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Prevalence of clinical and subclinical endometritis and their impact on reproductive performance in grazing dairy cattle in Buenos Aires Province, Argentina

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'I don't write a book so that it will be the final word; I write a book so that other books are possible, not necessarily written by me'.

Michel Foucault, 1971

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ABBREVIATIONS

AI	artificial insemination
BCS	body condition score
CI	confidence interval
CE	clinical endometritis
dpp	days post partum
exam 1	examination 1
exam 2	examination 2
HR	hazard ratio
IR	interquartile range
OR	odds ratio
PMN	polymorphonuclear cells
RR	relative risk
VDS 0- 3	vaginal discharge score 0- 3

1 INTRODUCTION

Management of the periparturient period is the key challenge of successful dairy farming. In the focus of interest are aspects of uterine health, resumption of ovarian cyclicity after parturition as well as nutrition and housing conditions. The complex interactions at individual and herd level have been the objectives of investigations for years.

In the first two weeks after calving in 80 to 100% of the cows the uterus was found to be contaminated with bacteria (Elliott et al. 1968; Griffin et al. 1974; Sheldon et al. 2002b), which in the normal postpartum period are eliminated within three weeks after calving (Sheldon et al. 2004). Persisting bacterial infection leads to chronic clinical and subclinical endometritis and impaired subsequent reproductive performance (Sheldon et al. 2006).

Clinical endometritis has been defined as an inflammation of the endometrium later than three weeks postpartum. It is associated with a chronic bacterial infection and characterized by mucupurulent or purulent uterine discharge detectable in the vagina (Földi et al. 2006; LeBlanc 2008; Sheldon et al. 2006). Character and odor of vaginal mucus has been shown to be correlated with bacterial load of the uterus and immune response (Williams et al. 2005). Most relevant pathogen bacteria involved are *Arcanobacterium pyogenes, Escherichia coli, Fusobacterium necrophorum* and *Prevotella melaninogenicus* (Huszenicza et al. 1999). Recently, bovine herpesvirus 4 (BoHV-4) has been demonstrated to play a role in the etiology of clinical endometritis as well (Donofrio et al. 2007). Furthermore, in recent years several publications have reported a negative impact of subclinical endometritis on reproductive performance (Barlund et al. 2008; Gilbert et al. 2005; Kasimanickam et al. 2004; Lenz et al. 2007). Subclinical endometritis has been defined as an inflammation of the endometrium in the absence of clinical signs of endometritis (Sheldon et al. 2006). Reported prevalence of clinical endometritis vary from 17% under intensive production conditions (LeBlanc et al. 2002) to 21 to 29% in extensive dairy farming (De la Sota et al. 2008; Mee et al. 2009). Prevalence of subclinical endometritis ranges from 11% to 53% under intensive housing conditions (Barlund et al. 2008; Gilbert et al. 2005; Kasimanickam et al. 2004). Only few information is available about the prevalence of subclinical endometritis under extensive housing conditions. One study revealed a prevalence of 10% (Madoz et al. 2008).

Examination of the vagina and evaluation of vaginal mucus is the most reliable method for the diagnosis of clinical endometritis (LeBlanc et al. 2002; Sheldon and Noakes 1998). Vaginal examination can be performed manually withdrawing vaginal mucus with a gloved hand (Sheldon et al. 2002a), using the metricheck device (McDougall et al. 2007) or by vaginoscopy, inspecting the vaginal lumen (LeBlanc et al. 2002). Recently, Pleticha et al. (2009) found significantly more cows affected with vaginal discharge using the metricheck device, than by examination with a speculum or the gloved hand (47.5 vs. 36.9 and 36.8%).

Diagnosis of subclinical endometritis can be performed by uterine cytology or ultra-sonography. For uterine cytology, samples are obtained by lavage technique (Barlund et al. 2008; Gilbert et al. 2005; Kasimanickam et al. 2005a) or with the cytobrush method (Barlund et al. 2008; Kasimanickam et al. 2004; Kasimanickam et al. 2005a; Kasimanickam et al. 2005b). Cytological smears are evaluated for their proportion of polymorphonuclear cells (PMN) by endometrial cells present in a sample. The threshold value for the proportion of PMN to define subclinical endometritis is controversial and ranges from 4% to 18% (Barlund et al. 2008; Galvao et al. 2009a; Gilbert et al. 2005; Kasimanickam et al. 2004; Raab 2003).

Negative effects of clinical as well as subclinical endometritis on reproductive performance have been reported from several studies, e.g. prolonged days open and a reduced risk of conception at first AI (Barlund et al. 2008; Fourichon et al. 2000; Gilbert et al. 2005; Kasimanickam et al. 2004; LeBlanc et al. 2002; McDougall et al. 2007).

Most data on the prevalence and impact of clinical and subclinical endometritis, however, has been obtained from herds in confinement housing systems in the western industrialized countries resulting in recommendations for dairy farming worldwide. Only little information exists about the relevance of clinical and subclinical endometritis in dairy cows kept in extensive housing systems. It can be hypothesized that prevalence and impact of the diseases vary with different housing, climatic and feeding conditions. The objective of the presented studies was to investigate the prevalence of clinical and subclinical endometritis and their impact on reproductive performance in a pasture-based, extensive dairy farming system in Argentina.

2 RESEARCH ARTICLES

Original research articles about the studies have been published in the peer reviewed Journals `Reproduction in Domestic Animals` (Impact Factor 2009: 1.606) and `Animal Reproduction Science` (Impact factor 2009: 2.107).

Plöntzke, J, Madoz, L, De la Sota, RL, Heuwieser, W and Drillich, M. (2010). Prevalence of clinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina. Reproduction in Domestic Animals.
http://dx.doi.org/ 10.1111/j.1439-0531.2010.01700.x

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http://dx.doi.org/10.1016/j.anireprosci.2010.07.006

The two articles are presented in the format outlined in the guide for authors of the respective journal.

2.1 ARTICLE I

Prevalence of clinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina

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Prevalence of clinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina

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Abstract

The objective of the present study was to evaluate the prevalence of clinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina to compare data with previous reports from herds kept in confinement housing systems. A total of 243 Holstein dairy cows from three commercial dairy farms in Buenos Aires Province (Argentina) were examined for signs of clinical endometritis 18 to 38 days postpartum (dpp) by external inspection and manual vaginal examination. Vaginal discharge was scored into the categories VDS 0 (transparent, clear mucus), VDS 1 (mucupurulent discharge), VDS 2 (purulent discharge), and VDS 3 (purulent discharge with fetid odor). Cows diagnosed with VDS 1 to VDS 3 were regarded as affected with clinical endometritis and cows with VDS 0 as free of clinical endometritis. All cows were reexamined 14 days later following the same examination protocol.

Prevalence of clinical endometritis 18 to 38 dpp was 35% and decreased to 18% at reexamination. Cows with no palpable ovarian structures or periparturient disorders were at higher risk for clinical endometritis. Hazard for pregnancy was significantly lower in cows with purulent or fetid odor discharge compared with reference cows with no discharge (HR = 0.49; P = 0.01), resulting in a lower proportion of cows pregnant by 360 dpp (66% vs 78%). Furthermore, number of services per pregnancy was higher for cows with clinical endometritis than for cows without clinical endometritis (4.4 vs 3.1; P = 0.04). Cows with clinical endometritis were 1.6 times as likely to be culled as cows with no signs of clinical endometritis. In conclusion the prevalence and impact of clinical endometritis in a pasture-based, extensive dairy production system in Argentina were similar to previously published data from dairy farms with confinement production systems

Keywords: clinical endometritis, prevalence, manual vaginal examination, mucus score, grazing dairy cattle

Introduction

The key approach of successful dairy farming is the management of the periparturient period. Aspects of uterine health, resumption of ovarian cyclicity after parturition as well as nutrition and housing conditions are in the focus of interest. The complex interactions at individual and herd level have been object of investigations for years.

In the first two weeks after calving, the uterus of 80 to 100% of the cows is contaminated with bacteria (Elliott et al. 1968; Griffin et al. 1974; Sheldon et al. 2002b). In the normal postpartum period bacteria are eliminated within three weeks postpartum (Sheldon et al. 2004). Persisting bacterial infection leads to metritis and endometritis and impaired subsequent reproductive performance (Griffin et al. 1974; LeBlanc et al. 2002). Metritis is an acute disease in the early postpartum period, associated with redish-brown, watery and fetid vulvar discharge and systemic signs of illness, e.g. fever and depressed attitude. Clinical endometritis can be defined as an inflammation of the endometrium later than three weeks postpartum (Sheldon et al. 2006). It is associated with a chronic bacterial infection and characterized by mucupurulent or purulent uterine discharge detectable in the vagina, with no systemic signs of illness (Földi et al. 2006; LeBlanc 2008; Sheldon et al. 2006). Williams et al. (2005) showed that character and odor of vaginal mucus is correlated with bacterial load of the uterus and immune response. Several risk factors for clinical endometritis such as retained fetal membranes, assisted calving, stillbirth, vulval angle, primparity and male offspring have been identified (Potter et al. 2010). Most relevant pathogen bacteria involved are *Arcanobacterium pyogenes, Escherichia coli, Fusobacterium necrophorum* and *Prevotella melaninogenicus* (Huszenicza et al. 1999). Recently, bovine herpesvirus 4 (BoHV-4) has been demonstrated to play a role in the etiology of endometritis (Donofrio et al. 2007). Prevalence of clinical endometritis reported vary from 17% under intensive production conditions (LeBlanc et al. 2002) to 21 to 29% in extensive dairy farming (De la Sota 2008; Mee et al. 2009).

Examination of the vagina and evaluation of vaginal mucus is the most reliable method for the diagnosis of endometritis (LeBlanc et al. 2002; Sheldon and Noakes 1998). Vaginal examination can be performed manually withdrawing vaginal mucus with a gloved hand, using the metricheck device (a silicon hemisphere screwed on a rod) (McDougall et al. 2007) or by vaginoscopy, inspecting the vaginal lumen (LeBlanc 2002). Recently, Pleticha et al. (2009) found significantly more cows affected with vaginal discharge using the metricheck device, than by examination with a speculum or a gloved hand (47.5 vs. 36.9 and 36.8%).

Negative effects of clinical endometritis on reproductive performance have been shown in several studies. Prolonged days open, decreased first service conception and pregnancy rates and a higher risk of culling were reported (Fourichon et al. 2000; LeBlanc et al. 2002; McDougall et al. 2007). The impact of clinical endometritis on fertility is caused by a delayed uterine involution (Sheldon and Noakes 1998) and altered ovarian activity and hormonal pattern (Huszenicza et al. 1999; Sheldon and Dobson 2004; Sheldon et al. 2002b; Williams et al. 2007).

Most data on the prevalence and effects of clinical endometritis were obtained from herds in confinement housing systems resulting in recommendations for dairy farming worldwide. Only little information exists about the relevance of clinical endometritis in dairy cows kept in extensive housing systems. It can be hypothesized that prevalence and impact of the disease vary with different housing, climatic and feeding conditions. Thus, the objective of this study was to describe the occurrence and factors associated with the prevalence of clinical endometritis diagnosed by manual vaginal examination and its impact on reproductive performance in a pasture-based, extensive dairy production system in Argentina.

Materials and methods

Study farms

The study was conducted in two periods of 4 months (September to December) in two consecutive years (2006 and 2007) on three commercial dairy farms in Buenos Aires Province, Argentina. The surface area of the three dairy farms was 10,000; 6,400 and 236 hectare and numbers of milking cows were 1,545 (farm 1), 1,200 (farm 2) and 96 (farm 3) per herd. All cows were of commercial Holstein breed type. The average daily milk yield was 29, 22 and 18 liter per cow, respectively. Cows were kept in extensive pasture system on grasslands, rotating for fresh pasture lots when grazed, as typical for this region. Calving areas of approximately 0.5 hectare were close to the accommodation of farm personnel. Feed consisted of mixed pastures with alfalfa, festuca, lolium or bromegrass and summer annual grasses, e.g. white and red clover, ryegrass or soybeans. Feeding was supplemented with sorghum or corn silage according to animals' requirements. Concentrates were offered twice daily during milking. Veterinarians visited the farms routinely every week or every second week for fertility service. This included diagnosis of pregnancy and examination of non-pregnant cows by rectal palpation, followed by treatment according to the diagnosis, e.g. induction of estrus with prostaglandin $F_{2\alpha}$ (Bioprost D, BiotayS.A., Grand Bourg, Argentina). After a voluntary waiting period of 40 to 45 dpp, cows were bred by artificial insemination (AI) after observed estrus or by timed insemination after estrus synchronization with prostaglandin $F_{2\alpha}$ and intravaginal progesterone releasing devices (CIDR, Pfizer S. R. L., Buenos Aires, Argentina) as a part of the fertility service by the veterinarians. Pregnancy diagnosis was performed by transrectal palpation of the uterus and its contents later than 35 days after artificial insemination. Relevant data were recorded with a herd-management software (Syscord-Tamb, Lincoln, Argentina, and Protambo Master, Santa Fe, Argentina).

Study design

Two study periods were conducted between September and December in both 2006 and 2007. Farms were visited every 14 days. Enrollment of the cows, clinical examination and evaluation and data collection were performed by the same investigator.

Lactating Holstein cows were enrolled at a first visit (**exam 1**) between 18 and 38 dpp. Animals were reexamined at a second visit (**exam 2**) 14 days later.

Clinical examination

In each cow a clinical examination of the reproductive tract was performed by manual vaginal examination and transrectal palpation of the uterus and the ovaries.

Before manual vaginal examination, the vulva was cleaned with water and an arm-length glove was taken from a dispenser box. The gloved hand (guantes largos descartables, Flex, Buenos Aires, Argentina), lubricated with gel for ultrasound examinations (Gel para ultrasonido, PharmaClean, Buenos Aires, Argentina) was inserted into the vaginal lumen. The open hand was moved in a circular direction to collect vaginal mucus, closed, withdrawn and with spread fingers, vaginal discharge was scored on a scale from 0 to 3. Clear, transparent mucus without any particles of pus was equivalent to a vaginal discharge score of 0 (**VDS 0**), indicating cows not affected by clinical endometritis (**CE-)**. Cows diagnosed with mucupurulent discharge (VDS 1), purulent discharge (VDS 2), and purulent discharge with fetid odor (VDS 3) were regarded as affected by clinical endometritis (**CE+**). Transrectal palpation was performed immediately after vaginal examination. The gloved hand was inserted into the rectum, ovaries were located and palpated with the thumb, while fixing the ovary with the fingers. The presence of follicles, a corpus luteum or the absence of those was recorded. Body condition of all cows was scored according to a 5-point scale with 0.25 intermediary steps (Edmonson et al. 1989). At second visit 14 days later cows were examined following the same examination protocol.

Data

All data were recorded on case report forms on farm and transferred into a spreadsheet (Excel 2003, Microsoft Corporation, Redmond, WA). Data included results from vaginal examination and transrectal palpation findings of the ovaries (corpus luteum, follicle, acyclic). Animal specific data (parity, date of parturition), periparturient disorders (assisted calving, retained fetal membranes, hypocalcaemia, and abortion) and reproductive data (first insemination, last insemination, number of inseminations, outcome of pregnancy diagnosis, and date of culling) were obtained from the herd management software.

Reproductive performance was characterized by days to first service, conception at first AI (number of cows pregnant after first AI divided by number of cows inseminated × 100), days to pregnancy, proportion of cows pregnant (number of cows documented to be pregnant at 360 dpp divided by number of cows enrolled × 100), services per pregnancy (total number of inseminations divided by number of pregnant cows), and proportion of cows culled within 360 dpp. Follow-up period was set at 360 dpp to compensate for a two month interruption of the breeding period in April and May, which is common in Argentina to avoid calvings during the hot summer months.

Statistical analysis

Data were analyzed using SPSS software (Version 16.0, SPSS Inc., Munich, Germany). Prevalence of clinical endometritis was calculated for all cows enrolled and separately for herds. Further analysis was conducted in cows diagnosed with clinical endometritis between 18 and 38dpp (exam 1). Relative risks (**RR**) were calculated for herd prevalence of clinical endometritis, with farm 1 (highest number of animals) as reference. Frequency distribution of VDS showed that only five cows were categorized as VDS 3. As previous reports (LeBlanc et al. 2002) did not reveal differences between purulent and foul smelling discharge on reproductive performance, for further analyses VDS 2 and VDS 3 were collapsed into one category VDS 2+3. Relative risks for the diagnosis of clinical endometritis in cows examined at 18 to 38 dpp and binary logistic regression models for the chance of conception at first AI as outcome variables were calculated. Survival analyses for the chance of insemination and pregnancy within 360 dpp were performed using Kaplan-Meier survival analysis and Cox regression, censoring for cows not inseminated and not pregnant, respectively. For logistic regression models and Cox regression, VDS (with VDS 0 as reference), reported periparturient disorder (0 = no, 1 = yes), parity (0 = primiparous, 1 = multiparous), body condition (0=BCS less than 2.75; 1=BCS 2.75 to 5.00), palpable ovarian structures (0 = corpus luteum or follicle palpable, 1 = no palpable corpus luteum or follicle) and study period (0 = September to December 2006, 1 = September to December 2007) were included as factors. The number of services per conception was analyzed using Kaplan-Meier survival analysis, regarding each service as a "time period" and pregnancy as the principal outcome, as described by Gilbert et al. (2005), log rank test was performed to compare between CE- and CE+ cows. Adjusted RR, odds ratios, hazard ratios, confidence intervals (**CI**), and *P*-values are reported. For RR, logistic regression and survival analyses, CI was set at 95%. For all statistical analyses level of significance was set at $\alpha = 0.05$.

Results

During the two study periods, a total of 243 Holstein cows between 18 and 38 dpp were enrolled in this study, 107 cows in the first study period in 2006 and 136 in the second study period in 2007. Six cows were enrolled in both study periods. Follow up of all cows was completed 360 days after the end of the second observation period in December 2008. Cow parity ranged from 1 to 9, with a median of 2.0 (Interquartile range: 1 – 3).

Prevalence

The overall prevalence of clinical endometritis (**CE**) on the three commercial dairy farms was 34.6%. Stratified by farm, prevalence was 31.9% (60/188), 46.2% (18/39), and 37.5% (6/16), on farm 1, 2 and 3, respectively. The likelihood for clinical endometritis did not differ between farms (farm 2: RR = 1.45, 95% CI = 0.97-2.15; farm 3: RR = 1.18, 95% CI = 0.60-2.29, with farm 1 as reference). Proportion of cows with clinical endometritis was 36.4% (28/77) and 33.7% (56/166) in primiparous and multiparous cows and 37.4% (40/107) and 32.4% (44/136) in the study period of 2006 and 2007, respectively.

The frequency distribution of vaginal discharge scores was 65.4% (159/243), 18.5% (45/243), and 16.0% (39/243) for VDS 0, 1, and 2+3, respectively.

At clinical examination 2, 80.2% (195/243) of the cows enrolled at first visit were reexamined. A total of 19.8% (48/243) of the cows enrolled at first visit were not reexamined because they had died or were sold or other reasons. The overall prevalence of clinical endometritis had decreased to 17.9% (35/195). Of 159 CE- cows at first visit, 72.3% (115/159) remained CE- at reexamination; 6.9% (11/159) were found CE+, while 20.8% (33/159) were not reexamined. Of 84 cows with clinical endometritis at first visit, 28.6% (24/84) remained CE+, 53.6% (45/84) were found CE- and 17.9% (15/84) were not reexamined.

Cows with clinical endometritis had a higher likelihood to be diagnosed with no palpable corpus luteum or follicle at examination than cows without clinical endometritis (57.7% vs 39.4% of cows; P= 0.01, Table 1). Proportion of cows with periparturient disorders was 18.9% (30/159) and 31.0% (26/84) in CE- and CE+ cows, respectively. Periparturient disorder was a significant risk factor for clinical endometritis (RR: 1.50, 95% CI: 1.05 – 2.13, P= 0.03, Table 1).

Impact on reproductive performance

Descriptive reproductive performance traits are shown in Table 2. Conception at first AI was 25.7% (18/70) and 31.9% (43/135) in CE+ and CE- cows, respectively. Further analyses by logistic regression revealed that conception at first AI was not affected by form of VDS (P = 0.21, Table 3). Risk for conception at first service was only numerically lower for VDS 2+3 compared to VDS 0 (OR = 0.37, 95% CI: 0.12 – 1.11, P = 0.08, Table 3).

The outcome of Cox Regression for insemination and pregnancy are reported in Table 4. The chance of pregnancy was affected by VDS (P = 0.03), with VDS 2+3 cows at lower chance for pregnancy than reference cows with VDS 0 (HR = 0.49, 95% CI: 0.28 – 0.84, P = 0.01). The chance of insemination was affected by study period (HR = 1.69, 95% CI: 1.21 – 2.37, P = 0.002). Survival curve for CE+ and CE- cows pregnant is illustrated in Figure 1.

Kaplan-Meier curve shows number of services per pregnancy (Figure 2). Cows with clinical endometritis required significantly more services per pregnancy than CE- cows (4.4 vs 3.1 services, Log rank test: χ^2 = 4.69, P = 0.03). Cows with clinical endometritis were 1.6 times as likely to be culled as CE- cows (RR = 1.57, 95% CI: 1.04 – 2.38, P = 0.04).

Tables and Figures

Table 1.

Relative risk for the diagnosis of clinical endometritis in cows examined 18 to 38 dpp, considering periparturient disorders, parity class, body condition, palpable ovarian structures and study period as factors.

Factor	RR[1]	CI[2]95%	*P*
Periparturient disorders[3]	1.50	1.05-2.13	0.03
Parity class[4]	0.93	0.63-1.35	0.70
BCS group[5]	0.80	0.57-1.13	0.21
Ovarian structure[6]	1.62	1.10-2.37	0.01
Study period[7]	0.87	0.61– 1.22	0.41

[1] RR = Relative risk.
[2] CI = Confidence interval.
[3] Periparturient disorders: 0 = no periparturient disorders; 1 = periparturient disorders.
[4] Parity class: 0 = primiparous; 1 = multiparous.
[5] BCS group: 0=BCS less than 2.75; 1=BCS 2.75 to 5.00.
[6] Ovarian structure: 0 = palpable follicle or corpus luteum; 1 = no follicle or corpus luteum palpable.
[7] Study period: 0 = September to December 2006; 1 = September to December 2007.

Table 2.

Descriptive reproductive performance traits of 243 cows examined 18 to 38 dpp for clinical endometritis.

Trait	Clinical endometritis	
	Yes	No
Number of cows	84	159
Cows inseminated, % (95% CI[1])	83.3 (74.8 – 90.7)	84.9 (79.0 – 90.2)
Median days to first AI (IR[2])	59.0 (53.8 - 66.0)	58.0 (53.0 – 63.0)
Days to first AI (Min – Max)	41 - 144	21 - 203
First service conception rate, % (95% CI)	25.7 (14.8 – 35.2)	31.9 (23.6 – 39.3)
Services per pregnancy	4.4	3.1
Median days to pregnancy (IR)	123.0 (62.0 – 233.0)	125.0 (60.0 – 212.5)
Days to pregnancy (Min – Max)	41 - 359	21 - 356
Cows pregnant, % (95% CI)	65.5 (54.7 – 75.0)	78.0 (71.2 – 84.1)
Cows culled, % (95% CI)	34.5 (23.8 – 44.1)	22.0 (15.3 – 28.1)

[1] CI = Confidence interval.
[2] IR = Interquartile range.

Table 3.

Results of binary logistic regression analysis for the risk of conception at first AI, in cows examined for clinical endometritis between 18 and 38 dpp.

	Conceiving at first AI		
Factor	OR[1]	CI[2] 95%	*P*
Vaginal discharge score[3]			0.21
Score 0		Reference	
Score 1	0.85	0.37–1.95	0.69
Score 2+3	0.37	0.12–1.11	0.08
Periparturient disorders[4]	2.14	0.98-4.67	0.06
Parity class[5]	1.16	0.56-2.41	0.69
BCS group[6]	0.88	0.42-1.85	0.75
Ovarian structure[7]	1.73	0.88-3.40	0.11
Study period[8]	0.78	0.38-1.60	0.50
Constant	0.52		0.32

[1] OR = Odds ratio.
[2] CI = Confidence interval.
[3] Vaginal discharge score: 0 = clear, transparent mucus; 1 = mucupurulent discharge, 2+3 = purulent and fetid odor discharge.
[4] Periparturient disorders: 0 = no periparturient disorders; 1 = periparturient disorders.
[5] Parity class: 0 = primiparous; 1 = multiparous.
[6] BCS group: 0=BCS less than 2.75; 1=BCS 2.75 to 5.00.
[7] Ovarian structure: 0 = palpable follicle or corpus luteum; 1 = no follicle or corpus luteum palpable.
[8] Study period: 0 = September to December 2006; 1 = September to December 2007.

Table 4.

Results of the survival analyses (Cox-Regression) for the risk of insemination and pregnancy in cows examined for clinical endometritis at 18 to 38 dpp.

	Insemination			Pregnancy		
Factor	HR[1]	CI[2] 95%	*P*	HR	CI 95%	*P*
Vaginal discharge score[3]			0.99			0.03
Score 0		Reference			Reference	
Score 1	1.02	0.69–1.51	0.94	0.80	0.51-1.20	0.28
Score 2+3	0.97	0.61–1.54	0.90	0.49	0.28-0.84	0.01
Periparturient disorders[4]	1.06	0.75–1.51	0.75	1.25	0.86-1.81	0.25
Parity class[5]	0.77	0.55–1.08	0.13	0.72	0.51-1.02	0.06
BCS group[6]	1.03	0.73-1.45	0.86	1.06	0.73-1.53	0.76
Ovarian structure[7]	0.81	0.59–1.22	0.21	1.18	0.84-1.66	0.33
Study period[8]	1.69	1.21-2.37	0.002	0.74	0.52-1.05	0.09

[1] HR = Hazard ratio.
[2] CI = Confidence interval.
[3] Vaginal discharge score: 0 = clear, transparent mucus; 1 = mucupurulent discharge, 2+3 = purulent and fetid odor discharge.
[4] Periparturient disorders: 0 = no periparturient disorders; 1 = periparturient disorders.
[5] Parity class: 0 = primiparous; 1 = multiparous.
[6] BCS group: 0=BCS less than 2.75; 1=BCS 2.75 to 5.00.
[7] Ovarian structure: 0 = palpable follicle or corpus luteum; 1 = no follicle or corpus luteum palpable.
[8] Study period: 0 = September to December 2006; 1 = September to December 2007

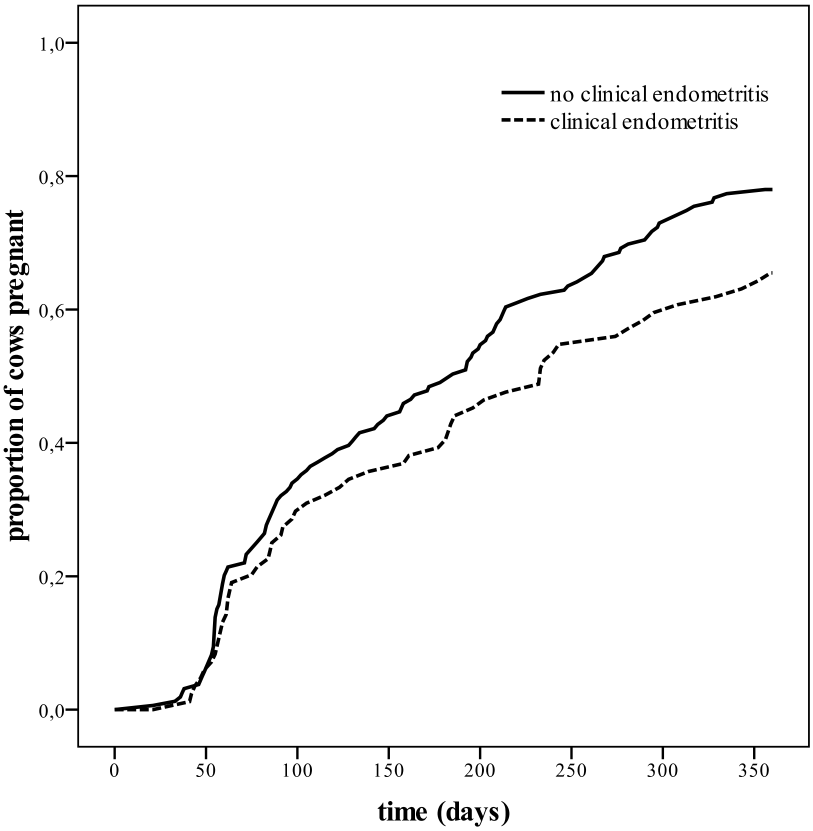

Figure 1.

Kaplan–Meier survival curves for the proportion of cows pregnant by clinical endometritis status (clinical endometritis: n = 124; no clinical endometritis: n = 55). The proportion of cows censored was 34.5% and 22.0% for cows with clinical endometritis and with no clinical endometritis, respectively.

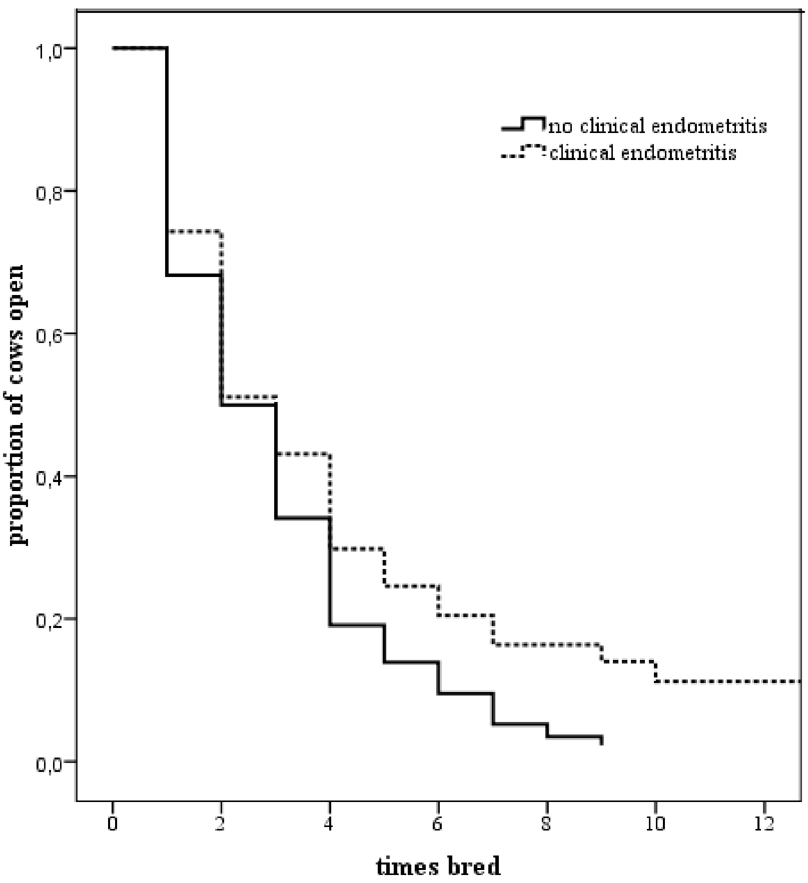

Figure 2.

Survival analysis for times bred per conception by clinical endometritis status (clinical endometritis: n = 124; no clinical endometritis: n = 55). Log rank test: $\chi^2 = 4.69$, $p = 0.03$. The proportion of cows censored was 34.5% and 22.0% in cows with clinical endometritis and with no clinical endometritis, respectively.

Discussion

Prevalence of clinical endometritis

This study is one of few reports on clinical endometritis in Holstein dairy cows under extensive housing conditions in Argentina.

The prevalence of clinical endometritis in the present study was 35%. In the discussion of prevalence of endometritis in different studies the time of examination postpartum, definition criteria of clinical endometritis, i.e. quality of vaginal discharge, and diagnostic technique have to be considered. In the herds in the present study the voluntary waiting period was relatively short. Thus, it was necessary to examine cows earlier postpartum than in some other studies. Examination of cows between 18 and 38 dpp may have resulted in the inclusion of cows that not have completed the uterine involution process.

The prevalence of endometritis in our study was higher than in previous reports. In intensive dairy farming in North America LeBlanc et al. (2002) reported a prevalence of 17% diagnosed by vaginoscopy and cervical diameter between 20 and 33 dpp. Previous Argentinean reports described prevalence of 21 to 22 % between 15 and 62 dpp diagnosed by manual vaginal examination (De la Sota 2008; Madoz et al. 2008). Recently, Mee et al. (2009) reported for 62 pasture-based, seasonal calving dairy herds in Ireland with a total of 5751 cows an average prevalence of endometritis of 29.4%. In that study cows were examined by ultrasound > 14 days postpartum. The overall prevalence of clinical endometritis at the second visit 32 to 52 dpp was 18%. This decrease in prevalence with increasing time postpartum is in accordance with other studies from confinement production systems (Griffin et al. 1974; LeBlanc et al. 2002; Williams et al. 2005) and with findings from Argentina (Mejia and Lacau-Mengido 2005). In the present study, purulent and fetid odor discharge, but not mucupurulent discharge, was associated with a decreased chance for pregnancy compared with cows with no discharge. This result is in accordance with findings from confinement housing conditions (LeBlanc et al. 2002). Contrary, Williams et al (2005) reported for cows with mucupurulent discharge diagnosed at 28 dpp a prolonged calving to conception interval. The predictive value of different uterine discharge categories for subsequent reproductive performance depends on the interval from calving to diagnosis (LeBlanc et al. 2002). Results of the present study indicate that purulent or fetid odor discharge after 18 dpp may identify cows at risk for reduced subsequent reproductive performance in a pasture based extensive housing system. For the same housing conditions, De la Sota et al. (2008) reported impaired reproductive performance for cows diagnosed with any vaginal discharge between15 and 30 dpp. LeBlanc et al. (2002) found that purulent vaginal discharge or a cervical diameter > 7.5 cm after 20 dpp or mucupurulent discharge after 26 dpp identifies cows at risk for reduced subsequent reproductive performance in confinement housing.

The technique of manual vaginal examination applied to diagnose clinical endometritis is simple to perform and with high repeatability (Sheldon et al. 2002a). Pleticha et al. (2009) reported that different techniques of vaginal examination, vaginoscopy, manual examination or examination with the metricheck device can be used interchangeably. Any technique to detect uterine discharge in the vagina, however, may lead to false positive results caused by vaginitis, cervicitis, cystitis or purulent nephritis, or to false negative results. For the diagnosis of clinical endometritis vaginal examination is more sensitive and similarly specific than transrectal palpation (LeBlanc et al. 2002).

Significant differences in prevalence between herds as reported for confinement housing from North America (LeBlanc et al. 2002) were not present in our study. It could be hypothesized that in confinement housing the pathogen density in the calving areas varies more between herds, dependent on various factors such as quality of bedding material or crowding of cows. Further studies on the herd prevalence of clinical endometritis in extensive housing condition, however, with a larger number of farms are necessary to confirm our findings.

Relative risk analysis did not reveal a significant effect of parity or body condition on the occurrence of clinical endometritis. In contrast to our findings, a higher risk for endometritis has been reported for cows in third or higher lactation from confinement housing (LeBlanc et al. 2002) and contrary for primiparous cows from extensive farming (De la Sota 2008). Bell and Roberts (2007) found calving assistance to be highly associated with subsequent uterine infections. In the present study, reported periparturient disorders, including assisted calving had a significant effect on the occurrence of endometritis. Acyclic cows, i.e. cows with no palpable corpus luteum or follicle, were at higher risk for clinical endometritis. It has been demonstrated that cows with vaginal discharge are at higher risk for delayed resumption of ovarian cyclicity or prolonged postpartum luteal phases (Opsomer et al. 2000). A suggested mechanism for anovulatory conditions is that the endotoxin LPS produced by uterine pathogen *Escherichia coli* interfere with the hypothalamus-pituitary–ovarian axis (Battaglia et al. 1999) and enhance luteotropic prostaglandin E2 synthesis (Herath et al. 2009).

Impact of clinical endometritis on reproductive performance

For a successful and economically efficient reproductive management, it is necessary to achieve a high proportion of cows pregnant in an adequate period of time (Dijkhuizen et al. 1985; Plaizier et al. 1997). Therefore, at the end of voluntary waiting period the ovaries should have resumed cyclicity and the uterus should be completely involuted, free of inflammation or infection in order to be capable for conception, implantation and embryo survival. There is general agreement that clinical endometritis impairs subsequent reproductive performance of dairy cows kept under intensive housing conditions (LeBlanc et al. 2002; Sheldon et al. 2006). Recent research has demonstrated that not only clinical but also subclinical endometritis has a negative impact on fertility (Kasimanickam et al. 2004), which could be associated to the production of local cytokines and may help to explain long-term effects of endometritis on fertility (Fischer et al. 2010; Gabler et al. 2009). In several studies, the negative effect of endometritis was demonstrated, although cows received a treatment with prostaglandin F2α or local antibiotics (LeBlanc et al. 2002; Sheldon et al. 2006). Our findings for cows kept on pasture-based, extensive housing conditions showed that the negative impact of clinical endometritis on reproductive performance was similar for most reproductive traits as previously reported for intensive housing systems. Cows with clinical endometritis were at lower chance for pregnancy and required more services to become pregnant than cows without clinical endometritis. A meta-analysis by Fourichon et al. (2000) summarized effects of endometritis on reproductive performance from 24 studies in confinement housing conditions. The impact of endometritis on reproductive performance was quantified as a decrease of 20% for conception at first service, an increase of additional 19 days open, a 31% decrease in risk of pregnancy at 150 dpp, and a 6% decrease in overall pregnancy rate. A large scale study by LeBlanc et al. (2002) in Canada found that cows with clinical endometritis had an additional 28 days open, decreased pregnancy and first service conception rates, and increased number of inseminations per pregnancy. The decrease in first service conception rate of 6.2 percentage points in the present study was less pronounced than described for confinement housing systems of 8.3 percentage points (Fourichon et al. 2000) and 8.1 percentage points (LeBlanc et al. 2002). In a large scale study conducted in a seasonal breeding dairy system in New Zealand (McDougall et al. 2007) cows with clinical endometritis had a reduced first service conception rate, decreased pregnancy rate and an increase from 7 to 27 days from the start of the breeding program to pregnancy. In a study from Argentina, De la Sota et al. (2008) described an increase in days open and a significantly lower proportion of cows pregnant at 100 and 200 dpp in cows with clinical endometritis. Although culling reasons were not recorded, in the present study cows with clinical endometritis were 1.6 times as likely to be removed from herd as cows without clinical endometritis. Similarly, LeBlanc et al. (2002) found cows with clinical endometritis to be 1.7 times more likely to be culled for reproductive failure.

Conclusions

The prevalence and impact of clinical endometritis in a pasture-based, extensive dairy production system in Argentina was similar to previously published data from dairy farms with confinement production systems. Cows with periparturient disorders or no palpable ovarian structures were at higher risk of clinical endometritis. Affected cows were less likely to become pregnant, required more services per conception, and were more likely to be removed from the herd. Further studies should evaluate the infectious agents involved in the development of clinical endometritis and proof treatment recommendations under extensive dairy farming conditions.

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2.2 ARTICLE II

Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina

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Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina

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Abstract

Recently several studies have reported that subclinical endometritis impairs reproductive performance in cattle. Most of the studies were conducted in western industrialized countries under intensive housing conditions. The objective of the present study was to determine the prevalence of subclinical endometritis and its impact on reproductive performance outcomes in clinically healthy postpartum dairy cows in a pasture based extensive dairy farming system in Argentina. Lactating Holstein cows (n = 201) at 18 to 38 days postpartum (dpp) from three commercial dairy farms in Buenos Aires Province, Argentina, were examined for signs of clinical endometritis by external inspection and manual vaginal examination. Only cows without signs of clinical endometritis i.e. no vaginal discharge were enrolled in this study and examined for subclinical endometritis using the cytobrush technique. Cows with ≥ 5% polymorphonuclear cells (PMN) in the cytological sample were regarded as affected by subclinical endometritis. All cows were reexamined 14 days later following the same examination protocol. Prevalence of subclinical endometritis 18 to 38 dpp was 38% and decreased to 19% at reexamination. The proportion of cows pregnant at first service was 29% and proportion of cows pregnant at 360 dpp was 73% and 75% in cows with subclinical endometritis and those without, respectively. The probability of conception at first service, hazards of insemination and pregnancy, respectively were not affected by subclinical endometritis. Primiparous cows had a greater chance for insemination (HR = 0.66; 95% CI = 0.47 to 0.92) and pregnancy (HR = 0.63; 95% CI = 0.45 to 0.90) than multiparous cows. In conclusion subclinical endometritis did not affect reproductive performance outcomes in a pasture-based, extensive dairy farming system in Argentina.

Keywords: subclinical endometritis, prevalence, cytobrush, reproductive performance, grazing dairy cattle.

Introduction

Diagnosing and monitoring subclinical diseases such as subclinical mastitis, ketosis and rumen acidosis has become a substantial part of modern health management in dairy cattle (LeBlanc et al., 2006). In recent years, several publications have reported a negative impact of subclinical endometritis in dairy cattle on subsequent reproductive performance (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004; Lenz et al., 2007).

Subclinical endometritis is defined as an inflammation of the endometrium in the absence of clinical signs of endometritis (Sheldon et al., 2006). Diagnosis of subclinical endometritis can be performed by uterine cytology or ultrasonography. For uterine cytology, samples are obtained by lavage technique (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2005a) or cytobrush method (Barlund et al., 2008; Kasimanickam et al., 2004; Kasimanickam et al., 2005a, b). Cytological smears are evaluated for their proportion of polymorphonuclear cells (PMN) by endometrial cells present in a sample. An increased proportion of PMN is prognostic for impaired subsequent reproductive performance (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004). The threshold value for the proportion of PMN to define subclinical endometritis is controversial and ranges from 4% to 18% (Barlund et al., 2008; Galvao et al., 2009; Gilbert et al., 2005; Kasimanickam et al., 2004; Raab, 2003). The reported prevalence of subclinical endometritis ranges from 11% to 53% (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004) and decreases with increasing days post partum (Gilbert et al., 2005; Madoz et al., 2008). In previous studies, cows diagnosed with subclinical endometritis had prolonged days open and a reduced probability of conception at first AI compared with cows without subclinical endometritis (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2004).

The majority of data, however, is based on studies conducted in dairy herds under confinement housing conditions. There are few data published on the occurrence of subclinical endometritis in South America. Madoz et al. (2008) reported a prevalence of subclinical endometritis of 10.1% in Argentina. It can be hypothesized that prevalence and impact of the disease vary with different housing, climatic and feeding conditions. The objective of the present study was to determine prevalence of subclinical endometritis and its impact on reproductive performance outcomes for a pasture based, extensive, dairy farming system.

Materials and Methods

Study farms

The study was conducted on three commercial dairy farms in Buenos Aires Province, Argentina, between September 2006 and December 2007. The land base of the dairy farms was 10,000 (farm 1), 6,400 (farm 2) and 236 (farm 3) ha and the number of milking cows were 1,545, 1,200 and 96, respectively. The average daily milk yield was 29, 22 and 18 liters per cow respectively and cows were kept on extensive grasslands, rotating for fresh pasture lots. Calving areas of approximately 0.5 ha were close to the accommodation of farm personnel. Feed was composed of mixed pastures (alfalfa, festuca, lolium, brome grass) and summer annual grasses (white and red clover, ryegrass, and soybean) and supplemented with sorghum and corn silage according to animals' requirements. Concentrates were offered twice daily during milking. Veterinarians visited the farms routinely every week or every second week for reproductive examination.

Calving occurred year round except for the hot summer months (January, February) as common for this region. The voluntary waiting period was set at 40 to 45 days post partum (**dpp**). Cows observed in estrus were artificially inseminated. Cows not observed in estrus were submitted to prostaglandin F2α (Bioprost D, Biotay S.A., Grand Bourg, Argentina) and intravaginal progesterone releasing insert (CIDR, Pfizer S. R. L., Buenos Aires, Argentina) synchronisation protocols. Pregnancy diagnosis was performed by transrectal palpation of the uterus and its contents between 35 and 48 days post insemination. Relevant data were managed with herd management software (Syscord-Tamb, Lincoln, Argentina; Protambo Master, Santa Fe, Argentina).

Study design

Two replicates were conducted between September and December in 2006 and 2007. The owners of the farms and the veterinarians were informed about the relevant characteristics of the study and agreed with the study design. The farms were visited every 14 days. Enrolment of cows, clinical examination, sampling and evaluation were performed by the same investigator.

Cows were examined at first visit (**exam 1**) between 18 and 38 dpp. In each cow a clinical examination of the reproductive tract was performed by manual vaginal examination and transrectal palpation of the uterus and the ovaries. Cows with vaginal discharge were diagnosed as affected by clinical endometritis and excluded from the study. Animals without vaginal discharge were enrolled in this study and examined with the cytobrush method. Body condition of all cows was scored according to a 5-point scale (Edmonson et al., 1989). All animals were re-examined at a second visit (**exam 2**) 14 days later following the same examination protocol.

Clinical examination

Before manual vaginal examination the vulva was cleaned with water and an arm-length glove (Guantes largos descartables, Flex, Buenos Aires, Argentina) was lubricated and inserted into the vaginal lumen. Evaluation of the cervix was performed by smooth introduction of the index finger. The open hand was moved in a circular direction in the vagina, withdrawn and with spread fingers vaginal mucus was scored. Clear, transparent mucus without any particles of pus was regarded as indicative for cows not affected by clinical endometritis. In these cows uterine cytological samples were taken by the cytobrush method.

Cytological samples

A small brush (2.2 cm length, 0.5 cm in diameter; Medibrush, Medical Engineering Co. S. A., Buenos Aires, Argentina), mounted on the tip of a metal rod (45 cm length) and protected by a disposable catheter (Condor, Buenos Aires, Argentina) was inserted under transrectal control through the vagina, into the uterine lumen. Inside the uterine body the brush was pushed gently forward until emerging out of the catheter. Cells were collected by rotating the cytobrush while in contact with the uterine wall. For protection, the brush was retracted into the catheter during the passage through the genital tract. Smears were prepared by rolling the brush onto a microscopic slide, dried and fixed (Roby, Argencos S.A., San Martin, Argentina) immediately after collection. In the laboratory of the Institute for Theriogenology of the University of La Plata (UNLP) samples were stained (Tincion 15, Biopur S.R.L., Rosario, Argentina) and evaluated by 400x magnification (Nikon, E200, Japan). A total of 200 endometrial cells were counted to determine the proportion of polymorphonuclear cells (**PMN**). The threshold value for the proportion of PMN indicating subclinical endometritis was set at 5% (Gilbert et al., 2005; Raab, 2003).
Farmers were not informed about the results of cytological examinations, to exclude bias on interventions given to enrolled cows.

Data management and statistical methods

All data were recorded on case report forms on farm and transferred into a spreadsheet (Excel 2003, Microsoft Corporation, Redmond, WA). Data recorded included results of the vaginal and cytological examinations. Animal specific data (parity, date of parturition), periparturient disorders (assisted calving, retained fetal membranes, hypocalcaemia, and abortion), and reproductive performance outcomes (first insemination, last insemination, number of inseminations, outcome of pregnancy exams, and date of culling) were obtained from the herd management software.

Reproductive performance outcome was characterized by days to first AI (days from parturition to first service), conception at first AI (number of cows pregnant after first AI divided by number of cows inseminated × 100), days to pregnancy (days from parturition to pregnancy), proportion of cows pregnant (number of cows documented to be pregnant at 360 dpp divided by number of cows enrolled × 100), services per pregnancy (total number of inseminations divided by number of pregnant cows) and proportion of cows culled within 360 dpp. A follow-up period was set at 360 dpp to compensate for a two month interruption of the breeding period in April and May, which is common in Argentina to avoid calvings during the hot summer months.

Data were analyzed using SPSS software (Version 16.0, SPSS Inc. Munich, Germany). The correlation between dpp and percentage PMN was analyzed by Spearman-Rho test. The relative risk (RR) for the diagnosis of subclinical endometritis was calculated with farm 1 (larger number of cows) as reference. In a next step, RR for the diagnosis of subclinical endometritis at 18 to 38 dpp and binary logistic regression models for the risk of conception at first AI as outcome variables were calculated. Survival analyses for the hazards of insemination and pregnancy within 360 dpp were performed using Kaplan-Meier survival analysis and Cox regression, censoring cows that were not inseminated and not pregnant, respectively. For calculation of RR, logistic regression models and Cox regression, subclinical endometritis (0 = no, 1 = yes), reported periparturient disorders (0 = no, 1 = yes), parity (0 = primiparous, 1 = multiparous), body condition (0 = BCS < 2.75, 1 = BCS ≥ 2.75), and replicate (0 = September to December 2006, 1 = September to December 2007) were included as factors. Adjusted RR, odds ratios, hazard ratios, confidence intervals (**CI**) for HR and OR, medians with interquartile ranges (IR), and *P*-values are reported. For RR, logistic regression and survival analyses, CI was set at 95%. For all statistical analyses level of significance was set at $\alpha = 0.05$.

Results

During the two replicates, a total of 201 cows without any vaginal discharge, were enrolled in the study. Follow-up of all cows was completed 360 days after the end of the second observation period in December of 2008. Cytological samples eligible for final analyses were obtained from 194 cows. In four cows the cytobrush could not be passed through the cervix and three samples were not readable.

Spearmans correlation analysis revealed a negative correlation between dpp and percent of PMN in the cytological sample (r = -0.17; *P* = 0.02). The overall prevalence of subclinical endometritis at exam 1 was 38% (74/194). Herd prevalence was 39% (63/163), 36% (9/25) and 33% (2/6) on farms 1, 2 and 3, respectively. The likelihood for subclinical endometritis did not differ between farms, with farm 1 as reference (farm 2: RR = 0.93, 95% CI = 0.53-1.63; farm 3: RR = 0.86, 95% CI = 0.27-2.72). The overall percentage of enrolled primiparous cows was 29%. Prevalence of subclinical endometritis in primiparous and multiparous cows was 35 and 39%, respectively. The prevalence of periparturient disorders was 21 and 24% in cows with and without subclinical endometritis, respectively. Relative risk analysis did not reveal any significant effect of parity class, periparturient disorders, body condition or replicate on the prevalence of subclinical endometritis (Table 1).

At exam 2 (32 to 52 dpp) 114 cows were reexamined. Overall prevalence of subclinical endometritis decreased from 38% at exam 1 to 19% at exam 2. Of 43 reexamined cows with subclinical endometritis at exam 1, 21% remained ≥ 5% PMN at exam 2, 60% were found with < 5% PMN and 19% were diagnosed with clinical endometritis. Of 71 reexamined cows negative for subclinical endometritis at first visit 80% remained with < 5% PMN at exam 2, 15% were found with > 5% PMN, and 4% were diagnosed with clinical endometritis.

Descriptive statistics of reproductive performance outcomes are presented in Table 2. Conception at first AI was affected by periparturient disorders (OR = 2.41; 95% CI = 1.10 to 5.29; *P* = 0.03), but not by subclinical endometritis (OR = 0.91; 95% CI = 0.45 to 1.87; *P* = 0.80) at exam 1 (Table 3). The hazards for insemination and pregnancy are reported in Table

4. Parity class affected the likelihood for insemination (HR = 0.66; 95% CI = 0.47 to 0.92; *P* = 0.01) and pregnancy (HR = 0.63; 95% CI = 0.45 to 0.90; *P* = 0.01), with a lower hazard for primiparous than for multiparous cows. Subclinical endometritis did not affect the likelihood of insemination or pregnancy (Figure 1).

Tables and Figures

Table 1.

Relative risk analysis for the diagnosis of subclinical endometritis in cows 18 to 38 dpp with periparturient disorders, parity class, body condition and study period as factors.

Factor	RR[1]	CI[2]95%	*P*
Parity[3]	0.89	0.59-1.34	0.57
Periparturient disorders[4]	1.13	0.75-1.70	0.57
BCS group[5]	1.14	0.79-1.64	0.48
Replicate[7]	1.20	0.82-1.74	0.35

[1] RR = Relative risk.
[2] CI = Confidence interval.
[3] Parity class: 0 = primiparous, 1 = multiparous.
[4] Periparturient disorders: 0 = no periparturient disorder; 1 = periparturient disorder.
[5] BCS group: 0 = BCS 0.25- 2.50, 1 = BCS 2.75-5.00.
[7] Replicate: 0 = September to December 2006, 1 = September to December 2007.

Table 2.

Descriptive reproductive performance outcomes of 194 cows examined 18 to 38 dpp for subclinical endometritis by uterine cytology.

Trait	Subclinical Endometritis	
	Yes	No
Number of cows	74	120
Cows inseminated, % (CI[1]95%)	85.1 (76.4 – 92.6)	86.7 (80.2 – 92.3)
Median days to first AI (IR[2])	56.0 (50.0 – 62.0)	56.0 (53.0 – 60.0)
Conception at first AI, % (CI 95%)	28.6 (16.6 – 38.9)	28.8 (19.7 – 37.1)
Median days to pregnancy (IR)	160.0 (58.5 – 237.8)	113.0 (61.5 – 204.0)
Services per pregnancy	3.7	3.4
Cows pregnant, % (CI 95%)	73.0 (62.2 – 82.4)	75.0 (66.8 – 82.3)
Cows culled, % (CI 95%)	27.0 (16.2 – 36.5)	25.0 (16.8 – 32.3)

[1] CI = Confidence interval.
[2] IR = Interquartile range.

Table 3.

Results of binary logistic regression analysis for the risk of conception at first AI, in cows examined for subclinical endometritis at 18 to 38 dpp by uterine cytology, including subclinical endometritis, periparturient disorders, parity, body condition and study period as covariates.

	Conceiving after first AI		
Factor	OR[1]	CI[2] 95%	*P*
Subclinical endometritis[3]	0.91	0.45 – 1.87	0.80
Periparturient disorders[4]	2.41	1.10 – 5.29	0.03
Parity class[5]	1.19	0.56 – 2.52	0.65
BCS group[6]	0.62	0.28 – 1.36	0.23
Replicate[7]	1.31	0.60 – 2.84	0.50
Constant	0.35		0.02

[1] OR = Odds ratio.
[2] CI = Confidence interval.
[3] Subclinical endometrtis: 0 = no subclinical endometritis; 1 = subclinical endometritis.
[4] Periparturient disorders: 0 = no periparturient disorder; 1 = periparturient disorder.
[5] Parity class: 0 = primiparous; 1 = multiparous.
[6] BCS group: 0 = BCS 0.25- 2.50; 1 = BCS 2.75-5.00.
[7] Replicate: 0 = September to December 2006; 1 = September to December 2007.

Table 4.

Results of Cox-Regression for the hazard of insemination and pregnancy in cows examined for subclinical endometritis 18 to 38 dpp by endometrial cytology.

	Insemination			Pregnancy		
Factor	HR[1]	CI[2] 95%	*P*	HR	CI 95%	*P*
Subclinical endometritis[3]	0.94	0.69-1.29	0.70	0.92	0.66-1.30	0.65
Periparturient disorders[4]	0.98	0.68-1.42	0.92	1.07	0.72-1.59	0.74
Parity class[5]	0.66	0.47-0.92	0.01	0.63	0.45-0.90	0.01
BCS group[6]	1.30	0.92-1.82	0.13	1.27	0.87-1.86	0.21
Replicate[7]	1.27	0.92-1.76	0.15	0.66	0.47-0.95	0.02

[1] HR = Hazard ratio.
[2] CI = Confidence interval.
[3] Subclinical endometrtis: 0 = no subclinical endometritis; 1 = subclinical endometritis.
[4] Periparturient disorders: 0 = no periparturient disorder; 1 = periparturient disorder.
[5] Parity class: 0 = primiparous; 1 = multiparous.
[6] BCS group: 0 = BCS 0.25-2.50; 1 = BCS 2.75-5.00.
[7] Replicate: 0 = September to December 2006; 1 = September to December 2007.

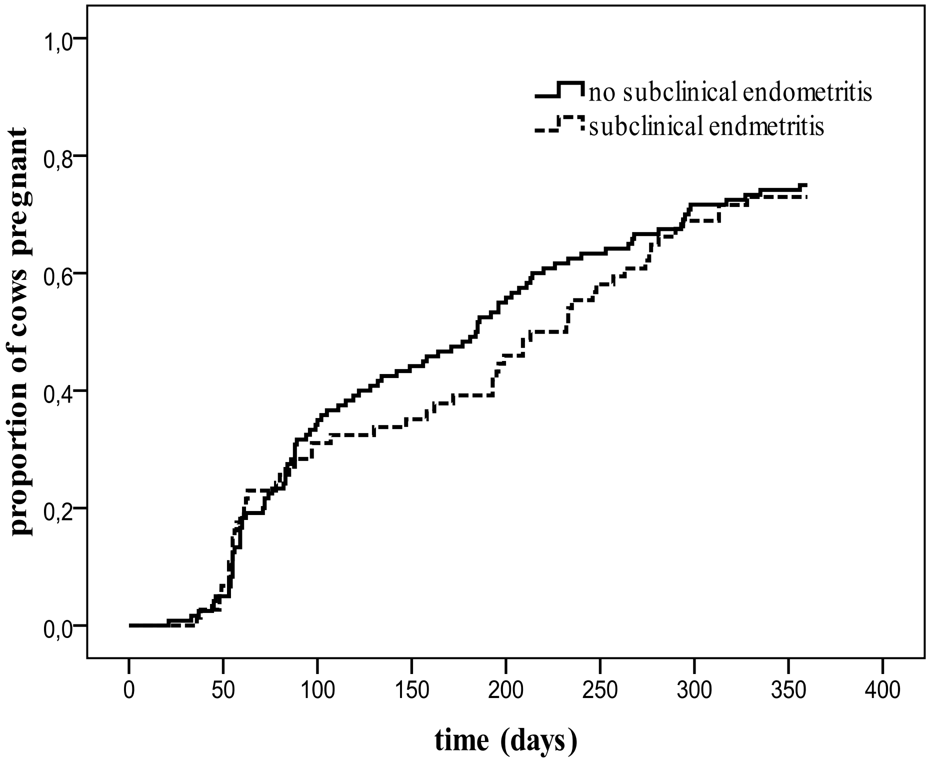

Figure 1.

Kaplan-Meier survival curves for proportion of cows pregnant by subclinical endometritis status. The proportion of cows censored was 27 and 25% for cows with and without subclinical endometritis, respectively.

Discussion

Prevalence of subclinical endometritis

The present study is one of the first studies on the prevalence of subclinical endometritis and its impact on reproductive performance in clinically healthy postpartum dairy cows in a pasture based extensive housing system.

The overall prevalence of subclinical endometritis in this study was 38% in a pasture based dairy farming system. That result is in accordance with previously published data from confinement housing systems. Kasimanickam et al. (2004) diagnosed subclinical endometritis by endometrial cytology in clinically healthy dairy cows and described a prevalence of 35% and 34% for 20 to 33 dpp and 34 to 47 dpp, respectively. Gilbert et al. (2005) reported a prevalence of 53% in cows examined 40 to 60 dpp. The timeframe chosen by Gilbert et al. (2005) is overlapping with the second visit of our study, for which the prevalence was 19%. The authors observed a large range of prevalence varying from 37 to 74% between 5 herds. In our study we did not find significant differences between the three herds. Gilbert et al. (2005) performed endometrial cytology by uterine lavage in cows without previous vaginal examination for uterine discharge. Therefore it is likely that some cows with clinical endometritis were enrolled in that study. Madoz et al. (2008) reported a prevalence of subclinical endometritis in Argentina of only 10.1%. In that study, however, cows were examined later (i.e. 21 to 62 dpp) with the majority of cows examined after 38 dpp and the PMN threshold used to identify cows with subclinical endometritis by endometrial cytology was 18% (21 to 33dpp), 10% (34 to 47dpp) and 5% (≥ 48dpp) as proposed by Kasimanickam et al. (2004). Using those thresholds in our data set prevalence of subclinical endometritis would have been 18% and 9% at first (18 to 38 dpp) and second (32 to 52 dpp) examination, respectively.

Prevalence at the second visit was less than at first visit. Several studies on dairy cows (Gilbert et al., 2005; Kasimanickam et al., 2004; Kasimanickam et al., 2005a), beef cows

(Santos et al., 2009), and zebu-friesian crossbred cows (Bacha and Regassa, 2009) are in agreement with our findings that the prevalence of subclinical endometritis decreased with increasing time post partum. Interestingly, 10% of the cows were found with signs of clinical endometritis at reexamination. Similar findings were reported by Kasimanickam et al. (2004), who diagnosed 9% of cows with uterine discharge within 24 hours after a cytobrush examination. It remains unclear if these cows were affected by clinical endometritis, but vaginal discharge was not identified at first examination (Pleticha et al., 2009), or if examination by cytobrush itself caused clinical endometritis. Kaufmann et al. (2009) showed that the collection of the samples with the cytobrush did not have an effect on conception after first AI.

Relative risk analysis did not reveal an association between parity, body condition or periparturient disorders on the occurrence of subclinical endometritis. In contrast, Kasimanickam et al. (2004) reported that cows with periparturient events (i.e. twins, assisted calving, retained placenta) were three times more likely to be diagnosed with subclinical endometritis. Recently, Santos et al. (2009) described that beef cows with twin calves have a higher risk for subclinical endometritis. Additional risk factors for subclinical endometritis are decreased dry matter intake prepartum, elevated prepartum plasma NEFA concentrations, and impaired PMN function prior to calving (Hammon et al., 2006).

Threshold values from 4% to 18% PMN have been suggested to define subclinical endometritis by uterine cytology (Barlund et al., 2008; Gilbert et al., 2005; Kasimanickam et al., 2005a; Raab, 2003; Santos et al., 2009). Recent studies on endometrial mRNA expression profiles reported a dys-regulated cytokine and prostaglandin profile in cows with ≥ 5% PMN in the endometrial sample (Fischer et al., 2010; Gabler et al., 2009) supporting the assumption that 5% PMN is a suitable threshold for the diagnosis of subclinical endometritis. These results demonstrate that biomolecular techniques may be appropriate to help to define subclinical endometritis in the future.

Impact on Reproductive performance

In accordance with recently published findings in beef cattle (Santos et al., 2009), an impact of subclinical endometritis on reproductive performance was not observed in the present study for grazing dairy cows in an extensive housing system. Days to first service, conception at first AI, days open, proportion of cows pregnant and services per pregnancy did not differ between cows with and without subclinical endometritis. In contrast, Madoz et al. (2008) reported an increase of 52 days open and a reduced proportion of pregnant cows at 120 dpp. In that study the time frame for cytological examination varied widely (21 to 62 dpp), with the majority of cows being examined after 38 dpp and three PMN thresholds based on dpp (18%, 10%, 5%) were used as described by Kasimanickam et al. (2004). The same authors observed a decreased proportion of cows pregnant, increased days open (29 and 62 days), and a reduced conception at first AI for cows with subclinical endometritis kept under intensive farming conditions (Kasimanickam et al., 2004). Applying the threshold of Kasimanickam et al. (2004) to our data set, no difference was observed for time to pregnancy by Kaplan-Meier survival analysis between cows with or without subclinical endometritis (log rank test: $\chi 2 = 0.59$, $P = 0.44$). Similar to the present study, an effect on days to first insemination has not been reported by Kasimanickam et al. (2004).

Although the prevalence of subclinical endometritis was similar to reports from confinement housing systems a negative effect on reproductive performance outcomes as previously reported (Barlund et al., 2008; Galvao et al., 2009; Gilbert et al., 2005; Kasimanickam et al., 2004; Madoz et al., 2008) was not observed. It can be speculated that diminished bacterial load in the uterus or a more effective immune system for cows in extensive, pasture-based systems did improve self cure rate compared to cows in confinement housing systems. Furthermore, the effects of other factors such as feeding, the design of calving areas, management practices such as synchronization of estrus should be evaluated by specifically designed studies.

Conclusion

The prevalence of subclinical endometritis in a pasture-based, extensive dairy production system in Argentina was similar to previously published data for confinement production systems. In this study, however, subclinical endometritis did not have a negative impact on reproductive performance outcomes.

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3 DISCUSSION

Chronic clinical endometritis is highly prevalent in the postpartum dairy cow, impairing subsequent reproductive performance outcomes. Causes, diagnosis, definitions and treatment are in the focus of research since decades. Since a couple of years furthermore subclinical endometritis has been described to have a negative impact on subsequent reproductive performance. Mainly, studies have been conducted with cows kept under confinement housing conditions in the western industrialized countries, leading to recommendations for dairy farming worldwide. Few studies have been conducted to describe occurrence and impact of chronic clinical and subclinical endometritis under extensive production conditions.

The objective of the presented studies was to determine prevalence of clinical and subclinical endometritis and their impact on reproductive performance in dairy cows kept in pasture based extensive housing systems in Argentina.

3.1 Prevalence of clinical and subclinical endometritis

The prevalence of clinical endometritis between 18 and 38 dpp diagnosed by manual vaginal examination in the presented study was 35%. To discuss prevalence of clinical endometritis defining criteria, i.e. quality of vaginal discharge, the time of examination postpartum and diagnostic technique have to be considered. In the presented study the prevalence of clinical endometritis between 18 and 38 dpp was higher than in previous reports. In intensive dairy farming in North America LeBlanc et al. (2002) reported a prevalence of 17% diagnosed by vaginoscopy and cervical diameter between 20 and 33 dpp. Previous Argentinean reports described prevalence of 21 to 22 % between 15 and 62 dpp diagnosed by manual vaginal examination (De la Sota et al. 2008; Madoz et al. 2008). Recently, Mee et al. (2009) reported for 62 pasture-based, seasonal calving dairy herds in Ireland with a total of 5751 cows an average prevalence of endometritis of 29%. In that study cows were examined by ultrasound > 14 days postpartum.

At second examination 32 to 52 dpp the overall prevalence of clinical endometritis was 18%. This decrease in prevalence with increasing time postpartum is in accordance with other studies from confinement production systems (Griffin et al. 1974; LeBlanc et al. 2002; Williams et al. 2005) and with findings from Argentina (Mejia and Lacau-Mengido 2005).

In the presented study, purulent and fetid odor discharge, but not mucupurulent discharge, was associated with a decreased chance for pregnancy compared with cows with no discharge. This result is in accordance with findings from confinement housing conditions (LeBlanc et al. 2002). Contrary, Williams et al. (2005) reported for cows with mucupurulent discharge diagnosed at 28 dpp a prolonged calving to conception interval. The predictive value of different uterine discharge categories for subsequent reproductive performance depends on the interval from calving to diagnosis (LeBlanc et al. 2002). Results of the presented study indicate that purulent or fetid odor discharge after 18 dpp may identify cows at risk for reduced subsequent reproductive performance in a pasture based extensive housing system. For the same housing conditions, De la Sota et al. (2008) reported impaired reproductive performance for cows diagnosed with any vaginal discharge between 15 and 30 dpp. LeBlanc et al. (2002) found that purulent vaginal discharge or a cervical diameter > 7.5 cm after 20 dpp or mucupurulent discharge after 26 dpp identifies cows at risk for reduced subsequent reproductive performance in confinement housing.

The overall prevalence of subclinical endometritis in clinically healthy cows between 18 and 38 dpp was 38% in the presented study. In the discussion of the prevalence of subclinical endometritis the time post partum at examination and the PMN threshold to identify subclinical endometritis have to be considered. Madoz et al. (2008) reported a prevalence of subclinical endometritis of 10.1% in Argentina. In that study cows were examined later (i.e. 21 to 62 dpp) than in the presented study and the PMN threshold to identify cows with subclinical endometritis by endometrial cytology was 18% (21 to 33 dpp), 10% (34 to 47 dpp) and 5% (≥ 48 dpp) as suggested by Kasimanickam et al. (2004). Using those thresholds in our data prevalence of subclinical endometritis would have been 18% and 9% at first (18 to 38 dpp) and second (32 to 52 dpp) examination, respectively. Kasimanickam et al. (2004) diagnosed subclinical endometritis by endometrial cytology in clinically healthy cows and reported a prevalence of 35% and 34% at 20 to 33 and 34 to 47 dpp, respectively. Gilbert et al. (2005) found a prevalence of subclinical endometritis of 53% in cows examined 40 to 60 dpp using a PMN threshold of 5%, as in the presented study. The timeframe in that study was overlapping with the second visit of our study (i.e. 32 to 52 dpp), where the prevalence was 19%. Gilbert et al. (2005) performed endometrial cytology by uterine lavage without previous vaginal examination for uterine discharge. Therefore it is likely that some cows with clinical endometritis were enrolled. Prevalence of subclinical endometritis at the second examination was lower than at first examination. In agreement with the findings of the presented study, decreasing prevalence with increasing time post partum has been reported in several studies in dairy cows (Gilbert et al. 2005; Kasimanickam et al. 2004; Kasimanickam et al. 2005a), beef cows (Santos et al. 2009), and zebu-friesian crossbred cows (Bacha and Regassa 2009). Interestingly, in the presented study 10% of the cows without signs of clinical endometritis at first examination were found with signs of clinical endometritis at reexamination. Similarly Kasimanickem et al. (2004) observed 9% of cows with uterine discharge within 24 hours after a cytobrush examination. It remains unclear if these cows were affected by clinical endometritis, but had not been detected with vaginal discharge at first examination, or if examination with the cytobrush itself caused clinical endometritis. Kaufmann et al. (2009), however, showed that the collection of the samples with the cytobrush had no effect on conception after first AI.

Contrary to previous reports from confinement housing systems (Gilbert et al. 2005; LeBlanc et al. 2002), no significant differences in the prevalence of clinical and subclinical endometritis have been found between herds in the presented studies. It could be hypothesized that in confinement housing the pathogen density in the calving areas varies more between herds, dependent on various factors such as quality of bedding material or crowding of cows. It has to be mentioned that herd size varied widely in the presented studies, thus further studies with a larger number of farms are necessary to confirm this findings.

3.2 Risk factors

In the transition period numerous changes in the cows` organism e.g. metabolic, energetic or hormonal, changes in the cows` environment e.g. group, food, routine and variables of calving as calving assistance or twins can contribute to following development of disease. An optimal management of risk factors can help to prevent disease and enhance performance. Several risk factors associated with the development of clinical and subclinical endometritis are known, e.g. metabolic disorders, retained fetal membranes, calving assistance, twins, and parity. In the presented studies the factors body condition, parity (primiparous vs multiparous) and periparturient disorders (assisted calving, retained fetal membranes, hypocalcaemia, and abortion) have been included in the analysis. The influence of ovarian activity (presence of a corpus luteum or follicle, or absence of those) at time of examination on clinical endometritis has been evaluated furthermore.

The occurrence of clinical endometritis in the presented study was not affected by parity or body condition. In contrast to this findings, a higher risk has been described for cows in the third or higher lactation from intensive dairy farming (LeBlanc et al. 2002) and contrary for primiparous cows in extensive farming conditions (De la Sota et al. 2008). Bell and Roberts (2007) reported that calving assistance is highly associated with the occurrence of uterine infections postpartum. In the presented study, reported periparturient disorders, including assisted calving had a significant effect on the occurrence of clinical endometritis. Acyclic cows, i.e. cows without palpable corpus luteum or follicle at examination, were at higher risk to be diagnosed with clinical endometritis. In cows with vaginal discharge a higher risk for delayed resumption of ovarian cyclicity or prolonged postpartum luteal phases has been demonstrated (Opsomer et al. 2000). A suggested mechanism for postpartum anovulatory conditions is that endotoxin produced by the uterine pathogen *Escherichia coli* interfere with the hypothalamus-pituitary-ovarian axis (Battaglia et al. 1999) and enhance luteotropic prostaglandin E2 synthesis (Herath et al. 2009).

The occurrence of subclinical endometritis in the presented study was not associated with parity, body condition or periparturient disorders. In contrast, Kasimanickam et al. (2004) found cows in confinement housing with periparturient events, i.e. twins, assisted calving, retained placenta, three times more likely to be diagnosed with subclinical endometritis. Santos et al. (2009) found beef cows with twin calves at higher risk for subclinical endometritis. It can be speculated that for cows in extensive housing systems conditions of feeding, climatic exposure or cow exercise are more impacting than in intensive dairy farming. Those variables may have contributed to the development of disease and have not been included in the analysis. Recently a study from Ethiopia (Bacha and Regassa 2009) found cows with lack of regular exercise at higher risk for subclinical endometritis. Further studies, however, are required to identify risk factors for subclinical endometritis under extensive housing conditions.

Prepartum measurable risk factors for development of clinical and subclinical endometritis have been identified, e.g. decreased dry matter intake, elevated plasma NEFA concentrations, and impaired PMN function (Hammon et al. 2006), but were not included in the analysis because such data were not available.

3.3 Diagnosis

In the presented study manual vaginal examination has been applied to detect uterine discharge in the vaginal lumen. This technique is simple to perform and with high repeatability (Sheldon et al. 2002a). Recently Pleticha et al. (2009) found significantly more cows affected with vaginal discharge using the metricheck device, than by examination with a speculum or a gloved hand (47.5 vs. 36.9 and 36.8%). In the same study treatment with prostaglandin F2α in all examined groups did not result in better reproductive performance outcome for cows examined with the metricheck device compared to the other diagnostic methods. Any technique to detect uterine discharge in the vagina, however, may lead to false positive results caused by vaginitis, cervicitis, cystitis or purulent nephritis, or to false negative results. For the diagnosis of clinical endometritis, however, vaginal examination is more sensitive and similarly specific than transrectal palpation (LeBlanc et al. 2002). Recently, Westermann et al. (2010) found 17 to 29% of the cows to be diagnosed false positive for endometritis by vaginoscopy considering the presence of aerobic uterine pathogens and elevated PMN in endometrial cytology as indicative for endometritis.

For the diagnosis of subclinical endometritis a gold standard has not been established yet. In the presented study subclinical endometritis was diagnosed by determining the proportion of PMN in the endometrial cytological sample, which was obtained with the cytobrush technique in cows without signs of clinical endometritis. Several studies (Bacha and Regassa 2009; Barlund et al. 2008; Gilbert et al. 2005; Kasimanickam et al. 2004; Madoz et al. 2008) demonstrated a predictive value of PMN proportion in endometrial cytology for subsequent reproductive performance. Only few studies, however, described an impact of subclinical endometritis on reproductive performance, when uterine cytology was performed in clinically healthy cows, that were previously examined for signs of clinical endometritis (Bacha and Regassa 2009; Kasimanickam et al. 2004; Madoz et al. 2008).

Different threshold values for PMN, ranging from 4% to 18% have been suggested to define subclinical endometritis by uterine cytology (Barlund et al. 2008; Gilbert et al. 2005;

Kasimanickam et al. 2005a; Raab 2003; Santos et al. 2009). Recent studies on endometrial mRNA expression profiles reported a dys-regulated cytokine and prostaglandin profile in cows with > 5% PMN in the endometrial sample (Fischer et al. 2010; Gabler et al. 2009) supporting the assumption that 5% PMN is suitable threshold for the diagnosis of subclinical endometritis.

3.4 Impact on reproductive performance

For a successful and economically efficient reproductive management, it is necessary to achieve a high proportion of cows pregnant in an adequate period of time (Dijkhuizen et al. 1985; Plaizier et al. 1997). Therefore, at the end of voluntary waiting period the ovaries should have resumed cyclicity and the uterus should be completely involuted, free of inflammation or infection in order to be capable for conception, implantation and embryo survival. Clinical and subclinical endometritis have been shown to impair subsequent reproductive performance in dairy cows kept under intensive housing conditions (Kasimanickam et al. 2004; LeBlanc et al. 2002).

3.4.1 Clinical Endometritis

Findings of the presented study for cows kept under pasture-based, extensive housing conditions demonstrated that the negative impact of clinical endometritis on reproductive performance was similar for most reproductive traits as previously reported from intensive dairy farming. Cows with clinical endometritis had a lower chance to become pregnant than cows without clinical endometritis. Furthermore, cows with clinical endometritis required more services per pregnancy. The decrease in conception after first AI of 6.2 percentage points in the presented study was less pronounced than described for confinement housing systems of 8.3 percentage points (Fourichon et al. 2000) and 8.1 percentage points (LeBlanc et al. 2002). A recent study (Mee et al. 2009) on dairy cows in pasture-based, seasonal calving herds, reported a negative effect of endometritis on conception after first AI. A meta-analysis by Fourichon et al. (2000) summarized effects of endometritis on reproductive performance from 24 studies in confinement housing conditions. The impact of endometritis on reproductive performance was quantified as a decrease of 20% for conception at first service, an increase of additional 19 days open, a 31% decrease in risk of pregnancy at 150 dpp, and a 6% decrease in overall pregnancy rate. A large scale study conducted by LeBlanc et al. (2002) in Canada revealed that cows with clinical endometritis had an additional 28 days open, decreased pregnancy rate and conception after first AI, and increased number of inseminations per pregnancy. In a study conducted in a seasonal breeding dairy system in New Zealand (McDougall et al. 2007) cows with clinical endometritis had reduced conception after first AI, decreased pregnancy rate and an increase in 7 to 27 days from the start of the breeding program to pregnancy. In a study from Argentina, De la Sota et al. (2008) described an increase in days open and a significantly lower proportion of cows pregnant at 100 and 200 dpp in cows with clinical endometritis. In the presented study cows with clinical endometritis were 1.6 times as likely to be removed from herd as cows without clinical endometritis. Similarly, LeBlanc et al. (2002) reported cows with clinical endometritis to be 1.7 times more likely to be culled for reproductive failure.

3.4.2 Subclinical Endometritis

In accordance with recently published findings in beef cattle (Santos et al. 2009) an impact of subclinical endometritis on reproductive performance was not observed in the presented study for dairy cows in a pasture-based housing system. Days to first service, conception after first AI, days open, proportion of cows pregnant and services per pregnancy did not differ between cows with or without subclinical endometritis. In contrast, Madoz et al. (2008) found an increase of 52 days open and a reduced pregnancy rate at 120 dpp under extensive housing conditions. In that study the time frame for cytological examination varied widely (21 to 62 dpp) with the majority of cows being examined after 38 dpp and three PMN thresholds based on dpp (18%, 10%, 5%) as described by Kasimanickam et al. (2004) were used. Kasimanickam et al. (2004) reported a decreased pregnancy rate, increased days open (29 and 62 days), and reduced conception after first AI for cows with subclinical endometritis housed under intensive farming conditions. Applying the threshold of Kasimanickam et al. (2004) to our data set, no difference was observed for time to pregnancy by Kaplan-Meier survival analysis between cows with or without subclinical endometritis (log rank test: $\chi2 = 0.59$, $P = 0.44$). Similar to findings of the presented study, an effect on days to first insemination has not been found by Kasimanickam et al. (2004).

Although the prevalence of subclinical endometritis in the presented study was similar to reports from confinement housing systems a negative impact on reproductive performance as previously reported (Barlund et al. 2008; Galvao et al. 2009b; Gilbert et al. 2005; Kasimanickam et al. 2004; Madoz et al. 2008) has not been observed. It can be speculated if a diminished bacterial load in the uterus or a more effective immune competence for cows in a pasture-based extensive farming systems has improved cure rates compared with cows in confinement housing systems.

3.5 Conclusion

The prevalence of clinical and subclinical endometritis in a pasture-based, extensive dairy production system in Argentina was similar to previously published data from intensive dairy farming. Cows without palpable ovarian structures or periparturient disorders were at higher risk for clinical endometritis. Cows with clinical endometritis were less likely to become pregnant, required more services per conception, and were more likely to be removed from herd than cows not affected by clinical endometritis. Clinical endometritis is a common problem in extensive dairy farming as it has been described for intensive dairy farming negatively impacting subsequent reproductive performance. Subclinical endometritis did not have a negative impact on reproductive performance.

4 SUMMARY

Prevalence of clinical and subclinical endometritis and their impact on reproductive performance in grazing dairy cattle in Buenos Aires Province, Argentina

Several studies have described prevalence and impact of chronic clinical and subclinical endometritis on reproductive performance in dairy cows. Most of these studies have been conducted in western industrialized countries under intensive housing conditions, giving recommendations for dairy farming worldwide. The objective of the present study was to determine the prevalence of chronic clinical and subclinical endometritis and their impact on reproductive performance in grazing dairy cattle in Argentina.

The study was conducted on three commercial dairy farms in Buenos Aires Province, Argentina. A total of 243 Holstein dairy cows were examined for signs of clinical endometritis between 18 and 38 days postpartum (dpp) by manual vaginal examination. Vaginal discharge was scored into the categories VDS 0 (transparent, clear mucus), VDS 1 (mucupurulent discharge), VDS 2 (purulent discharge), and VDS 3 (purulent discharge with fetid odor). Cows diagnosed with VDS 1 to VDS 3 were regarded as affected with clinical endometritis and cows with VDS 0 as free of clinical endometritis. Cows without signs of clinical endometritis (VDS 0) were examined for subclinical endometritis with the cytobrush technique. Cows with ≥ 5% polymorphonuclear cells (PMN) in the cytological sample were regarded as affected by subclinical endometritis. All cows were reexamined 14 days later following the same examination protocol.

Prevalence of clinical endometritis 18 to 38 dpp was 35% and decreased to 18% at re-examination. Cows without palpable ovarian structures at examination or history of periparturient disorders were at higher risk for clinical endometritis. Hazard for pregnancy was lower in cows with VDS 2 and 3 compared with reference VDS 0 (HR = 0.49; *P* = 0.01), resulting in a lower proportion of cows pregnant by 360 dpp (66% vs 78%). Furthermore, number of services per pregnancy was higher for cows with clinical endometritis than for cows with VDS 0 (4.4 vs 3.1; P = 0.04). Cows with clinical endometritis were 1.6 times as likely to be culled as cows with no signs of clinical endometritis.

Prevalence of subclinical endometritis 18 to 38 dpp was 38% and decreased to 19% at reexamination. Subclinical endometritis did not affect the risk of conception after first service, hazards of insemination and pregnancy, respectively.

In conclusion in a pasture-based, extensive dairy farming system in Argentina the prevalence of clinical and subclinical endometritis was similar as previously reported from intensive dairy farming systems. Clinical endometritis is a common problem in extensive dairy farming as it has been described for intensive dairy farming negatively impacting subsequent reproductive performance. Subclinical endometritis had no negative impact on reproductive performance.

5 ZUSAMMENFASSUNG

Prävalenz klinischer und subklinischer Endometritiden und deren Auswirkung auf die Fruchtbarkeit bei extensiv gehaltenen Milchkühen in der Provinz Buenos Aires, Argentinien

In den letzten Jahren berichteten mehrere Studien über das Vorkommen klinischer und subklinischer Endometritiden und deren Auswirkung auf die folgende Fruchtbarkeit bei Milchkühen. Die Mehrzahl dieser Studien wurde in den westlichen Industrieländern unter intensiven Haltungsbedingungen durchgeführt. Über Vorkommen und Auswirkung der Erkrankung in Regionen mit extensiven Haltungssystemen ist nur wenig bekannt.
Die vorliegende Studie hatte das Anliegen, die Prävalenz klinischer und subklinischer Endometritiden sowie deren Auswirkung auf die nachfolgende Fruchtbarkeit bei extensiv gehaltenen Milchkühen in Argentinien zu ermitteln. Die Studie wurde auf drei Milch produzierenden Betrieben in der Provinz Buenos Aires, Argentinien, durchgeführt. Insgesamt 243 Milchkühe der Rasse Holstein Friesian wurden zwischen 18 und 38 Tagen nach der Geburt (dpp) mittels manueller vaginaler Untersuchung auf Anzeichen einer klinischen Endometritis untersucht. Das bei der manuellen vaginalen Untersuchung vorgefundene Sekret wurde in die Kategorien VDS 0 (klar, durchscheinend), VDS 1 (mucupurulent), VDS 2 (purulent) und VDS 3 (purulent, übel riechend) eingeteilt. Kühe mit VDS 1, VDS 2 und VDS 3 wurden als an einer klinischen Endometrtitis erkrankt, Kühe mit VDS 0 als frei von klinischer Endometritis angesehen. Bei nicht an einer klinischen Endometritis erkrankten Tieren (VDS 0) wurde mit der Cytobrush Methode ein Zellabstrich aus dem Uterus zur Diagnose einer subklinischen Endometritis gewonnen. Eine Kuh galt als an einer subklinischer Endometritis erkrankt, wenn in der endometrialen Zellprobe mehr als 5% polymorphkernige neutrophile Granulozyten (PMN) gezählt wurden. Alle Kühe wurden 14 Tage später nach dem gleichen Schema erneut untersucht.

Die Prävalenz klinischer Endometritiden betrug 35% und fiel zur Wiederholungsuntersuchung auf 18%. Das Risiko der Diagnose einer klinischen Endometritis war höher bei azyklischen Kühen ohne palpierbare Follikel oder Gelbkörper zum Untersuchungszeitpunkt als bei zyklischen Tieren. Ebenso erhöhten peripartale Störungen das Risiko der Erkrankung. Kühe mit VDS 2+3 hatten eine geringere Chance tragend zu werden und der Anteil tragender Kühe war geringer in dieser Gruppe nach 360 dpp (66% vs 78%) verglichen mit der Referenzgruppe VDS 0 (HR = 0.49; $P = 0.01$). Kühe mit einer klinischen Endometritis benötigten eine künstliche Besamung mehr um tragend zu werden als Kühe mit VDS 0 (4.4 vs 3.1; $P = 0.04$). Für Kühe mit einer klinischen Endometritis war es 1.6-mal wahrscheinlicher aus der Herde auszuscheiden als für nicht betroffene Herdengenossinnen.

Die Prävalenz subklinischer Endometritiden zwischen 18 und 38 dpp betrug 38% und fiel zur Wiederholungsuntersuchung auf 19%. Subklinische Endometritiden hatten keinen Einfluss auf den Erstbesamungserfolg, Rastzeit und Güstzeit.

Zusammenfassend lässt sich sagen, dass die Prävalenz klinischer und subklinischer Endometritiden unter extensiven Haltungsbedingungen in Argentinien vergleichbar mit der unter intensiven Haltungsbedingungen war. Klinische Endometritiden hatten einen signifikanten Effekt auf die Fruchtbarkeit, nicht jedoch subklinische Endometritiden.

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Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 5. November 2010

Julia Plöntzke