final main effects model.

The fit of the multivariable model was evaluated and observations that did not fit the model well (outliers) or that had an undue influence on the model were identified. The overall goodness-of-fit of multivariable model was assessed using Hosmer–Lemeshow goodness-of-fit tables and statistics (Hosmer *et al.* 2013). Outliers were identified by an analysis of the residuals, and models with and without the influential observations were compared.

Following fitting of the final multivariable model, estimated marginal means of risk factors were computed

using Stata's margins post-estimation command. This command generated estimates of predicted outcomes for the levels of the added variable of interest after adjusting for the effects of all the other terms in the model. Standard errors were obtained using the delta method. Differences between estimated marginal means across levels of each risk factor or interaction term were estimated and statistically compared using linear combinations of estimators and pairwise comparisons respectively, using Stata pwcompare post-estimation command.

Population attributable fractions

The population attributable fraction for each of the risk factors retained in the final multivariable model was estimated to provide a measure of the relative importance for each risk factor contained within the final multivariable model. After recoding the outcome variable to reflect cows not becoming pregnant by 4 months after calving rather than P4M, a logistic regression model, clustered by herd, containing the main explanatory factors that were retained in the multivariable model and not including any interactions, was used to estimate the population attributable fractions for each risk factor by using Newson (2010) Stata command punafcc.

Effects of risk factors not contained in the final model

Infectious disease risk factors, which were derived from cross-sectional antibodies in blood and vaginal mucus samples performed in two of the three main study production years, had >40% missing values and therefore were ineligible for inclusion in the main multivariable modelling. Therefore, the potential effects of risk factors summarising management group prevalence of seropositives and management group prevalence of recent infection with BVDV, *N. caninum*, BEF virus, *L. hardjo*, *L. pomona*, and *C. fetus* subsp. *venerealis* were estimated by solely adding each risk factor to the final model. Additionally, due to sampling procedures in management groups of >300 cows, hip height, which was measured at the first pregnancy-diagnosis muster, also contained >40% missing values and was not included in the model-building process.

The risk factor describing a herd's genotype was initially considered, by using three categories (<50% *B. indicus*, 50–75% *B. indicus* and >75% *B. indicus*). However, as herds in the Northern Forest were mostly high-grade Brahman and there were no herds in the country type that had <50% *B. indicus* content, a two-level risk factor ($\leq 75\%$ and $>75\%$ *B. indicus*) was considered in the overall multivariable model-building process, with the factor not being identified as a statistically significant determinant in the final model. Because of the well established association between genotype and reproductive performance, an alternative exploratory model was developed to estimate

the effect in the current dataset. This exploratory model varied from the final model in that it specified genotype as a three-level categorical variable and was restricted to a subset of the data that contained female performance records only for those country types where all three levels of genotype category (<50% *B. indicus*, 50–75% *B. indicus* and >75% *B. indicus*) were represented. This meant that the Northern Forest was omitted from this exploratory model.

Ethical clearance

Ethical clearance (AEC approval number SVS/756/08/MLA) was obtained from the University Animal Ethics Committee (Production and Companion Animal), The University of Queensland.

Results

Description of study population

The starting dataset contained 35 902 rows of data representing a production year for an individual cow. On average, each individual cow and heifer contributed 1.3 (95% CI, 1.3–1.4) and 1.5 (95% CI, 1.4–1.5) animal-production years of data for which a valid P4M outcome was ascribed. Seventy-three herds contributed information to the starting dataset with a median of 293 (interquartile range, 188–502) P4M outcomes relating to an individual herd.

The population-averaged P4M, with adjustment for clustering at the herd level, was 41.6% (95% CI, 32.3–50.5%) of cows per production year, using the null model. P4M varied greatly among country types, with means of 65.4% (95% CI, 53.1–77.7%) for the Southern Forest, 57.5% (95% CI, 44.8–70.2%) for the Central Forest, 61.8% (95% CI, 48.1–75.6%) for the Northern Downs and 16.4% (95% CI, 10.7–22.1%) for the Northern Forest. The proportion of the variation explained at the herd level in the null model for P4M was 0.38.

Univariable associations

The candidate risk factors that were considered during univariate screening and potentially progressed into multivariate model-building process are presented in Table 1. Three risk factors that were found to have statistically significant associations with P4M in univariable analyses, but not retained in the final multivariate model, were average wet-season dietary CP content of the pasture (<7%/≥7%), herd BVDV vaccination policy (heifers/herd/not vaccinated) and the reported bull:female ratio (<3:100/≥3:100) during the breeding season.

Multivariable model results

The final model included data representing 25 070 animal production years from 58 herds; 30.2% of animal

Table 2. Adjusted odds ratios (OR), 95% confidence intervals and P-values from a multivariable logistic regression model of risk factors for probability of lactating beef cows being pregnant within 4 months of calving (P4M) in northern Australia.

Variable	Coefficient	s.e.	Adjusted OR	95% CI of OR		P-value
				Lower	Upper	
Country type						<0.0001
Northern Forest	Ref					
Southern Forest	3.17	0.45	23.78	9.78	57.82	<0.01
Central Forest	2.10	0.44	8.14	3.44	19.25	<0.01
Northern Downs	1.46	0.45	4.30	1.78	10.35	<0.01
Year observed						<0.0001
2009	Ref					
2010	0.14	0.10	1.15	0.95	1.40	0.15
2011	0.49	0.11	1.62	1.31	2.02	<0.01
Cow-age class						<0.0001
First-lactation cows	Ref					
Second-lactation cows	0.22	0.21	1.25	0.82	1.90	0.30
Mature cows (≥ 4 to ≤ 8 years old)	1.20	0.12	3.33	2.65	4.19	<0.01
Aged cows (> 8 years old)	1.18	0.14	3.25	2.45	4.30	<0.01
Estimated period of calving expressed as predicted period when the cow calved						<0.0001
July–September	Ref					
October–November	1.49	0.06	4.43	3.95	4.97	<0.01
December–January	2.20	0.07	8.98	7.87	10.25	<0.01
February–March	1.84	0.08	6.29	5.37	7.38	<0.01
April–June	1.38	0.11	3.96	3.18	4.94	<0.01
BCS at the pregnancy diagnosis muster						0.0007
1–2	Ref					
2.5	0.45	0.20	1.57	1.06	2.31	0.02
3	0.72	0.19	2.06	1.43	2.96	<0.01
3.5	0.63	0.19	1.88	1.29	2.74	<0.01
4–5	0.76	0.20	2.14	1.45	3.14	<0.01
BCS change between pregnancy diagnosis and weaning/branding						<0.0001
Maintained or lost	Ref					
Gained	0.384	0.05	1.47	1.34	1.61	<0.01
Average DMD:CP during wet season						<0.0001
$\geq 8:1$	Ref					
$< 8:1$	0.36	0.06	1.44	1.27	1.63	<0.01
Average ratio of FecP:ME during wet season						<0.0001
< 500 g P:1MJME	Ref					
≥ 500 g P:1MJME	0.96	0.11	2.62	2.12	3.23	<0.01
Interaction: country type \times cow age class						0.005
Southern Forest: second-lactation cows	0.51	0.26	1.67	1.00	2.79	0.05
Central Forest: second-lactation cows	0.71	0.26	2.04	1.22	3.40	<0.01
Northern Downs: second-lactation cows	0.83	0.25	2.29	1.41	3.72	<0.01
Southern Forest: mature cows	−0.34	0.17	0.71	0.51	1.00	0.05
Central Forest: mature cows	−0.23	0.16	0.79	0.58	1.09	0.15
Northern Downs: mature cows	0.05	0.15	1.05	0.78	1.41	0.74

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Table 2. (Continued).

Variable	Coefficient	s.e.	Adjusted OR	95% CI of OR		P-value
				Lower	Upper	
Southern Forest: aged cows	−0.23	0.23	0.79	0.50	1.25	0.32
Central Forest: aged cows	−0.13	0.21	0.88	0.58	1.32	0.54
Northern Downs: aged cows	0.13	0.18	1.14	0.80	1.62	0.48
Interaction: Country type × body condition score at the pregnancy diagnosis muster						<0.0001
Southern Forest: 2.5	−0.46	0.26	0.63	0.38	1.04	0.07
Southern Forest: 3	−0.30	0.24	0.74	0.47	1.17	0.20
Southern Forest: 3.5	−0.07	0.24	0.93	0.58	1.49	0.76
Southern Forest: 4–5	0.11	0.25	1.12	0.69	1.81	0.65
Central Forest: 2.5	0.11	0.26	1.12	0.67	1.87	0.66
Central Forest: 3	0.09	0.24	1.09	0.68	1.75	0.71
Central Forest: 3.5	0.39	0.24	1.48	0.92	2.39	0.11
Central Forest: 4–5	0.33	0.25	1.39	0.86	2.24	0.18
Northern Downs: 2.5	0.02	0.22	1.02	0.65	1.58	0.95
Northern Downs: 3	0.10	0.21	1.10	0.73	1.66	0.64
Northern Downs: 3.5	0.66	0.21	1.94	1.27	2.95	<0.01
Northern Downs: 4–5	0.63	0.22	1.88	1.23	2.89	<0.01
Interaction: cow age class × average FecP:ME during wet season						0.0001
Second-lactation cows: ≥500 g P:IMJME	−0.93	0.16	0.40	0.29	0.54	<0.01
Mature cows: ≥500 g P:IMJME	−0.86	0.11	0.42	0.34	0.53	<0.01
Aged cows: ≥500 g P:IMJME	−0.63	0.15	0.53	0.40	0.71	<0.01
Intercept	−5.54	0.33				<0.001
Random effect	s. d.			95% CI		
				Lower	Upper	
Level 2 (property)	1.00			0.82	1.22	
Rho (ICC)	0.23			0.17	0.31	

Predicted percentages are based on the estimated marginal means generated from the multivariable logistic regression model and are adjusted for all other factors contained in the model. Bold values are generalised Wald-test *P*-values; others are Wald-test *P*-values.

BCS, body condition score; FecP:ME, ratio of faecal phosphorus to metabolisable energy; DMD:CP, ratio of dry-matter digestibility to dietary crude protein.

production years and 20.5% of herds with valid entries for the outcome P4M were not represented in the final model because of missing values for one or more risk factors. In the final multivariable model (Table 2), there was an effect of country type, production year, cow age class, estimated period of calving, body condition score at the time of pregnancy diagnosis and its change through to subsequent weaning/branding of the calf and average ratios of FecP to dietary ME and of dietary CP to DMD of pastures measured across the wet season.

The fixed part of the final multivariable model fitted the data only partially well, with fewer cases of P4M than expected at lower probabilities. The *P*-value for the Hosmer–Lemeshow goodness-of-fit statistic was <0.001, indicating a poor fit. All attempts to improve the fit of the model did not result in changes to the overall significance of covariates or direction of the coefficients for the risk

factors. An inspection of covariate values showed all values to be plausible, and, as a result, no observations were removed from the dataset.

The predictive abilities of the final model were modest; the area under the receiver operating curve was 0.75 (s.e. 0.01). Sensitivity was high (>0.90) at very low cut points (<0.2), while specificity was high (>0.90) only at cut points of >0.8.

In the final model, predicted mean P4M (Table 3) was significantly (*P* < 0.05) lower in the Northern Forest than in the other country types; however, the effects of country type were dependant on cow age class (Table 2). Apart from the Southern Forest country type, P4M was lower in first-lactation cows than in either mature or aged cows (*P* < 0.05). Within the Southern Forest, first-lactation cows performed similarly to second-lactation and mature cows (~4.5 to <9 years), and aged cows (>9 years) had 13.7 (95% CI, 6.4–21.0%) percentage point higher P4M than did

Table 3. Predicted mean (%) P4M for each level of risk factor or pair of risk factors identified in the final multivariable model.

Variable	n	Mean P4M	95% CI of mean	
			Lower	Upper
Year observed				
2009	1522	35.5	27.5	43.4
2010	13 221	38.8	32.0	45.6
2011	10 327	47.2	40.1	54.3
Estimated period of calving expressed as predicted window when the cow calved				
July–September	3619	14.6	10.8	18.3
October–November	8755	43.0	35.9	50.2
December–January	9195	60.5	53.5	67.5
February–March	2722	51.8	44.1	59.4
April–June	779	40.3	32.1	48.6
BCS change between pregnancy diagnosis and weaning/branding				
Maintained or lost	19 681	35.9	29.2	42.6
Gained	5389	45.1	37.8	52.4
Average DMD:CP during wet season				
>8:1	6884	36.1	29.0	43.2
≤8:1	18 186	44.8	37.8	51.9
Interaction: country type × BCS at the pregnancy diagnosis muster				
Northern Forest: 1–2	318	6.0	2.9	9.2
Northern Forest: 2.5	679	9.2	5.0	13.3
Northern Forest: 3	1784	11.7	6.9	16.5
Northern Forest: 3.5	1401	10.8	6.2	15.4
Northern Forest: 4–5	1341	12.1	7.0	17.2
Southern Forest: 1–2	431	60.2	45.1	75.2
Southern Forest: 2.5	378	59.8	44.7	74.9
Southern Forest: 3	1062	69.6	56.9	82.4
Southern Forest: 3.5	1130	72.5	60.5	84.5
Southern Forest: 4–5	1289	78.3	68.0	88.6
Central Forest: 1–2	409	36.4	22.2	50.5
Central Forest: 2.5	460	50.1	35.3	65.0
Central Forest: 3	1563	56.3	42.2	70.3
Central Forest: 3.5	1557	61.4	47.9	74.9
Central Forest: 4–5	2170	62.9	49.7	76.2
Northern Downs: 1–2	800	26.2	13.8	38.7
Northern Downs: 2.5	896	36.1	21.4	50.8
Northern Downs: 3	2570	44.7	29.1	60.2
Northern Downs: 3.5	2294	56.4	40.9	71.9
Northern Downs: 4–5	2538	58.9	43.6	74.2
Interaction: cow-age class × average FecP:ME during wet season				
First-lactation cows: <500 g P:IMJME	2283	21.4	16.2	26.6

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Table 3. (Continued).

Variable	n	Mean P4M	95% CI of mean	
			Lower	Upper
First-lactation cows: ≥500 g P:IMJME	2452	41.7	34.1	49.2
Second-lactation cows: <500 g P:IMJME	1112	36.3	28.6	44.0
Second-lactation cows: ≥500 g P:IMJME	1382	37.1	29.0	45.2
Mature cows: <500 g P:IMJME	8898	44.3	37.0	51.7
Mature cows: ≥500 g P:IMJME	5192	46.8	39.2	54.4
Aged cows: <500 g P:IMJME	2630	45.5	37.6	53.5
Aged cows: ≥500 g P:IMJME	1121	53.8	45.4	62.1
Interaction: country type × cow-age class				
Northern Forest: first-lactation cows	1344	7.1	3.8	10.3
Northern Forest: second-lactation cows	203	5.6	2.5	8.8
Northern Forest: mature cows	3072	14.1	8.6	19.6
Northern Forest: aged cows	904	15.2	8.9	21.5
Southern Forest: first-lactation cows	1035	60.9	46.5	75.3
Southern Forest: second-lactation cows	647	67.2	53.6	80.8
Southern Forest: mature cows	2205	70.6	58.3	82.8
Southern Forest: aged cows	403	74.6	62.6	86.6
Central Forest: first-lactation cows	1464	42.6	28.6	56.6
Central Forest: second-lactation cows	794	54.4	39.8	68.9
Central Forest: mature cows	3326	56.0	42.1	69.9
Central Forest: aged cows	575	60.7	46.5	74.8
Northern Downs: first-lactation cows	892	30.2	16.7	43.6
Northern Downs: second-lactation cows	850	43.7	27.9	59.5
Northern Downs: mature cows	5487	49.5	33.9	65.2
Northern Downs: aged cows	1869	53.7	37.9	69.5

Predicted percentages are based on the estimated marginal means generated from the multivariable logistic regression model and are adjusted for all other factors contained in the model. BCS, body condition score; FecP:ME, ratio of faecal phosphorus to metabolisable energy; DMD:CP, ratio of dry-matter digestibility to dietary crude protein.

first-lactation cows (Table 3). Within the Northern Forest, first-lactation cows were also found to perform similarly to second-lactation cows. However, within both the Central Forest and Northern Downs, P4M was higher in second-lactation cows than in first-lactation cows ($P < 0.05$), with 11.8 (95% CI, 5.9–17.7%) and 13.5 (95% CI, 7.6–19.5%)

percentage point differences respectively. Aged cows were found to perform similarly to mature cows within each country type, excepting the Northern Downs where P4M for aged cows was 4.2 (95% CI, 0.6–7.8%) percentage points higher than that for mature cows ($P < 0.05$).

P4M was lowest for those cows predicted to calve between July and September and highest for those cows predicted to calve between December and January. The interaction between estimated period of calving and country type as a predictor of percentage P4M was not able to be assessed in the final model because the widespread use of controlled mating in the Southern and Central Forest resulted in very few cows calving between February and June in these country types.

Generally, P4M increased as body condition at the pregnancy diagnosis muster increased. However, the effects of body condition on P4M were dependent on country type. Within each country type apart from the Southern Forest, those cows with a body condition score (BCS) of 1–2 (poor to backward condition) had lower ($P < 0.05$) P4M than did cows in all other categories of body condition (Table 2). Within the Southern Forest, cows that had a BCS of 1–2 had a P4M similar to those with a BCS of 2.5. Within all country types, cows that had a BCS of 3.0 at the time of pregnancy diagnosis subsequently had a significantly higher P4M than did those cows with a BCS of 2.5. However, within the Northern Forest, those cows with a BCS of 3.5 performed similarly to those with a BCS of 2.5. Also, in the Northern Forest, predicted P4Ms for cows with BCS of 3.0, 3.5 and 4–5 were not significantly different. However, the predicted mean P4M of cows with a BCS of 3.5 was 5.1 percentage points (95% CI, 0.9–9.4) higher in the Central Forest and 11.7 percentage points (95% CI, 8.4–15.0) higher in the Northern Downs, than those for cows with a BCS of 3 (Table 3).

P4M of cows grazing protein-adequate pasture throughout the wet season (mean DMD:CP ratio of $<8:1$; Dixon and Coates 2005) was 12.7 percentage point higher ($P < 0.001$) than that for cows grazing poorer-quality pastures. Cows gaining body condition between pregnancy diagnosis and the subsequent annual branding/weaning muster had a 9.2 percentage points higher P4M than did those that either maintained or lost condition during this period ($P < 0.001$; Tables 2, 3).

Across cow age-class cohorts, average P4M where FecP:ME was <500 was 0.8–20.3 percentage points lower than it was where FecP:ME ≥ 500 (Table 3). The difference was higher in first-lactation cows at 20.2 percentage points (95% CI, 15.4–25.1; $P < 0.001$) than in aged cows (>8 years old) at 8.3 percentage points (95% CI, 2.2–14.3; $P < 0.01$), but negligible in second-lactation and mature cows (0.8%, $P = 0.78$ and 2.5%, $P = 0.16$ respectively; Tables 2, 3).

Estimates of the proportional reduction in P4M due to significant risk factors (Table 4) should be interpreted with caution. Because the final model used to estimate population-attributable fraction (PAF) omitted interaction

Table 4. Estimated population-attributable fraction (PAF) for cows that failed to become pregnant within 4 months of calving for all risk factors retained in the full multivariable model.

Risk factor	PAF (%)	95% CI	
		Lower	Upper
Country type	62.5	41.9	75.8
Estimated period of calving	40.4	38.3	42.4
BCS at the pregnancy diagnosis muster	26.2	22.6	29.6
BCS change between pregnancy diagnosis muster and weaning/branding muster	26.0	20.6	31.0
Cow age class	24.6	18.9	30.0
Average FecP:ME during wet season	17.6	11.9	23.0
Year observed	15.2	10.2	19.9
Average DMD:CP during wet season	7.0	3.7	10.1

Note: the population-attributable fractions for individual risk factors are estimated using a variation of the final model where interaction terms have not been specified. It is suggested that interpretation should be directed towards the likely relative importance individual risk factors rather than the absolute PAF estimates presented (i.e. proportional reduction in P4M). BCS, body condition score; FecP:ME, ratio of faecal phosphorus to metabolisable energy; DMD:CP, ratio of dry-matter digestibility to dietary crude protein.

terms, models with dummy-coded interaction terms were tested. Moderate changes to the estimated proportional reduction in P4M were evident although the overall ranking of risk factors was comparable. Emphasis is suggested on the likely relative importance of different risk factors rather than the absolute PAF estimates presented.

Effects of risk factors not contained in the full multivariable model

Cow hip height (<125 cm/ ≥ 125 to <140 cm/ ≥ 140 cm) was found to be significantly ($P < 0.0001$) associated with P4M. However, its inclusion in a candidate final model removed seven herds and 8384 animal production years from the analysis and, therefore, was not retained in the final model. Overall, mean P4M for cows within hip-height categories of <125 cm, ≥ 125 to <140 cm and ≥ 140 cm was predicted, after adjustment for the effects of all the other terms in the final model, to be 47.0% (95% CI, 37.4–56.6%), 40.0% (95% CI, 32.1–47.9%) and 36.2% (95% CI, 28.4–43.9%) respectively.

The additional exploratory analysis to assess the impact of genotype-category model showed that there was a statistically significant ($P < 0.001$) association with the occurrence of P4M. Cows that were $<50\%$ *B. indicus* (68.3%) were predicted to have a significantly higher occurrence of P4M than for cows that were either 50–75% *B. indicus* (52.9%) or $>75\%$ *B. indicus* (51.1%; $P < 0.05$).

The inclusion of infectious disease risk factors resulted in 17 herds and 13 880 animal production years being removed from the modelling and, therefore, some caution must be

exercised in interpreting results when including these factors. With the exception of BVDV, the association between P4M and management group seroprevalence for infectious disease risk factors for *Neospora caninum*, BEF virus infection, *Leptospira hardjo* and *pomona*, and *Campylobacter fetus* subsp. *venerealis* were found to be either not statistically significant or biologically implausible, or both. The mean predicted P4M for those cows within groups categorised as having a low (<20%), moderate (≥ 20 to $\leq 80\%$) and high seroprevalence (>80%) for BVDV infection was 57.3% (95% CI, 43.8–70.9%), 43.2% (95% CI, 26.2–60.1%) and 34.3% (95% CI, 17.0–51.6%) respectively ($P = 0.03$), with the primary difference of 23.0 percentage points (95% CI, 7.1–39.0) being between low- and high-prevalence groups ($P = 0.007$).

Discussion

This unique study of reproductive performance of commercially managed beef cattle located across major beef-cattle breeding regions of northern Australia found that at the herd level, only 42% of lactating cows were P4M. This finding highlighted a likely substantial constraint for weaner production in this region, as it clearly demonstrated the low efficiency by which cows conceive while lactating in northern Australian beef herds, compared with other parts of Australia. Prolonged lactational anoestrus intervals for this region have previously been reported and are highlighted as the main cause of the low reproductive rates observed for lactating cows in northern Australia (Entwistle 1983; Frisch *et al.* 1987; Teleni *et al.* 1988; Burns *et al.* 2010). Country type was shown to have the greatest influence on P4M, with period of calving, BCS and BCS changes around and after calving, cow age class and wet-season nutritional risk factors other top-order determinants.

Consistent with the findings of the current study, the effect of geographical location on reproduction performance is well established in the literature (Entwistle 1983; O'Rourke *et al.* 1992; Bortolussi *et al.* 2005b; Gleeson *et al.* 2012). These results were consistent with the previously reported generalisation that pregnancy percentages tend to decrease in a northerly direction (Entwistle 1983). The Northern Forest, compared with that in the other country types, is known to contain large areas of relatively low soil fertility; despite annual rainfall being high and pasture growing conditions adequate, the soils of Northern Forest limit animal production through pasture growth and quality, a consequence of nutrient dilution (Ash *et al.* 1997).

The impact of country type, as it is reported in the present study, is likely to be understated, because how country type is specified is likely to represent differences in the prevalence of other regionally associated factors that are not elsewhere represented in the final model. Diverse beef production

systems exist across northern Australia, ranging from low-intensity grazing on native rangelands to intensive grazing on productive rainfed improved pastures, which differ for several factors previously been shown to influence reproductive performance, including *B. indicus* content (Morris *et al.* 1993), mating management, genetic selection for fertility (Johnston *et al.* 2014), climate, soil fertility (Ash *et al.* 1997), pasture production and nutritive value (Bortolussi *et al.* 2005c). Even though the ability of herd managers to control the effect of country type is constrained by the physical attributes of the pastoral resource, the large variance that existed within country types in the present study emphasises the substantial opportunity that exists to improve the reproductive performance of females within northern Australia. Focusing herd selection on fertility traits associated with lactation anoestrus (Johnston *et al.* 2014) by substantial further investment into improving the pastoral resource or modifying the structure of the beef production systems adopted in the region (Bell *et al.* 2014) are examples of potential strategies to achieve such improvements; however, they require careful whole-of-business economic assessment prior to implementation.

Even though larger magnitude of effects was often predicted for disease and animal risk factors, the dominating effects of nutritional and environmental factors on performance from a population perspective were demonstrated in the present study. These results are potentially explained by the fact that exposure to a small risk of a large proportion of the cattle population is likely to yield a greater impact on performance than is exposure to a large risk of a small proportion. Even though among-year variation existed, the consistent and broad reaching impact of nutrition was highlighted in the present study. With the majority of cows in northern Australian beef production systems typically lactating and conceiving during the wet season (Sullivan and O'Rourke 1997), the importance of the detected nutritional factors relating to this period (DMD:CP ratio $\leq 8:1$ and FecP:ME ratio) is not surprising. The increased demand for nutrients, particularly energy and protein, to produce colostrum, physically give birth and produce an adequate supply of milk and its association with cows returning to oestrus is well established (Sawyer *et al.* 1991; Fordyce *et al.* 1997; Sullivan and O'Rourke 1997). Inadequate energy or protein intake prior or following calving extends the postpartum anoestrus interval and reduces the likelihood of pregnancy in lactating beef cattle (Fitzpatrick 1994).

This study used the ratio of the conception of P in the faeces to the metabolisable energy content of the diet as a mob-level indicator of the dietary P content, which at the time of developing this research activity was recommended as a diagnostic test to determine the likely response of cattle to P supplementation (Jackson *et al.* 2012). Recommendations from research activities completed since suggest that while there is some evidence that FecP:ME is related to the P

content of the diet, further validation of the threshold values of FecP:ME is required (Quigley *et al.* 2015), findings that are consistent with those of the current study. The present study did demonstrate that an association between FecP:ME and reproductive performance did exist at a population level, which is consistent with effects on reproductive performance relating to voluntary feed intake (Wadsworth *et al.* 1990) and energy balances of animals rather than the direct effects of nutrients, such as P (Dixon *et al.* 2011). However, this finding should be interpreted with some degree of caution as further validation of FecP:ME across a much wider range of vegetation and land types has been recommended and is useful only when specific conditions where met (Quigley *et al.* 2015). For instance, in animals that have been consuming a relatively similar diet for a reasonable length of time, which is potentially the case in some grazing situations, FecP:ME may provide some indication of P intake (Quigley *et al.* 2015). This is further evidenced by the observed interaction between cow age class and FecP:ME in the present study, which is consistent with the previously reported varying dietary requirements of female cattle in northern Australia (Miller *et al.* 1990). Irrespective to the current uncertainty around the validity of the threshold values for the FecP:ME, the findings of the current study should be considered as further evidence that managers of breeding herds grazing P-deficient areas of northern Australia should be encouraged to offer P supplements to cattle over the wet season, when P is the first limiting nutrient.

The threshold value of ≥ 8 for average ratio of DMD:CP, measured by NIRS, during the wet season was determined as having some association with P4M in the present study. Even though the calibration equations predicting CP and DMD from NIRS analysis of faeces of cattle grazing tropical pastures are considered robust (Dixon and Coates 2010), the use of this ratio to describe wet-season dietary conditions is uncommon. The ratio of DMD:CP is a particularly useful tool to evaluate whether cattle are likely to respond to supplemental N during the dry season when protein content of the pasture often is the primary limiting nutrient and forbs represent a relatively low proportion of the selected diet (Dixon and Coates 2005). Regardless, this finding is consistent with the unconfirmed reports of observed production responses from low levels of urea supplemented to cattle grazing high-quality wet season pastures in central Queensland (Entwistle and Jephcott 2013) and highlights that the risk of inadequate protein constraining production is not only restricted to the dry season. However, studies have also shown that supplementation of nitrogen during rainy seasons can provide metabolic benefits by improving the efficiency of ME use (Lazzarini *et al.* 2016). These results highlight that an opportunity to improve the reproductive performance of females potentially exists under some wet-season conditions through the safe provision of additional protein, providing that all other all other

nutritional deficiencies are met. It is probably that these responses are likely to occur during the wet–dry transition when the protein content of the pasture is declining.

Lower P4M in first- and second-lactation cows reported in this study is consistent with previous reports and because of nutritional demands associated with lactation while still growing themselves (Entwistle 1983; Teleni *et al.* 1988; Lalman *et al.* 1997; Schatz and Hearnden 2008; Burns *et al.* 2010). The limiting nutrition affecting reproduction in beef cattle is typically energy, and high mobilisation of body reserves during the peripartum period by primiparous cows is associated with delayed postpartum first ovulation (Guedon *et al.* 1999). Interventions made to reduce the loss of body reserves by improving the diet by providing energy-rich supplements have resulted in substantial improvements in reproduction, particularly in first-lactation cows (Fordyce *et al.* 1997). Alternative strategies include weaning the calves from first-calf cows 1 month earlier than from multiparous cows in the main herd, so that the duration of lactation is reduced and additional time is provided for first-lactation cows to grow and recover. The increased magnitude of effect for this risk factor observed in the Northern Forest demonstrates the increased importance for such strategies to be adopted in this country type to achieve and maintain high levels of reproductive performance.

Despite adjustment for other terms included in the final model, large among-year variation for P4M existed in the present study. This effect appeared to be independent as there was an absence of interactions between season and other important explanatory factors. Typical of northern Australia, extended dry periods and large rainfall events causing widespread flooding were experienced in all regions during the present study, highlighting the large seasonal variability and likely differences in environmental and nutritional conditions among years. To experience the typical range in seasons when conducting studies in rainfall-dependent systems of northern Australia, it has been recommended that they continue for 6–8 years of observation (Taylor and Tulloch 1985). However, the absence of interactions among other important explanatory factors in the present study appears to suggest that the major factors identified by these analyses had a consistent effect across a range of seasons experienced during this study.

The results of the present study were generally consistent with the optimal calving period for an area corresponding to the time when improvements in feed conditions were expected, matching the peak nutritional demands of the cow with the time when suitable feed is available. High P4M was observed for cows that were predicted to calve between December and January compared with all other periods in the present study and corresponded to the period when the onset of the wet season had typically occurred in all country types and available nutrition was usually highest (McCosker *et al.* 2020). However, when considering

the overall performance of the cow–calf unit, in terms of reproductive performance of the cow and the pre-weaning growth of the progeny, it is generally recommended that calving should commence 6–8 weeks prior to the time when improvement in feed quality is expected. This is because nutritional demand is strongly associated with milk production, which is lowest during the month of calving and dramatically increases to peak lactation during the second and third month after calving (McCarter *et al.* 1991). Consistent with this, the calving window desired by herd managers within Southern Forest often included the July–September calving period so that they could take full advantage of the pasture-growing season and avoid calves being born after December when they are thought to often grow poorly, increasing the variability in liveweight at weaning and age at turn off. For this to be a sustainable management practice in this country type, other outcomes such as high weaner liveweight and value may achieve business benefits and may counter the much-lower ability of cows to wean a calf in consecutive years when calving before October.

The identification of risk factors representing energy intake and body condition in the current study as being important explanatory variables for variation in reproductive performance is supported by scientific literature (Hess *et al.* 2005). These findings highlight the importance of herd managers employing sound nutritional management practices when most females within management groups are in their last trimester of pregnancy and during lactation, so as to sustain high levels of reproductive performance. The positive association between body condition at the pregnancy muster and P4M is consistent with numerous studies reporting associated effects of body condition on postpartum anoestrus (Entwistle 1983; Blanc and Agabriel 2008; Burns *et al.* 2010) and is considered to represent the capacity for cows to mobilise energy reserves to meet the increased nutritional demands associated with lactation and parturition. Likewise, the additive negative effect of cows not being able to sufficiently meet the nutritional demands to maintain body condition after calving being associated with P4M is also well established in the literature, with lactating cows remaining acyclic for longer when in negative energy balance (Randel 1990; Short *et al.* 1990; Lalman *et al.* 1997; Ciccioli *et al.* 2003; Waldner and García Guerra 2013). Cows calving at greater body condition are at a higher risk of negative energy balance after calving, which may contribute to reduced fertility (Montiel and Ahuja 2005). This is likely to explain the observed reduced effect of body condition of P4M in the Northern Forrester where nearly all lactating cows lose weight due to the restrictive nutritional conditions.

The finding that assessment of body condition at the time of pregnancy diagnosis (approximately mid-pregnancy) explains a greater proportion of variation for P4M than that at other time points in the reproductive cycle is supported by the scientific literature and is likely to be largely explained by the proximity of assessment to when the

majority of cows were expected to calve (D'Occhio *et al.* 2019) and its partial representation of the nutritional management of cows during late pregnancy (Hess *et al.* 2005). It is also possible that due to cows needing to be restrained for pregnancy diagnosis, increasing the time for technicians to visually appraise cows, there was less error in the assessment of BCS than at other time points when cows were restrained, if at all, only for short periods of time.

While the potential risk of information bias resulting from only a proportion of the available data being used to assess the predicted effects of hip height, genotype and infectious disease using alternative exploratory models is acknowledged and warrants some caution being exercised when interpreting it, the results from these analyses strongly align to existing scientific literature. Lower hip height being associated with higher P4M is likely to be explained by the larger nutrient requirements for maintenance of larger frame-sized cows, with smaller cows of acceptable body condition likely to conceive quicker after calving (Vargas *et al.* 1999). While these findings appear to suggest that to sustaining high levels of reproductive performance and profitability, herd managers should maintain small to moderate frame-size herds, individual whole-of-business analysis for this recommendation requires careful consideration prior to intervening. Business benefits from premiums from the sale of large-framed cows and increased growth rate of progeny potentially offset these production gains in some situations (Marshall *et al.* 2021).

The identified association for females with <50% *B. indicus* content to have a higher occurrence of P4M than for cows with higher levels of *B. indicus* was thought to be independent of the effects of hip height in the current study and further supports existing scientific literature with females with a higher *B. indicus* content having a tendency for longer lactational anoestrus intervals (Mackinnon *et al.* 1989; Johnston *et al.* 2014). The high *B. indicus* content of northern Australia beef herds is a consequence of their ability to cope with the environmental conditions within northern Australia and overseas after live export, and the preferences held by live cattle importers. As reproduction traits for tropical beef genotypes are low to moderately heritable (Johnston *et al.* 2014), these results highlighted the existing opportunity for managers of herds, particularly of high *B. indicus* content, to improve reproductive performance by selecting for traits such as lactation anoestrus interval.

The inclusion of infectious-disease risk factors resulted in 17 herds and 13 880 animal production years being removed from the modelling and, therefore, some caution must be exercised in interpreting results when including these factors.

The association between high prevalence of previous infection with BVDV, as indicated by antibody concentration, and lower P4M is partially consistent with a previous report (McGowan *et al.* 1993). However, as the present study did not establish an association between P4M and prevalence

of recent BVDV infection, as indicated by high agar gel immunodiffusion (AGID) antibody titres, this effect is not easily explained and warrants further investigation. The most likely explanation for the latter difference is that animals were typically sampled 6–7 months after the start of mating, by which time the titre concentrations would have waned to levels not indicative of recent infection.

It could be expected that antibody prevalence for other infectious diseases would be associated with P4M only if their impact is on fertilisation or early stage pregnancy. This is the case for leptospirosis. Although neosporosis is a major cause of abortion in high-productivity systems, the finding of the present study of no impact on P4M is fully consistent with the findings of another recent study (Fordyce et al. 2013b), also showing no impact on fertility in extensively managed herds in northern Australia. The lack of association between bovine ephemeral fever antibody concentrations and P4M was not unexpected, as there has been no previously reported significant association for northern Australia.

Although the impact of *Campylobacter fetus* subsp. *venerealis* infection is considered to be primarily on fertilisation success and embryo survival (Clark 1971), a high prevalence of antibody was not associated with P4M. A possible explanation for this is that high antibody concentrations may be associated with infection over a number of years and are indicative of a higher herd immunity. Also, although infection rates may be higher where antibody concentrations are higher, there will be fewer susceptible animals in these groups, which is the opposite of what occurs in groups with a low prevalence of infection, thus disguising the effect of the infectious agent. As maiden heifer groups had not previously calved, the outcome P4M was unable to be ascribed and therefore they were precluded from this analysis. However, the main impact of infection on conception is likely to be seen in this group, as previously reported (Schatz et al. 2006).

The final model included data representing 25 070 animal production years from 58 herds; 30.2% of animal production years and 20.5% of herds with valid entries for the outcome P4M were not represented in the final model because of missing values for one or more risk factors. In the final multivariable model (Table 2), there was an effect of country type, production year, cow age class, estimated period of calving, BCS at the time of pregnancy diagnosis and its change through to subsequent weaning/branding of the calf and average ratios of FecP to dietary ME and of dietary DMD to CP of pastures measured across the wet season.

Despite our best efforts, bias cannot be eliminated from population-based studies. Commercially appropriate and practical methodology that minimised bias was employed and it has been identified where potential biases could not be avoided. The properties and herds selected for the study may not be absolutely representative of properties and herds in northern Australia. The herd managers enrolled in

the study were identified using several recruitment methods, including self-nomination, and were conducting or prepared to conduct an annual pregnancy diagnosis on all enrolled females, which is not routine practice in many areas of northern Australia. While individuals who volunteer to be involved in studies may possess different characteristics and management styles than does the general target population, there was large variability in performance and management represented in the current study. Potential for some selection and information bias also existed, given that 30.2% of animal production years and 20.5% of herds with valid entries for the outcome P4M were not represented in the final model because of missing values for one or more risk factors. If bias did exist as a result of the practical limitations of capturing data under commercial conditions, it may have resulted in marginal differences in the reported prevalence and effect sizes for levels of risk factors. However, the comparative impact of risk factors and overall direction of effect on P4M are not expected to be biased by the population used in this research.

Conclusions

This research has for the first time accurately quantified the primary factors influencing the ability of lactating cows to become pregnant within 4 months of calving (P4M) in commercial north Australian beef herds. The results reiterate the importance, from a population perspective, of risk factors of low to moderate impact on reproductive performance that operate on a large proportion of the northern Australia beef cattle population. Interventions targeting improvement of the reproductive performance of the northern Australia beef population should primarily focus on the development of strategies to cost-effectively address the misalignment between nutritional demand and dietary quality during the last trimester of pregnancy and during lactation, supported by selection of phenotypically and genetically appropriate females for the environment and adoption of strategic herd health programs. Extension programs targeting northern Australia herd managers should target the increased adoption of well recognised practices such as controlled mating and segregation systems to ensure cows calve at the optimum time of the year, sound pasture management practices and strategic supplementation to rectify any nutritional deficiencies, particularly protein and P, or alternatively by cost-effective investments being made to improve the nutritional quality of the pastoral resource.

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Data availability. The data that support this study were obtained from individual enterprises in the northern Australia beef industry by permission. Data will be shared upon reasonable request to the corresponding author with permission from Meat and Livestock Australia and the University of Queensland.

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Author affiliations

^ADepartment of Industry, Tourism and Trade, PO Box 1346, Katherine, NT 0851, Australia.

^BAusVet Animal Health Services, PO Box 1278, Toowoomba, Qld 4350, Australia.

^CThe University of Queensland, School of Veterinary Science, Warrego Highway, Gatton, Qld 4343, Australia.

^DThe University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, Carmody Road, St Lucia, Qld 4072, Australia.

^EQueensland Institute of Medical Research, 300 Herston Road, Herston, Qld 4006, Australia.

^FPresent address: The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre for Animal Science, Warrego Highway, Gatton, Qld 4343, Australia.

^GPresent address: The University of Queensland, School of Veterinary Science, Warrego Highway, Gatton, Qld 4343, Australia.