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Purulent vaginal discharge in grazing dairy cows: Risk factors, reproductive performance, and prostaglandin $F_{2\alpha}$ treatment

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ABSTRACT

The objectives of this study were to assess the association of a 4-point scale of vaginal discharge score (VDS) with time to pregnancy to define criteria for a practical case of purulent vaginal discharge (PVD) in dairy cows, to test the risk factors for PVD, and, finally, the effect of a dose of $PGF_{2\alpha}$ on cure and reproductive performance. In experiment 1, grazing Holstein cows (n = 2,414) had their vaginal discharge scored at ~32 d in milk (DIM) on a 4-point scale, the effect of VDS on the hazard of pregnancy by 300 DIM was then assessed to derive a case definition of PVD. Risk factors for PVD and self-cure were also assessed. In experiment 2, grazing Holstein cows (n = 6,326) from 5 herds were checked for PVD at ~30 DIM. Cows with PVD were assigned to receive one dose of 500 μg of $PGF_{2\alpha}$ analog (Cloprostenol; Ciclase, Syntex SA, Buenos Aires, Argentina) per cow (odd ear tag number) or to remain untreated (even tag number). Cure was declared if cows presented clear normal vaginal discharge (VDS-0) at visit 2 (~62 DIM). Data were analyzed with Cox's regression and mixed logistic models. In experiment 1, cows with VDS ≥1 had lower hazard of pregnancy and longer calving to pregnancy interval than cows with VDS-0. This finding was not affected by the time at which the diagnosis was performed. Therefore, a cow ≥21 DIM and having VDS ≥1 was used to define a case of PVD. The odds of PVD were greater in primiparous cows compared with multiparous, in cows with abnormal calving compared with those with normal calving, and in those losing BCS peripartum. In experiment 2, $PGF_{2\alpha}$ treatment tended to slightly increase the hazard of pregnancy (adjusted hazard ratio = 1.13). Conversely, $PGF_{2\alpha}$ had no effect on the odds of cure of PVD [adjusted odds ratio (AOR) = 1.19], pregnancy at first service (AOR = 1.03), or pregnancy by 100 DIM (AOR = 0.89) or 200 DIM (AOR = 1.27). In conclusion, cows with VDS ≥1 can be considered to have PVD because of their lower hazard of pregnancy and longer calving to pregnancy interval (up to 48 d). Important risk factors are parity, calving, and body condition score loss peripartum. Optimal time of diagnosis is ≥28 to 35 DIM because cows experience a high self-cure rate. Self-cure is also affected by parity, prepartum BCS, and VDS. Finally, as treatment with one dose of $PGF_{2\alpha}$ had a small effect on the hazard of pregnancy and no effect on clinical cure, its therapeutic use in grazing dairy cows with PVD is not recommended.

Key words: dairy cow, purulent vaginal discharge, prostaglandin $F_{2\alpha}$ treatment, reproductive efficiency

INTRODUCTION

General consensus is that clinical endometritis (**CE**) has detrimental effects on reproductive performance of dairy cows (LeBlanc et al., 2002a; McDougall et al., 2007; Runciman et al., 2008; Gautam et al., 2009; Dubuc et al., 2010a, 2011b; Giuliodori et al., 2013a), but disease definition has varied among research groups. The first definition of CE based on its negative effect on reproductive performance was proposed by LeBlanc et al. (2002a). Those authors defined CE by the detection of a purulent or foul vaginal discharge, or cervical diameter >7.5 cm at ≥20 DIM, or muco-purulent discharge at ≥26 DIM (LeBlanc et al., 2002a). Based on that research, a group of researchers set a definition of CE as the presence of purulent (>50% pus) discharge in the vagina at ≥21 DIM, or by a muco-purulent (approx. 50% pus, 50% mucus) discharge in the vagina at ≥26 DIM (Sheldon et al., 2006). The prevalence of

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CE ranges from 5 to over 30% in some herds (LeBlanc et al., 2002a; Mejía and Lacau-Mengido, 2005; McDougall et al., 2007; Giuliodori et al., 2013a). It has recently been proposed to rename CE as purulent vaginal discharge (**PVD**), given that up to one third of cows having PVD have no evidence of endometritis (low count of polymorphonuclear cells; Dubuc et al., 2010a; Denis-Robichaud and Dubuc, 2015). In clinical practice, endometrial cytology is rarely evaluated, so no evidence exists as to whether cows had endometrial inflammation (i.e., endometritis). It is widely accepted that dystocia, stillbirth, retained placenta, and metritis are risk factors for PVD (Ghanem et al., 2002; LeBlanc et al., 2002a; Dubuc et al., 2010a; Gautam et al., 2010; Potter et al., 2010; Giuliodori et al., 2013a); another proposed risk factor is peripartum negative energy balance (**NEB**; Dubuc et al., 2010a; Kaufmann et al., 2010a; Giuliodori et al., 2013a). Treatment has received a lot of attention in recent decades and is still a controversial issue (Haimerl et al., 2012, 2013; Lefebvre and Stock, 2012). That controversy is mainly rooted in the fact that comparison among studies is difficult because of different case definitions, low numbers of cows per treatment, lack of negative controls, and treatment success based on clinical cure instead of on reproductive performance (Lefebvre and Stock, 2012).

Our hypotheses were that a modified 4-point scale of vaginal discharge score (**VDS**) is associated with reproductive performance of cows and can be used to define a case of PVD, that the lower the DIM at diagnosis the higher the prevalence of PVD, and that $PGF_{2\alpha}$ treatment increases cure rate and reproductive performance of treated cows. Our objectives were to assess (1) a 4-point scale of VDS to diagnose PVD in grazing Holstein dairy cows based on the reproductive performance of the cows (i.e., time to pregnancy), (2) the risk factors for PVD, (3) the optimal time to diagnose PVD, (4) the self-cure rate during the postpartum period, and (5) the effect of $PGF_{2\alpha}$ treatment on cure rate and reproductive performance of cows.

MATERIALS AND METHODS

Farm and Animal Management

The study involved 2 experiments that were conducted in 6 commercial dairy herds (35°37′S, 61°22′W, Argentina) enrolling a total of 8,740 grazing Holstein cows. Experiment 1 (effect of VDS) was performed on a single farm in years 2005 and 2006 (n = 2,414), whereas experiment 2 (effect of $PGF_{2\alpha}$) was run on 5 farms (A, B, C, D, and E) in years 2007 and 2008, enrolling a total of 6,326 cows (n = 936, 472, 2,386, 1,516, and 1,016 cows for farms A through E, respectively). On all farms, prepartum cows that were within 4 wk of expected calving were maintained on dry lots, fed a low-DCAD diet, and monitored for signs of calving by farm employees trained to assist parturition. After calving, cows were sent for 3 d to the fresh herd and kept on a dry lot. Lactating cows were at pasture in a rotational system (different paddock in each morning and afternoon). Feed was composed of mixed pastures (alfalfa, tall fescue) and winter annual grasses (ryegrass) and supplemented with TMR diets (corn silage, soybean pellets, and corn meal). Concentrates (40% soybean pellets and 60% corn meal) were offered twice daily during milking. The cows calved year-round and were milked twice daily with a herd average 305-d milk yield between 8,000 and 10,000 kg/cow. After a voluntary waiting period of 50 d, cows were observed for estrus twice daily and AI by using the a.m./p.m. rule. Pregnancy was diagnosed by rectal palpation after 35 d postinsemination, and pregnant cows were rechecked by transrectal palpation at 150 d of gestation. Veterinarians visited the farms monthly for routine reproductive examination.

Vaginal Discharge Evaluation and Treatments

Experiment 1 (Effect of VDS). Cows had their vaginal discharge scored twice, at visits 1 [32 (21–49) DIM; median (highest–lowest)] and 2 [62 (48–97) DIM], by direct inspection after manual examination of the vagina with a gloved hand (Sheldon et al., 2002). Samples were scored on a 4-point scale as VDS-0 = clear discharge; VDS-1 = clear discharge with flecks of pus; VDS-2 = muco-purulent not fetid discharge; and VDS-3 = watery, purulent or brown-colored, fetid discharge. In addition, body condition was scored (5-point scale; Ferguson et al., 1994) at each visit. As cows with VDS-1 and 2 at visit 1 were not treated, they were classified as self-cured if they had a VDS-0 at recheck at visit 2. Reproductive data were obtained from dairy records and used to assess the relationship between VDS and reproductive performance. Cows having VDS-3 were excluded from the statistical analysis assessing reproductive performance because they received 12 g of oxytetracycline systemically (Terramicina LA, Pfizer SRL, Buenos Aires, Argentina) plus 750 μg of Tiaprost (Ileren, Intervet Argentina, Buenos Aires, Argentina).

Experiment 2 (Effect of $PGF_{2\alpha}$). Cows also had their vaginal discharge sampled twice and scored as previously described at visits 1 [30 (21–50) DIM; median (highest–lowest)] and 2 [58 (49–84) DIM]. Purulent vaginal discharge was diagnosed when cows had VDS-1 to 3 at visit 1, whereas cows with VDS-0 were considered as healthy. Cows having a VDS-1 and 2 at visit 1 were assigned by ear tag number to receive one

i.m. injection of 500 μg of cloprostenol (Ciclase, Syntex SA, Buenos Aires, Argentina) per cow ($PGF_{2\alpha}$ group, odd tag number) or to remain untreated (control group, even tag number). Cure was declared if cows presented VDS-0 at visit 2 (~28 d later). Cows having VDS-3 were not included in the statistical analysis assessing $PGF_{2\alpha}$ effects on reproductive performance and cure rate because they received antibiotics, as described for experiment 1.

Statistical Analysis

Statistical significance was set at $P < 0.05$ and a tendency was set at $P < 0.10$. Proportional hazard models were analyzed with PROC PHREG (SAS, 2003), and logistic models were analyzed with PROC GLIMMIX (SAS, 2003) with binomial distributions and logit link functions. Modeling was performed by using a manual backward elimination method with an exclusion criteria set at $P > 0.10$, with the exception of VDS in experiment 1 and $PGF_{2\alpha}$ in experiment 2, which were forced to remain in models.

Defining PVD (Experiment 1). The effect of VDS [0 vs. 1 vs. 2 (cows having VDS-3 were excluded from analysis because they received antibiotics)] on the hazard of pregnancy by 300 DIM was evaluated by proportional hazard analysis, whereas the effect on the odds of pregnancy at first AI (**PFAI**), by 100 DIM (**P100**), and by 200 DIM (**P200**) were assessed by logistic regression analysis. The proportional hazard ratio model included the fixed effects of calving season [summer (December 21–March 21) vs. autumn (March 21–June 21) vs. winter (June 21–September 21) vs. spring (September 21–December 21)], parity (1 vs. ≥2), calving [normal vs. abnormal (dystocia, retention of placenta, and dead calf)], prepartum BCS (<2.75 vs. 2.75–3.25 vs. ≥3.50), postpartum BCS (<2.50 vs. 2.50–2.75 vs. ≥3.00, visit 1), and BCS loss (BCS prepartum minus BCS at visit 1: loss vs. no change or gain), and their 2-way interactions with VDS. Year of calving was included under the strata option. Time intervals (median ± 95% CI) and survival function estimates were obtained from PROC LIFETEST (SAS, 2003). Logistic models included the random effect of year of calving (2005 vs. 2006) and the same fixed effects already described.

Optimal Time to Diagnose PVD (Expeiment 1). The logistic model assessing the risk for PVD included the fixed effect of time at visit 1 (≤27 vs. 28 to 34 vs. ≥35 DIM) together with all the random and fixed effects described for risk factors analysis. Then, 2 proportional hazard models assessing the hazard of pregnancy by 300 DIM were run combining the effects of VDS (0, 1, or 2) and the time when diagnosis was performed (<28 vs. ≥28 DIM in model 1 and <35 vs. ≥35 DIM in model 2) as fixed effects.

Self-Cure Rate (Experiment 1). The logistic model assessing the risk of self-cure included the same random and fixed effects described for risk factors analysis. Cows having VDS-0 and -3 were excluded from this analysis.

Risk Factors for PVD (Experiment 1). The logistic model assessing risk factors included the random effect of year of calving (2005 vs. 2006), and the same fixed effects previously described (calving season, parity, calving, prepartum BCS, and BCS loss). Cows having VDS-3 were not excluded from this analysis.

$PGF_{2\alpha}$ Treatment Effects (Experiment 2). The proportional hazard models assessing the hazard of pregnancy by 300 DIM included the fixed effects of calving season [summer (December 21–March 21) vs. autumn (March 21–June 21) vs. winter (June 21–September 21) vs. spring (September 21–December 21)], parity (1 vs. ≥2), calving [normal vs. abnormal (dystocia, retention of placenta, and dead calf)], VDS (1 vs. 2), $PGF_{2\alpha}$ (yes vs. no), and their 2-way interactions. The effects of year of calving and farm were included under the strata option. Time intervals (median ± 95% CI) and survival function estimates were obtained from PROC LIFETEST (SAS, 2003). The logistic models assessing the effect of $PGF_{2\alpha}$ treatment (yes vs. no) on cure rate and reproductive performance (i.e., odds of PFAI, P100, and P200) included the random effect of year of calving (2007 vs. 2008) and farm (A, B, C, D, and E), and the same fixed effects already described for the proportional hazards regression model. Cows having VDS-0 and -3 were excluded from analysis because they were considered healthy (VDS-0) and did not receive $PGF_{2\alpha}$ or were treated with systemic antibiotics (VDS-3), as previously described.

RESULTS

In experiment 1, the percentages of cows having VDS-0, VDS-1, VDS-2, and VDS-3 at visit 1 were 80.4% (1,852/2,303), 10.3% (237/2,303), 7.8% (180/2,303), and 1.5% (34/2,303), respectively.

Defining PVD

In proportional hazards and logistic regression models, the hazard ratio and the odds ratio represent the instant risk and the risk of event (i.e., pregnancy) relative to cows without the factors of interest. For example, in Table 1, a hazard ratio of 0.74 means that cows having a VDS-1 had a risk of pregnancy per day only 74% of that of cows with VDS-0, and that cows with VDS-2 (hazard ratio of 0.57) had daily risk of pregnancy only

57% of that of cows showing VDS-0. Also, cows having VDS-1 and VDS-2 took 19 and 48 d longer to get pregnant, respectively, than cows with VDS-0 (Table 1, Figure 1). In addition, VDS was negatively associated with the odds of pregnancy ($P < 0.01$, Table 1), given that the odds of P100 were lower in cows VDS-1 and VDS-2 [odds ratio (**OR**) = 0.71 and 0.35, respectively] than in cows having VDS-0, and that the odds of P200 were also lower in cows with VDS-1 and VDS-2 (OR = 0.60 and 0.38, respectively) compared with cows having VDS-0. Thus, cows having VDS-1 or higher had lower reproductive performance than their counterparts with VDS-0 (Table 1), a VDS ≥1 was used to define a case of PVD.

Optimal Time to Diagnose PVD

The DIM at diagnosis had an effect on the detection of PVD, given that the proportion of positive cows were 32, 19, and 15% when the diagnosis was performed 21 to 27 versus 28 to 34 versus ≥35 DIM. The odds of detecting PVD were significantly higher in cows checked 21 to 27 DIM (OR = 3.40, 95% CI = 2.51–4.11) or 28–34 DIM (OR = 1.66, 95% CI = 1.23–2.25) compared with cows evaluated ≥35 DIM.

The hazard models of the combined effects of VDS (0, 1, and 2) and time at diagnosis [<28 vs. ≥28 DIM (model 1) and <35 vs. ≥35 DIM (model 2)] showed that cows having VDS-1 diagnosed <28 versus ≥28 DIM had 33 and 17% lower hazard of pregnancy, respectively, than cows with VDS-0, and that cows with VDS-2 detected <28 versus ≥28 DIM had 41 and 44% lower hazard of pregnancy, respectively, than cows with VDS-0 (Table 2). In addition, cows having VDS-1 diagnosed <35 versus ≥35 DIM had 25 and 19% lower hazard of pregnancy, respectively, than cows with VDS-0, and cows with VDS-2 checked <35 versus ≥35 DIM had 37 and 52.5% lower hazard of pregnancy, respectively, than cows with VDS-0 (Table 2).

Self-Cure Rate

The proportion of self-cured cows was 57% (188/330). Primiparous cows had higher odds of self-cure than multiparous [adjusted odds ratio (**AOR**) = 1.84, 95% CI = 1.13–3.02, $P = 0.02$] with 63 and 50% cured, respectively. Cows with VDS-1 had higher odds of self-cure than those having VDS-2 (AOR = 2.24, 95% CI = 1.34–3.76, $P = 0.01$) with 63 and 50% cured, respectively. Finally, the odds of self-cure were higher in cows diagnosed 21 to 27 DIM than in those assessed ≥35 DIM (AOR = 2.05, 95% CI = 1.16–3.62). Conversely, self-cure was similar for those checked 28 to 34 DIM to cows diagnosed ≥35 DIM (AOR = 1.19, 95% CI = 0.64–2.22).

Risk Factors

The odds of PVD were higher in cows calving in summer, winter, and spring than in autumn ($P < 0.01$, Table 3). Primiparous cows had much higher odds of PVD than multiparous ones ($P < 0.01$). Cows experiencing abnormal calving had greater odds of PVD than those with normal calving ($P < 0.01$, Table 3). In addition, the odds of PVD were higher in cows experiencing peripartum BCS loss ($P = 0.01$, Table 3).

Effect of $PGF_{2\alpha}$ Treatment

In experiment 2, the percentages of cows having VDS-0, VDS-1, VDS-2, and VDS-3 were 77.2% (4,092/5,300), 12.8% (679/5,300), 8.0% (424/5,300), and 2.0% (105/5,300), respectively. Purulent vaginal discharge was diagnosed in 22.7% (1,208/5,300) of cows. Cows having VDS-3 were excluded from the analysis because they received systemic antibiotic treatment, so that only cows with VDS-1 (n = 679) and VDS-2 (n = 424) were enrolled in the $PGF_{2\alpha}$ trial (n = 1,103).

The hazard of pregnancy up to 300 DIM was slightly higher in cows receiving $PGF_{2\alpha}$ treatment than in untreated cows (HR = 1.13, $P = 0.09$, Table 4). Median (95% CI) time from calving to pregnancy was 130 (120–137) and 128 (120–137) days for treated and untreated cows with PVD ($P = 0.07$). Treatment with $PGF_{2\alpha}$ had no effect on the odds of cure of PVD ($P = 0.26$, Table 4), with 74.3 and 70.9% cured for $PGF_{2\alpha}$ and untreated control groups, respectively (Table 4). Treatment with $PGF_{2\alpha}$ had no effect on the odds of PFAI ($P = 0.86$, Table 4), with 33.8 and 33.2% of treated and untreated cows pregnant, respectively. Treatment had no effect on the odds of P100 ($P = 0.51$, Table 4) due to the percentage of pregnant cows being 29.8 and 32.5% for treated and untreated cows, respectively. Treatment had no effect on the odds of P200 ($P = 0.17$, Table 4) because the percentage of pregnant cows was 77.2 and 72.8% in treated versus untreated cows, respectively.

DISCUSSION

The objectives of our study were to assess a 4-point scale of VDS for diagnosis of PVD and its association with reproductive performance, the risk factors for PVD in grazing Holstein dairy cows, the optimal time to diagnose PVD, and the effect of $PGF_{2\alpha}$ treatment of PVD.

Defining PVD

In agreement with our hypothesis, the 4-point scale of VDS is associated with reproductive performance.

Table 1. Defining purulent vaginal discharge in grazing Holstein dairy cows (n = 1,945) with a scale of vaginal discharge score evaluation

	K-M survival[2]				Hazard ratio of pregnancy			Odds ratio of pregnancy		
VDS[1]	Median	95% CI	%	no./no.	AHR[3]	95%CI	*P*-value	AOR[4]	95% CI	*P*-value
VDS-0	99	95–103			Referent					
VDS-1	118	101–127			0.74	0.64–0.86	<0.01			
VDS-2	147	134–162			0.57	0.47–0.68	<0.01			
Logistic regression										
At first AI										
VDS-0			38.6	623/1,615				Referent		0.67
VDS-1			34.5	67/194				0.87	0.63–1.19	
VDS-2			35.3	48/136				0.95	0.65–1.37	
By 100 DIM										
VDS-0			51.1	826/1,615				Referent		
VDS-1			40.7	79/194				0.71	0.52–0.96	<0.01
VDS-2			24.2	33/136				0.35	0.23–0.52	<0.01
By 200 DIM										
VDS-0			90.3	1,459/1,615				Referent		
VDS-1			83.5	162/194				0.60	0.40–0.93	<0.01
VDS-2			74.3	101/136				0.38	0.24–0.58	<0.01

[1]Vaginal discharge score: VDS-0 = clear discharge, VDS-1 = clear discharge with pus flecks, VDS-2 = muco-purulent not fetid discharge, and VDS-3 = watery, purulent or brown-colored, and fetid vaginal discharge. Vaginal discharge was scored at (mean ± SD) 35 ± 7 DIM. Cows diagnosed <21 DIM (n = 111), having VDS-3 (n = 34), culled (n = 255), and with missing data (n = 69) were removed from the statistical analysis [2,414 – (111 + 34 + 255 + 69) = 1,945].

[2]K-M survival = Kaplan–Meier survival analysis.

[3]AHR = adjusted hazard ratios.

[4]AOR = adjusted odds ratios.

Table 2. The effect of vaginal discharge score and the time at diagnosis on the reproductive performance in grazing Holstein dairy cows (n = 1,945)

			K-M survival[3]		Hazard of conception		
VDS[1]	Time[2]	n	Median	95% CI	AHR[4]	95%CI	*P*-value
VDS-0	≥28	1,336	100	96–104	Referent		<0.01
	<28	279	91	87–103	1.07	0.95–1.24	0.23
VDS-1	≥28	142	112.5	99–122	0.83	0.69–0.99	0.04
	<28	52	134	103–159	0.67	0.51–0.90	0.01
VDS-2	≥28	85	148	130–165	0.56	0.44–0.71	<0.01
	<28	51	146	104–180	0.59	0.44–0.79	<0.01
VDS-0	≥35	942	102	97–107	Referent		<0.01
	<35	673	94	89–100	1.14	1.03–1.26	0.01
VDS-1	≥35	87	105	95–123	0.81	0.65–1.01	0.06
	<35	107	125	103–151	0.75	0.61–0.92	<0.01
VDS-2	≥35	52	161	132–183	0.48	0.35–0.64	<0.01
	<35	84	146	115–154	0.63	0.50–0.79	<0.01

[1]Vaginal discharge score: VDS-0 = clear discharge, VDS-1 = clear discharge with pus flecks, VDS-2 = muco-purulent not fetid discharge (and VDS-3 = watery, purulent or brown-colored, and fetid vaginal discharge). Cows diagnosed <21 DIM (n = 111), having VDS-3 (n = 34), culled (n = 255), and with missing data (n = 69) were removed from the statistical analysis [2,414 – (111 + 34 + 255 + 69) = 1,945].

[2]Time at diagnosis (in DIM): before and after 28 DIM (model 1) and before and after 35 DIM (model 2).

[3]K-M survival = Kaplan–Meier survival analysis.

[4]AHR = adjusted hazard ratios.

Higher VDS was associated with reduced odds of pregnancy at first AI and longer time to pregnancy. Therefore, by using this 4-point scale of VDS, a case of PVD can be defined in cows ≥21 DIM having VDS-1 to -3, because these scores are significantly associated with poorer reproductive performance. Previous studies have reported the detrimental effects of PVD on reproduction (Fourichon et al., 2000; LeBlanc et al., 2002a; Williams et al., 2005; McDougall et al., 2007; Runciman et al., 2008; Gautam et al., 2009, 2010; Dubuc et al., 2010a, 2011b; Plöntzke et al., 2011; Giuliodori et al., 2013a), and our data agree with the fact that reproductive performance varies with VDS. It is generally accepted that PVD could reduce reproductive performance by delaying return to cyclicity (Mateus et al., 2002); by reducing progesterone production by luteal cells (Williams et al., 2007); by disrupting the uterine environment (BonDurant, 1999), and, lastly, by impairing embryo development (Soto et al., 2003). Irrespective of the explanation for the reduced fertility, it is important to note that the increase in pus content in the exudate in the vagina, and also the presence of fetid odor, are associated with decreased reproductive performance.

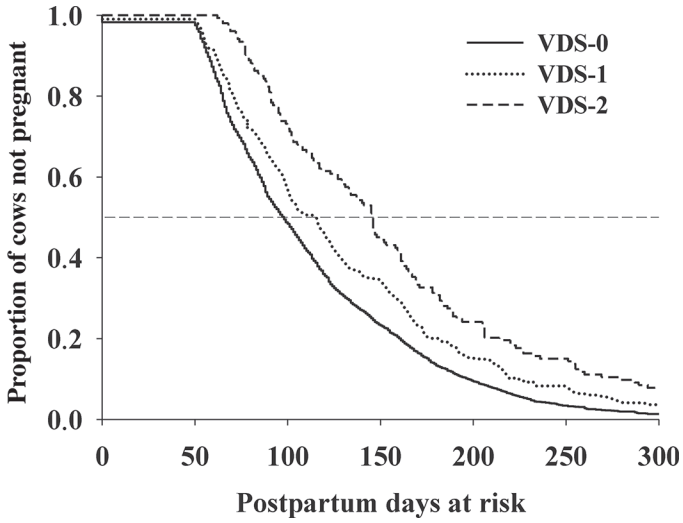

Figure 1. Effect of postpartum vaginal discharge score (VDS) on the hazard of pregnancy in grazing Holstein dairy cows (n = 1,945). VDS-0 = clear discharge, VDS-1 = clear discharge with pus flecks, VDS-2 = muco-purulent not fetid discharge, and VDS-3 = watery, purulent or brown-colored, and fetid vaginal discharge. Median (95% CI) days open were 99 (95–103) for cows with VDS-0 (n = 1,615), 118 (101–127) for cows with VDS-1 (n = 194), and 147 (134–162) for cows with VDS-2 (n = 136) (Log-rank test, $P < 0.01$). Cows diagnosed <21 DIM (n = 111), having VDS-3 (n = 34), culled (n = 255), and with missing data (n = 69) were removed from the statistical analysis assessing the effect of $PGF_{2\alpha}$ on cure rate and reproductive performance [2,414 – (111 + 34 + 255 + 69) = 1,945].

By using this definition, the prevalence of PVD (19.6% in experiment 1 and 22.8% in experiment 2) at visit 1 were similar to those previously reported (Ghanem et al., 2002; LeBlanc et al., 2002a; Mejía and Lacau-Mengido, 2005; Gautam et al., 2010; Potter et al., 2010; Plöntzke et al., 2011; Giuliodori et al., 2013a).

Optimal Time of Diagnosis

In agreement with our hypothesis, the proportion of cows with PVD is affected by the time (DIM) at which diagnosis is performed. The earlier the diagnosis

Table 3. Risk factors for purulent vaginal discharge in grazing Holstein dairy cows (n = 2,303)[1]

	Incidence		Odds ratio of PVD[2]		
Predictor	%	no./no.	AOR[3]	95% CI	*P*-value
Season[4]					0.07
Summer	20.9	133/637	Referent		
Autumn	15.6	121/774	0.64	0.45–0.91	
Winter	22.9	96/420	0.96	0.65–1.42	
Spring	21.4	101/472	0.94	0.66–1.34	
Parity					<0.01
1	26.1	263/1,007	Referent		
≥2	14.5	188/1,296	0.38	0.30–0.50	
Calving					0.02
Normal	16.6	265/1,597	Referent		
Abnormal	26.4	186/706	1.38	1.05–1.81	
BCS loss[5]					<0.01
No	12.7	109/858	Referent		
Yes	21.8	229/1,050	2.32	1.79–3.03	

[1]Cows diagnosed <21 DIM (n = 111) were removed from the statistical analysis (2,414 – 111 = 2,303).

[2]Purulent vaginal discharge (PVD): cows with VDS-1, -2 and -3 at visit 1 postpartum (approximately 32 DIM) were declared as having PVD (VDS-0 = clear discharge, VDS-1 = clear discharge with pus flecks, VDS-2 = muco-purulent not fetid discharge, and VDS-3 = watery, purulent or brown-colored, and fetid vaginal discharge).

[3]AOR = adjusted odds ratios.

[4]Season = calving season (summer vs. fall vs. winter vs. spring).

[5]BCS loss = prepartum BCS minus BCS at visit 1 postpartum (loss: no vs. yes). There were 113 cows with missing data regarding BCS pre- and postpartum.

is performed, the higher the proportion of cows that are test-positive. In the present work, the prevalence of PVD <28 DIM was double relative to ≥35 DIM (32 vs. 15%, respectively). This finding is in agreement with a previous report showing higher odds of PVD when cows were examined less than approximately 30 DIM (LeBlanc et al., 2002a; Gautam et al., 2010). As PVD was defined as those VDS that predicted reduced hazard of pregnancy, the combined effects of VDS and the time at which diagnosis was performed were examined for their associations with reproductive performance. Cows with VDS ≥1 had lower hazard of pregnancy and longer calving to pregnancy interval than cows with VDS-0 (Table 2). This finding was not affected by the time at which the diagnosis was performed (28 or 35 DIM); therefore, a cow ≥21 DIM and having VDS ≥1 was used to define a case of PVD.

Self-Cure

The observed high self-cure rate (57% in experiment 1 and 71% in experiment 2) could be the main reason why diagnosis should not be done too early postpartum. Some reports document spontaneous cure ranging from 63 to 77% (Gautam et al., 2010; Dubuc et al., 2011b; Plöntzke et al., 2011; Giuliodori et al., 2013a), but others found much lower self-cure rates (Brick et al., 2012; Maquivar et al., 2015). In addition, VDS has an effect on self-cure: the higher the score, the lower the odds of self-cure. This is in agreement with previous reports showing lower odds of clinical cure in treated cows with higher VDS (Kaufmann et al., 2010b; McDougall et al., 2013). Also, prepartum BCS could play a role in self-cure, given that cows having BCS 2.75 to 3.25 tended to show a higher spontaneous healing rate than cows having BCS <2.75 or ≥3.5; therefore, BCS before parturition would be a risk factor for persistent PVD. Similar results were found by our research group in Holstein dairy cows with metritis (Giuliodori et al., 2013b), where lower probability of cure was associated with prepartum BCS <2.75, low insulin, and high BHB plasma concentration. Heidarpour et al. (2012) reported lower BHB in cured cows 1 wk after therapy than in cows failing to respond to treatment of PVD. Thus, our work and previous work would suggest that NEB could have an effect not only on the odds of PVD but also on its cure.

Risk Factors

The odds of PVD were greater in grazing cows calving in spring, summer, and winter in primiparous cows and in those with abnormal calving. Direct seasonal effects have rarely been reported, and results depend mostly on geographic region and herd management. In a cold climate, with cows mostly housed in barns in Guelph, Canada, fall was the peak season for the incidence of PVD (Erb and Martin, 1978); however,

Table 4. Effect of the $PGF_{2\alpha}$ treatment on cure rate of purulent vaginal discharge[1] and on reproductive performance in grazing Holstein dairy cows (n = 1,103)

		K-M survival[2]				Hazard ratio of pregnancy			Odds ratio		
Item	$PGF_{2\alpha}$	Median	95% CI	%	no./no.	AHR[3]	95% CI	*P*-value	AOR[4]	95% CI	*P*-value
$PGF_{2\alpha}$[5]	No	128	120–137			Referent		0.09			
	Yes	130	120–137			1.13	0.98–1.31				
Logistic regression											
Cure[6]											
$PGF_{2\alpha}$[5]	No			70.9	406/573				Referent		0.26
	Yes			74.3	394/530				1.19	0.88–1.62	
Pregnancy at first AI											
$PGF_{2\alpha}$[5]	No			33.2	190/573				Referent		0.86
	Yes			33.8	179/530				1.03	0.77–1.38	
Pregnancy by 100 DIM											
$PGF_{2\alpha}$[5]	No			32.5	186/573				Referent		0.51
	Yes			29.8	158/530				0.89	0.62–1.28	
Pregnancy by 200 DIM											
$PGF_{2\alpha}$[5]	No			72.8	417/573				Referent		0.17
	Yes			77.2	409/530				1.27	0.90–1.78	

[1]Purulent vaginal discharge (PVD): cows with VDS-1, -2 and -3 at visit 1 postpartum (~30 DIM) were declared as having PVD (VDS-0 = clear discharge, VDS-1 = clear discharge with pus flecks, VDS-2 = muco-purulent not fetid discharge, and VDS-3 = watery, purulent or brown-colored, and fetid vaginal discharge). Cows diagnosed <21 DIM (n = 1,026), having VDS-0 (n = 4,092), or VDS-3 (n = 105) were removed from the statistical analysis [6,326 – (1,026 + 4092 + 105) = 1,103].

[2]K-M survival = Kaplan–Meier survival analysis.

[3]AHR = adjusted hazard ratios.

[4]AOR = adjusted odds ratios.

[5]PGF = cows having a VDS-1 and VDS-2 on visit 1 with odd ear tag number were assigned to receive one dose of 500 μg of $PGF_{2\alpha}$ analog (cloprostenol, Ciclase, Syntex SA, Buenos Aires, Argentina), whereas cows with even ear tag number remained as untreated controls.

[6]Cure was declared when cows have a VDS-0 at re-check.

in our study, with temperate climate conditions and under an outdoor grazing system, the lowest incidence of PVD was recorded in fall. It has been reported that the risk for retained placenta increases during Spring and Summer (Faye et al., 1986), and as this disease has been related to PVD (LeBlanc et al., 2002a; Sheldon et al., 2009), which could help to explain the higher prevalence observed for cows that calved in the hot season. Greater odds for PVD observed in primiparous cows and in cows with abnormal calving is in agreement with previous reports (Ghanem et al., 2002; LeBlanc et al., 2002a; Dubuc et al., 2010a; Gautam et al., 2010; Potter et al., 2010; Giuliodori et al., 2013a), and the explanation might be that primiparity, and especially dystocia, which increases the possibility of trauma, tissue damage, and bacterial contamination, could be leading to PVD (Sheldon et al., 2009). The odds of PVD are also affected by BCS loss peripartum given that cows losing BCS have higher odds for this disease than their counterparts maintaining or gaining BCS. This finding is in agreement with a previous report showing that that prepartum fatty acids and BHB are associated with increased odds of PVD (Dubuc et al., 2010b; Kaufmann et al., 2010a; Giuliodori et al., 2013a). Also, a recent report documents a negative association between prepartum BCS and PVD (Kadivar et al., 2014). Our results also support previous studies showing low BCS at wk −6, 0, 4, and 10 relative to parturition is associated with increased odds of PVD (Hoedemaker et al., 2009). Thus, cows experiencing peripartum BCS loss, high fatty acids prepartum, and high BHB postpartum, indicative of severe NEB or unsuccessful adaptation to NEB, might be reflecting a decrease in DMI at that time (Bell, 1995). In this regard, there is a probable link between peripartal metabolic changes in the dairy cow and immunosuppression (Williams, 2013; Ingvartsen and Moyes, 2015); for example, it has been proposed that NEB may have a detrimental effect on neutrophil functions and, therefore, on uterine health leading to PVD (Hammon et al., 2006), and that deep NEB can be associated with the increased expression of inflammatory genes in the endometrium of postpartum cows (Wathes et al., 2009). Thus, our findings show that primiparity, abnormal calving, and loss of BCS peripartum are all associated with the odds of PVD in grazing dairy cows in temperate climate conditions.

Effect of $PGF_{2\alpha}$

In disagreement with our hypothesis, $PGF_{2\alpha}$ had a tendency to slightly increase the hazard of pregnancy, but it had no effect on the odds of pregnancy by 100 and 200 DIM or of cure of PVD. Our data support the conclusion of meta-analysis studies failing to find any evidence of improvement in the reproductive performance of cows with PVD after treatment with $PGF_{2\alpha}$ (Haimerl et al., 2012, 2013; Lefebvre and Stock, 2012). It was proposed that inducing estrus with $PGF_{2\alpha}$ should clear the uterus and improve uterine health (Kasimanickam et al., 2005). Our study was not designed to test the effect of inducing estrus with $PGF_{2\alpha}$, but other studies also failed to find any benefit after a single injection of $PGF_{2\alpha}$ (LeBlanc et al., 2002b), after 2 injections of $PGF_{2\alpha}$ in cows with PVD (Dubuc et al., 2011b), or even following 3 injections of $PGF_{2\alpha}$ given every other week in cows with subclinical endometritis (Galvão et al., 2009). Additionally, detrimental effects of $PGF_{2\alpha}$ treatment on PFAI in cows without a corpus luteum have previously been reported by other researchers in some studies (LeBlanc et al., 2002b; Dubuc et al., 2011b) and by our group (Mejía and Lacau-Mengido, 2005). Thus, as no clear evidence exists of a positive effect of $PGF_{2\alpha}$ on reproductive performance in grazing Holstein cows, the use of one dose of $PGF_{2\alpha}$ for the treatment of PVD is not supported by our results or others.

CONCLUSIONS

In conclusion, PVD can be diagnosed in cows having pus in their vaginal discharge (VDS-1 to VDS-3) ≥21 DIM, given that VDS ≥1 is associated with lower hazard of pregnancy and longer calving to pregnancy interval (up to 48 d). Risk factors of PVD in grazing dairy cows are calving season, calving problems, and BCS loss. In addition, diagnosis of PVD should not be performed before 28 to 35 DIM because cows experience a high self-cure rate. Finally, as 1 dose of a $PGF_{2\alpha}$ analog (cloprostenol) had a negligible effect on the reproductive performance of grazing dairy cows with PVD, and no effect on the cure of PVD, we do not recommend the therapeutic use of 1 dose of $PGF_{2\alpha}$ in postpartum grazing dairy cows having PVD.

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