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Article

Farm-level risk factors associated with reproductive performance in small-scale dairy farms in Mexico

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Abstract:

The profitability of dairy farms is closely linked to reproductive performance. Identifying risk factors that compromise this performance is vital to implementing strategies to improve productivity. An analysis was done of the effects on reproductive performance of artificial insemination (AI) use, herd size and high seroprevalence of reproductive infectious diseases. Data on reproductive events were collected from 52 farms (10-100 cows; 959 lactations) over 18 mo (births 2011-2012). Neosporosis, bovine infectious rhinotracheitis (IBR) and bovine viral diarrhea (BVD) seroprevalences were documented at each farm. Multiple logistic regression analyses were applied to determine the degree of association (odds ratio, OR) between potential risk factors and reproductive variables. Herds of 33 or more cows (OR= 1.5) and high neosporosis seroprevalence (OR= 2.3) were risk factors for assisted calving. High IBR and BVD seroprevalences (OR= 1.3 and 1.9, respectively) were risk factors for days to first service over 70 d in milk (DFS>70). Artificial insemination was a common risk factor for DFS>70 (OR= 2.4) and days open over 110 days in milk (OR= 1.3). Herds of 33 or more cows was a risk factor for non-pregnant cows at first service (OR= 1.7). Artificial insemination, herds of 33 or more cows and high neosporosis, IBR and BVD seroprevalences are factors associated with reproductive performance in small-scale dairy farms in various geographical regions in Mexico.

Key words: Artificial insemination, Risk factors, Neosporosis, BVD, IBR.

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Introduction

Small-scale milk production systems improve food security and provide income in rural areas worldwide[1]. In Mexico, this production system accounts for approximately 23 %of livestock inventory[2], 30 % of national milk production(3), and 73 % of dairy farms[4]. Small scale dairy farms in Mexico are characterized by the use of family labor and specialized dairy breeds, the presence of few milking cows and medium-low technology levels[5-7]. Improved productive practices at small-scale dairy farms contribute to reducing poverty in rural areas[1], and promoting community development[8,9].

Dairy farm profitability is closely linked to efficient reproductive performance(10,11). Identifying risk factors that compromise reproductive performance is paramount to designing and implementing strategies that improve productivity. Studies of small-scale dairy herds indicate that, compared to natural breeding (NB), artificial insemination (AI) may affect the interval from birth to first postpartum service and the conception rate to first service compared to NB(12,13). In addition, studies of intensive production systems have shown that herd size influences reproductive performance(14-16),as do seroprevalences of reproductive infectious diseases such as neosporosis, bovine infectious rhinotracheitis (IBR) and bovine viral diarrhea (BVD)(17,18). The objective of the present study was to analyze the impact, as potential farm-level risk factors, of AI use, herd size and prevalence of infectious reproductive diseases on reproductive performance in small-scale dairy farms in Mexico. The working hypothesis was that these factors are associated with reproductive performance in dairy cows.

Material and methods

Farm selection and data collection

An observational prospective cohort study (959 records) was conducted in six states in Mexico with a substantial presence of small-scale dairy production systems. The study included 52 farms distributed among the six states: Jalisco (23); Estado de México (10); Tlaxcala (9); Guanajuato (4); Puebla (3); and Querétaro (3). Selection criteria included: primarily family labor used in production unit; 10 to 100 milking cows; milk production as primary objective of farm; and medium-low technology level. Holstein breed cows accounted for 91.3 % of the animals at the studied farms, and average number of cows per farm was 30.3 ± 2.4. The estimated culling rate was 26.4 %, and milk production per cow was 17.10 ± 0.5 kg/d. The farms included in the study meet the characteristics of small-scale production farms in Mexico(2,7,19). Data collection in the field was done for 18 mo, during which data on reproductive events were recorded: dates of parturition, service types (artificial or natural breeding) and dates, assisted calving or retained fetal membranes, and 50-d post-service gestation diagnosis results.

Events of interest and classification of potential risk factors linked to reproductive performance

Five events of interest were considered in the analysis: assisted calving (minor and major assistance pooled into the same category); retained fetal membranes (>12 h); days to first service over 70 days in milk (DFS> 70); days open over 110 days in milk (DO>110); and non-pregnant cows at first service (NP1S). Based on previous data from small-scale milk production systems, the >70 DFS and >110 DO limit values were established as indicators of reproductive failure[19].

Potential risk factors included use of AI, large herd size and high seroprevalence of neosporosis, IBR and BVD. Farms were classified by service type into AI, if at least 75 % of services were AI, and NB, if at least 75 % were NB. Herd size and seroprevalence values were established according to quartile distribution in the study sample[20]. Classification of herd size was done based on the average number of producing cows per farm during the field data collection period. The third quartile corresponded to 33 cows (classification <33 or ≥33), and was thus established as the limit to classify farms as high seroprevalence for neosporosis (≥84 %), IBR (≥38 %) and BVD (= 100 %) (Table 1).

Identification of neosporosis, IBR and BVD seropositive animals

Blood samples were taken by puncture of the coccygeal vein (vacutainer system) in a randomly selected 10 % of the producing cows in each studied herd. Samples were kept at 4 °C for 24 h, and centrifuged (2,500 xg for 10 min at 4 °C) to separate the serum, which was frozen at -20 °C until analysis.

Detection of *Neospora caninum* antibodies was done with a commercial ELISA test kit (IDEXX Laboratories), following manufacturer instructions. Serum analysis for BVD was run with a commercial ELISA kit for blocking (CIVTEST bovis BVD / Bd P80, Hipra Laboratories), following manufacturer instructions. The IBR analysis was done using the plate neutralization technique, with the MDBK cell line (bovine kidney cells), the IBR758 reference virus, and a $10^{5.6}$ $TCID^{50\%}$ titer at a dilution of 500-1000 infecting doses/ml. Sample positivity was determined by diluting the sera from 1:2 to 1:128, and observing the cytopathic effect produced by the virus[18]. Vaccination records were not available for each farm but this practice is common in the studied regions[13,18].

Statistical analysis

All analyzes were done using the SAS 9.3 statistical package (SAS Institute Inc., Cary, NC). Identification of risk factors was done with multiple logistic regression analysis (PROC LOGISTIC), following the methodology implemented by Potter *et al*[21]. Development of these models involved first running simple logistic regression tests between the events of interest and the potential risk factors. Factors with a value of P<0.35 were retained and analyzed later for collinearity[21]. Collinearity in multiple models was prevented by generating the correlation coefficients and applying paired χ^2 tests of the retained factors using the FREQ procedure with the CHISQ option. When the confidence limit of a factor pair's correlation coefficient did not include 0 and the P value of χ^2 was <0.05, both variables were not included in the same multiple model. Finally, parsimonious multiple models were generated with the BACKWARD option to retain significant variables at a P<0.1[21]. The final multiple models included only the main effects, and the odds ratio (OR) was used as a measure of association between the risk factors and the variables of interest.

Results

Events of interest and farm-level potential risk factors related to reproductive performance

Prevalence for events of interest were 13.2 % for assistance at delivery, 11.7 % for retained fetal membranes, 64.9 % for DFS>70, 46.4 % for DO>110 and 50.5 % for NP1S. Artificial insemination (AI) was a potential risk factor at 73.9 % of the farms while herd size ≥33 cows was one at 41.3 % of the farms. Seroprevalence for neosporosis, IBR and BVD varied widely among the farms (Table 1).

Table 1: Seroprevalences for neosporosis, infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) in 52 small-scale dairy farms

	Mean ± SE	Minimum	Quartile 1	Median	Quartile 3	Maximum
Neosporosis	52.7±4.5	0	33	50	84	100
IBR	23.3±1.8	0	0	23.5	38	75
BVD	59.7±3.2	0	28	75	100	100

Risk factors associated with reproductive performance failure

The simple logistic regression analyses (Table 2) and multiple models for each event of interest (Table 3) showed herd size ≥33 cows and high neosporosis seroprevalence to be risk factors (*P*<0.10) for the assisted calving variable (Table 4). No risk factors were identified (OR>1) for retained fetal membranes, although AI and high IBR seroprevalence were significant factors (OR<1, *P*<0.10; Table 4). For the DFS>70 variable the risk factors were AI and high IBR and BVD seroprevalences (*P*<0.10; Table 5). The only risk factor identified for DO>110 was AI (*P*<0.10, Table 5), while the only one identified for NP1S was herd size ≥33 cows (*P*<0.10).

Table 2: Probability (*P*) and odds ratio (OR) values for potential risk factors considering different events of interest; simple logistical regression analysis

	Events of interest				
Factors	AC (*P*;OR)	RFM (*P*;OR)	DFS>70 (*P*;OR)	DO (*P*;OR)	NP1S (*P*;OR)
AI	0.048; 0.67	0.072; 0.68	<0.001; 2.39	0.054; 1.32	0.162; 0.82
Herd size ≥33 cows	0.090; 1.52	0.821; NC	0.853; NC	0.823; NC	0.001; 1.69
High Neosporosis	<0.001; 2.29	0.099; 0.64	0.414; NC	0.357; NC	0.718; NC
High IBR	0.202; 1.32	0.005; 0.42	0.169; 1.25	0.484; NC	0.916; NC
High BVD	0.010; 0.46	0.793; NC	<0.001; 1.86	0.553; NC	0.049; 0.72

AC=assisted calving; RFM= retained fetal membranes; AI= artificial insemination; DFS>70 = days to first service over 70 d in milk; DO>110 = days open over 110 d in milk; NP1S = non-pregnant cows at first service; NC = OR not calculated due to lack of significance.

Table 3: Non-collinear potential risk factors for events of interest included in multiple models

Events of interest	Model	Potencial risk factors
Assisted calving	1	AI + High IBR
	2	Herd size ≥33 cows + High Neosporosis
	3	High BVD
Retained fetal membranes	1	AI + High neosporosis
	2	AI + High IBR
DFS>70	1	AI + High IBR
	2	High BVD + High IBR
DO>110	1	AI
NP1S	1	Herd size ≥33 cows
	2	High BVD
	3	AI

AI= artificial insemination; DFS>70= days to first service over 70 days in milk; DO>110= days open over 110 days in milk; NP1S= non-pregnant cows at first service; BVD= bovine viral diarrhea; IBR= infectious bovine rhinotracheitis.

Table 4: Effect of study variables on assisted calving and retained fetal membranes in multiple models

Variable	Effects	OR	CI95%	P
AC				
Model 1	Service type: NB	Ref.	N/A	N/A
	Service type: AI	0.67	0.45-0.99	0.048
Model 2	Herd size: <33 cows	Ref.	N/A	N/A
	Herd size: ≥33 cows	1.51	0.93-2.45	0.090
	Neosporosis: Remainder	Ref.	N/a	N/A
	Neosporosis: High	2.28	1.52-3.40	0.001
Model 3	BVD: Remainder	Ref.	N/A	N/A
	BVD: High	0.46	0.26-0.83	0.010
RFM				
Model 1	Service type: NB	Ref.	N/A	N/A
	Service type: AI	0.68	0.45-1.04	0.072
Model 2	Service type: NB	Ref.	N/A	N/A
	Service type: AI	0.66	0.43-1.01	0.055
	IBR: Remainder	Ref.	N/A	N/A
	IBR: High	0.41	0.23-0.75	0.004

P= probability value; AC= Assisted calving; RFM= Retained fetal membranes NB= natural breeding; AI= artificial insemination; BVD= bovine viral diarrhea; IBR= infectious bovine rhinotracheitis; CI= odds ratio confidence interval; OR= odds ratio; N/A= not applicable.

Table 5: Effect of study variables on days to first service (DFS>70), days open (DO>110) and non-pregnant cows at first service (NP1S) with different multiple models

Variable	Effects	OR	CI 95%	*P*
DFS>70				
Model 1	Service Type: NB	Ref.	N/A	N/A
	Service Type: AI	2.42	1.81-3.2	<0.001
	IBR: Remainder	Ref.	N/A	N/A
	IBR: High	1.32	0.95-1.84	0.097
Model 2	BVD: Remainder	Ref.	N/A	N/A
	BVD: High	1.86	1.31-2.64	<0.001
DO>110				
Model 1	Service Type: NB	Ref.	N/A	N/A
	Service Type: AI	1.32	0.99-1.8	0.054
NP1S				
Model 1	Herd size: <33	Ref.	N/A	N/A
	Herd size: ≥33	1.69	1.23-2.32	0.001
Model 2	BVD: Remainder	Ref.	N/A	N/A
	BVD: High	0.72	0.52-1.00	0.049

P= probability value; NB= natural breeding; AI= artificial insemination; BVD= bovine viral diarrhea; IBR= infectious bovine rhinotracheitis; CI= odds ratio confidence interval; OR= odds ratio; N/A= not applicable.

Discussion

Small-scale dairy systems in Mexico are highly heterogeneous in terms of productive, reproductive and health status[13,19]. The diseases neosporosis, IBR and BVD are associated with reproductive disorders[22,23]. In the present data, mean neosporosis seroprevalence values per farm were similar to those previously reported for small-scale dairy systems (51.7 %)[24], but higher than reported for intensive systems in Mexico (~43 %)[18,25]. Biosafety measures in small dairy farms may be less stringent, which could increase risk factors associated with the presence of Neospora, such as dogs in production units[26,27]. The seroprevalence of IBR in the present study was also similar to previous reports of small dairy farms in Mexico, with rates near 22 %[24,28], but was notably lower than the 69% seroprevalence reported in other small-scale production systems[18]. Average BVD seroprevalence per farm was within reported ranges (52-81%)[18,24]. Earlier studies have proposed that these three diseases are endemic and highly prevalent in dairy cattle in intensive, double-purpose and small-scale systems in Mexico[18,29,30]. Although these high reported seroprevalences may be due to IBR and BVD vaccine antibodies, their broad distribution and high prevalences in the present results confirm their importance in small-scale dairy farms.

It seems this is the first study reporting farm-level risk factors associated with reproductive performance in small-scale dairy farms in Mexico. Farms with high neosporosis seroprevalence were 128 % more probable to require calving assistance, while those with herds of 33 or more cows were 51 % more probable to require it. These results coincide with previous reports of a significant association between assistance in parturition and *Neospora*-seropositive animals[31]. Nonetheless, there are studies in which this association has not been identified[32], highlighting the need to clarify this potential association. A possible reason for assistance in parturition being more prevalent in farms with ≥33 cows may be that at larger farms larger sires (AI or NB) are used, or that problems exist with body condition at birth, which still requires confirmation. Effective management in the peripartum and correct obstetric care are some of the main factors in controlling problems of dystocia[33]. Labor at small-scale dairy farms usually consists only of family members, which can´t lead to possible labor shortages at critical moments such as parturition[6]. This could be particularly acute at farms with ≥33 cows.

At the farms with the highest BVD prevalence and which use AI, risk of the need for assistance was much lower. This result is to be expected since birth weight in calves positive to BVD antigens is 7 kg lighter than in BVD negative calves[34]. Moreover, use of AI can also influence the need for calving assistance because there is currently a wide variety of sires in the market offering multiple traits, such as calving ease[35,36]. Perhaps the studied farms had been selecting sires with just such a trait (pers. comm., MC Fernando Villaseñor). The farms using AI also exhibited a lower risk of retained fetal membranes (Table 4). Assisted calving is one of the most important risk factors associated with retained fetal membranes at the individual level[37]. Use of AI may therefore reduce the need for assistance and consequently placental retention. In the farms with high IBR seroprevalence risk of placental retention was lower, which is apparently counterintuitive since IBR has been associated with abortion and placental retention[38]. One possible explanation for the present results is that the high IBR seroprevalences observed here were due to vaccine antibodies[18], which would actually reduce rates of abortion due to IBR, consequently decreasing the risk of placental retention[39].

Cows at farms where AI was used were 142 % more likely to have DFS>70. The success of AI rests largely on how efficiently estrus is detected[40]. Although not recorded as part of the present study, estrus detection rates are commonly low at both small dairy farms[12], and intensive dairy production systems in Mexico[41]. Cows at farms with a high IBR seroprevalence were 32 % more likely to have DFS>70, whereas those at farms with high BVD seroprevalence were 86 % more likely to have DFS>70; in the case of IBR, this varied widely from a 5 % reduction in probability to an increase of 80 %. The reproductive consequences of BVD infection are well known(42,43), but there are no studies associating high IBR and BVD seroprevalences with days to first service. Indeed, from a pathological point of view, it is unclear how these diseases could increase DFS>70. One possible explanation is that high seroprevalences of these diseases could indirectly impact this indicator(44), or they may be correlated with other risk factors such as overcrowding or mismanagement of biological waste(45).

On average, cows at farms where AI was used were 32 % more likely to have DO>110, although this varied from a 1 % reduction to an 80 % increase. AI does allow for greater genetic selection but also has negative impacts on indicators such as days to first service, suggesting that estrus detection techniques are deficient(12,46). Estrus synchronization protocols have been implemented to counteract this tendency in intensive dairy production systems(47). Given the good fertility at first service in the studied system (49.5 %), implementation of fixed-time insemination protocols adapted specifically to this production system could improve reproductive performance(48).

Herd size >33 cows made them 69 % more likely to be NP1S. In other production systems, as herd size increases the capacity for effective reproduction management decreases(14-16). Although small-scale dairy production systems clearly have fewer animals to manage than intensive systems, the effect of herd size is still a telling indicator. Presence of BVD has been reported to cause early embryo death and subfertility in dairy cattle(49-51), but the present results showed herds with high BVD seroprevalence to a have lower risk of NP1S. This may seem contradictory, but these high seroprevalences could be due to vaccine antibodies(18).

Use of AI was a significant common factor influencing most of the events of interest. This genetic improvement technology reduces post-partum complications without affecting fertility at first service, but increases days at first service and days open (probably due to estrus detection deficiencies). Considering the good fertility rates observed at the studied small-scale dairy farms, one possible strategy to take full advantage of AI would be implementation of fixed-time synchronization protocols for the first service, with sexed semen. However, financial feasibility studies are needed before broad-scale recommendations can be made for this sector.

An obvious limitation in the present study is the uncertainty surrounding the source of the antibodies in the serological tests. No reliable vaccination history data were available in the data because the studied small-scale dairy farms did not keep exhaustive vaccination records, even though vaccination is common in the studied regions(13,18). This uncertainty limits the ability to make more accurate inferences based on the present results. However, interpretation of the results has been conservative and can function as a baseline for subsequent epidemiological and pathological studies.

Conclusions and implications

In small-scale dairy farms in Mexico, AI, herd size and high seroprevalences of neosporosis, IBR and/or BVD are factors associated with reproductive performance. The risk factors identified for assisted calvings were herds of 33 or more cows and high neosporosis seroprevalence. Those for DFS>70 were AI and high IBR and BVD seroprevalences, while for DO>110 it was AI. The single risk factor for NP1S was herds of 33 or more cows, and no risk factors were identified for retained fetal membranes. The present study also highlights the need to prevent neosporosis, IBR and BVD, all of which are widely distributed in Mexican dairy farms.

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Conflict of interest

The authors declare that they have no financial or personal relationships that could have inappropriately influenced development and reporting of this research.

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