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A clinical approach to determine false positive findings of clinical endometritis by vaginoscopy by the use of uterine bacteriology and cytology in dairy cows

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Abstract

Clinical endometritis in dairy cows is defined as mucopurulent or purulent vulvar discharge 21 days or more after parturition. The diagnosis of clinical endometritis is commonly based on vaginal examination. Techniques to reduce the proportions of false negative findings have been described. This paper discusses a clinical approach to determine the proportion of false positive findings that might occur by vaginal inspection. The consequences of false positive findings in dairy practice are unnecessary or inadequate treatments. In research, incorrect diagnoses have an impact on the interpretation of studies on the diagnosis and treatment of clinical endometritis. The objective of the present study was to compare intrauterine bacteriology and endometrial cytology in cows diagnosed with clinical endometritis with findings obtained by vaginoscopy. Clinical endometritis was defined as mucopurulent or purulent vulvar discharge. On two commercial dairy farms, cows were examined 21 to 28 d postpartum. Uterine samples (n = 230) were collected from cows with clinical endometritis with the cytobrush technique to determine the proportion of polymorphonuclear neutrophils (PMN) and to culture smears for aerobic bacteria. Two threshold values for the proportion of PMN (5 and 18%) were chosen as possible indicators for an inflamed endometrium. Common uterine pathogens *A. pyogenes* and *E. coli* were found in 33.5 and 10.4% of the samples, respectively. With increasing vaginal discharge score, proportion of samples positive for *A. pyogenes* increased significantly. The proportion of cows exceeding the thresholds for PMN increased with vaginal discharge score and the presence of *A. pyogenes*.

Considering only the presence of aerobic uterine pathogens and a proportion of PMN above the threshold values of 5 and 18% as indicative for endometritis, a proportion of 17.3 and 28.5%, respectively, of diagnoses by vaginoscopy were false positive.

Keywords: Clinical endometritis; Diagnosis; Bacteriology; Cytology

1. Introduction

During the first two weeks postpartum several species of bacteria can be isolated from the uterus in more than 90% of the cows [1–3]. Most of the cows eliminate these bacteria spontaneously [1,4]. Infection of the uterus in the early postpartum period with *Escherichia coli* appears to pave the way for subsequent infection with other bacteria or viruses [5–7]. Severe endometrial

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lesions, however, are mainly caused by *Arcanobacterium pyogenes* [8–10], which is the most prevalent bacteria in the late postpartum period [1,5,11] and acts synergistically with anaerobe pathogens, such as *Fusobacterium necrophorum* [3,12,13].

Clinical endometritis in dairy cows is defined as mucopurulent or purulent vulvar discharge 21 d or more after parturition, and not accompanied by systemic signs [14]. The diagnosis is usually based on vaginal examination with a speculum, a gloved hand, or the Metricheck device [15–17]. LeBlanc et al [18] suggested that a cervical diameter >7.5 cm be used as an additional predictor for endometritis and reduced reproductive performance. Despite the fact that transrectal palpation of the uterus is the most common means of diagnosis of endometritis, there is agreement that this method lacks accuracy to identify cows with endometritis and subsequent reduced fertility [11,18,19]. A four-point categorical scoring scheme based on character and odour of the uterine discharge in the vagina 21 d postpartum (dpp) or more has been developed to describe the severity of clinical endometritis [5,14]. Previous studies have reported a relationship between the vaginal mucus character and the presence of *A. pyogenes*, *E. coli*, *F. necrophorum*, and *Prevotella melaninogenicus* [5,11]. Findings in the vagina, however, do not necessarily represent the inflammatory status of the endometrium. Thus, the proportion of false positive and false negative findings is unknown. Recent approaches have focussed on diminishing false negative diagnoses by validation of vaginal examination techniques [16–18,20].

For dairy farmers, false positive diagnoses may result in unnecessary or inadequate treatments causing financial losses. In scientific studies, false positive diagnoses may result in incorrect evaluations of therapeutic interventions for endometritis. Enrollment of cows affected by vaginitis or cervicitis but not endometritis into such a study will result in overestimating or underestimating treatment effects. Incorrect diagnoses might also contribute to a different assessment of the effects of clinical endometritis on reproductive performance in literature [21].

To improve accuracy of diagnosis, new techniques such as uterine cytology have been introduced in bovine gynecology, mainly to detect subclinical endometritis in clinically healthy cows. The cytobrush technique and the low volume uterine lavage can be used to obtain endometrial cells from the uterus [20,22–24]. The proportion of polymorphonuclear neutrophils (PMN) in the total number of endometrial cells is indicative for subclinical endometritis. Different threshold values for the proportion of PMN have been suggested, varying from 5 to 18% [20,22,25,26]. Reports on the use of endometrial cytology for the diagnosis of clinical endometritis, however, are limited to one recent study that described endometrial cytology as the most reliable method of diagnosing endometritis in cattle [25]. Compared with endometrial cytology as the reference diagnostic test, vaginoscopy had a sensitivity of 53.9% and a specificity of 95.4%.

The objective of the present study was to compare the proportion of false positive diagnoses of clinical endometritis identified via vaginoscopy by using bacteriological and cytological findings as reference criteria for endometritis. Specifically we evaluated the relationship between intrauterine bacteriology and cytology in cows with clinical signs of endometritis.

2. Material and methods

2.1. Study farm

The study was conducted between June 2008 and January 2009 on two commercial dairy farms in Brandenburg, Germany, housing a total of 1,900 Holstein cows in free-stall facilities with cubicles, rubber mats and slotted floors. Maternity pens were straw bedded. On both farms, cows were fed a total mixed ration based on corn silage, grass silage, and concentrates. Herd average milk yield was 9,200 kg (fat 4.2%, protein 3.3%) and 9,300 kg per lactation (fat 4.2%, protein 3.3%) in herd A and B, respectively.

2.2. Study design

All cows were examined 21 to 27 dpp by vaginal inspection with a sterilized, single packed metal speculum. The vaginal discharge score (VDS) suggested by Williams et al [5] was used to classify vaginoscopic findings. Clear or translucent mucus was equivalent to VDS 0 for healthy cows. Vaginal discharge score 1 described mucus containing flecks of white or off-white pus, VDS 2 discharge containing less than 50% white or off-white mucopurulent material, VDS 3 discharge composed of more than 50% white or yellow pus.

Cows that had received an intrauterine or systemic antibiotic therapy prior to intrauterine sampling, e.g. for a treatment of acute metritis early postpartum were not included in the study. Cows with VDS 1 to 3 were enrolled in the study and uterine samples were collected with the cytobrush technique. Although it has been described that VDS 1 has no negative impact on reproductive performance, and thus should not be regarded

as indicative for endometritis [18], other studies found that even cows with VDS 0 but subclinical endometritis were affected by reduced fertility. Because this contradiction can not be solved yet, we decided to include VDS 1 in this study.

The cytobrush (Gynobrush, Heinz Herenz, Hamburg, Germany) used for collecting the endometrial samples was 20 mm in length and 6 mm in diameter. It was screwed on a metal rod of 70 cm length and protected by a disposable plastic catheter. The instrument was inserted via the cervix into the uterine body. To protect the brush from contamination the catheter was covered with a disposable plastic sleeve. Inside the uterus, the sleeve was retracted, and the brush was pushed gently forward and rolled along the uterine wall. Thereafter, the brush was retracted into the catheter to prevent contamination during the passage through the genital tract. The brush was rolled onto a sterile glass microscopical slide and stored in transport medium for bacteriological samples (Uni-Ter Amies CLR, Meus, Piove di Sacco, Italy). The slide was immediately fixed on farm and stained (LT-SYS, Labor und Technik, Berlin, Germany) in the laboratory. A total of 300 cells were counted under a microscope (× 400 magnification) to determine the proportion of PMN. The threshold value for the proportion of PMN as indicative for subclinical endometritis is still under discussion [20,22,25], therefore, analyses were performed for two thresholds of 5 and 18%. Because there is no reference on the proportion of PMN in cows with clinical endometritis, we assumed that these thresholds for subclinical endometritis should be the minimum proportions that may be expected in cows with clinical endometritis.

Bacteriological samples were cultured for aerobic bacteria by common methods for bacteriological testings [27,28]. Briefly, material from the brush was streaked onto sheep blood agar plates with a sterile disposable plastic eye. Plates were incubated for 48 h in aerobic conditions before bacterial speciation. Bacteria were identified on the basis of the characteristics of the colony, Gram stain, morphology, haemolysis, biochemical profile, and other standard tests. Plates containing one or more colony-forming units were considered a positive bacterial growth.

Cows with VDS 1 to 3 but with intrauterine samples negative for *A. pyogenes* or *E. coli* and <5 or <18% PMN were regarded as false positive findings.

2.3. Statistical analyses

The correlation between VDS and bacteriological findings, VDS and the proportion of PMN, and between bacteriological findings and proportion of PMN were analyzed by Spearman-Rho test. For analysis of correlation between *E. coli* and VDS and PMN, respectively, samples positive for *A. pyogenes* were excluded. For analysis of correlation between findings of coagulase-negative *Staphylococci* (CNS) or α-haemolytic *Streptococci* and VDS and PMN, respectively, samples positive for *A. pyogenes* or *E. coli* were excluded.

The proportions of samples exceeding the threshold values for PMN of 5 and 18%, respectively, were compared by χ^2-analysis considering intrauterine bacteriological findings. Relative risk ratios were calculated for bacteriological and cytological findings (with the given threshold values) and for false positive findings (defined by intrauterine bacteriology and cytology) in cows with VDS 2 and 3 with VDS 1 as a reference. Adjusted P-values and 95% confidence intervals (CI) are reported.

3. Results

A total of 1,164 Holstein-Friesian cows that had calved between June and November 2008 were examined by vaginoscopy 21 to 27 dpp (herd A = 657 cows, herd B = 507 cows). The prevalence of clinical endometritis was 39.7 and 42.2% in herd A and B, respectively. After withdrawing cows with VDS 0 (n = 690), cows meeting the exclusion criteria, i.e. previous antibiotic treatment (n = 193), and cows in which intrauterine sampling was not feasible (n = 51), a total of 230 cows with VDS 1 to 3 were eligible for final analyses.

3.1. Bacteriology and vaginal discharge score

In 45 samples (19.6%) bacteria were not found. In 37.3%, 29.8%, and 33.0% of bacteriological positive samples 1, 2, or ≥2 species were isolated, respectively. *A. pyogenes* and *E. coli* were found in 33.5 and 10.4% of the samples, respectively. Other frequently isolated bacteria were CNS (41.7%), and α-haemolytic *Streptococci* (33.5%). Further isolated bacteria were *C. bovis* (13.9%), *Bacillus* spp (15.2%), and others (<5%). Bacteriological findings were stratified by VDS 1 to 3 (Table 1).

The correlation between *A. pyogenes* and VDS was significant ($r = 0.4$; $P < 0.001$). Correlations between findings of *E. coli* and VDS ($r = -0.10$; $P = 0.21$), and between findings of CNS or α-haemolytic *Streptococci* and VDS ($r = -0.15$; $P = 0.09$) were not significant.

The relative risk for positive findings of *A. pyogenes* in monoculture or in mixed culture with CNS or α-haemolytic *Streptococci* was significantly greater for cows with VDS 2 or 3 compared with reference group VDS

Table 1
Intrauterine bacteriological findings in cows with vaginal discharge score 1 to 3 at 21 to 27 dpp.

Findings	VDS[a] 1 n = 88 % (n)	VDS 2 n = 77 % (n)	VDS 3 n = 65 % (n)
Bacteriologically positive	79.5 (70)	74.0 (57)	89.2 (58)
A. pyogenes			
in monoculture[b]	4.5 (4)	13.0 (10)	35.4 (23)
with *E. coli*	3.4 (3)	2.6 (2)	3.1 (2)
with CNS[c] or α-haemolytic *Streptococci*	6.8 (6)	14.3 (11)	24.6 (16)
Total	14.8 (13)	29.9 (23)	63.1 (41)
E. coli			
in monoculture[b]	4.5 (4)	1.3 (1)	0.0 (0)
with CNS or α-haemolytic *Streptococci*[d]	8.0 (7)	3.9 (3)	3.1 (2)
Total[d]	15.9 (14)	7.8 (6)	6.2 (4)
CNS			
in monoculture[b]	20.5 (18)	11.7 (9)	7.7 (5)
with *A. pyogenes* or *E. coli*	12.5 (11)	14.3 (11)	15.4 (10)
with α-haemolytic *Streptococci*	21.6 (19)	9.1 (7)	7.7 (5)
Total	55.7 (49)	35.1 (27)	30.8 (20)
α-haemolytic *Streptococci*			
in monoculture[b]	6.8 (6)	14.3 (11)	1.5 (1)
with *A. pyogenes* or *E. coli*	8.0 (7)	13.0 (10)	16.9 (11)
Total[d]	36.4 (32)	36.4 (28)	26.2 (17)

[a] Vaginal discharge score: VDS 1 = mucus containing flecks of white or off-white pus; VDS 2 = discharge containing less than 50% white or off-white mucopurulent material; VDS 3 = discharge composed of more than 50% white or yellow pus.
[b] Monoculture or combination with other bacteria than listed in the table.
[c] CNS: coagulase-negative *Staphylococci* (CNS).
[d] Combinations with bacteria that are listed before are not shown. Therefore numbers of isolates do not sum up to the total number of positive samples for this species.

1 (Table 2). The likelihood for positive findings of CNS was significantly lower in cows with VDS 2 or 3 compared with VDS 1.

3.2. Cytology and vaginal discharge score

In 16 cows, cytological samples had to be withdrawn because of a poor quality of the smears. Thus, a total of 214 samples were included in the final analyses (Table 3). The proportion of cows exceeding the threshold of 5 and 18% PMN was 78.0 and 61.7%, respectively ($P < 0.05$). The correlation between proportion of PMN and VDS was significant ($r = 0.30$; $P < 0.001$). The likelihood for PMN values exceeding the thresholds of 5 and 18% was greater for cows in VDS 2 and 3, respectively, compared with VDS 1 (Table 2).

3.3. Bacteriology and cytology

In 214 endometritic cows, intrauterine samples were analyzed both cytologically and bacteriologically. There was a significant correlation between findings of *A. pyogenes* and the proportion of PMN in endometrial samples ($r = 0.42$, $P < 0.001$). Correlations between proportion of PMN and findings of *E. coli* ($r = -0.09$, $P = 0.28$) and CNS or α-haemolytic *Streptococci* ($r = -0.11$, $P = 0.22$) were not significant.

In *A. pyogenes* positive cows, the proportion of samples exceeding the threshold values for PMN was significantly greater compared with bacteriologically negative cows, or with *A. pyogenes* negative cows but positive for other bacteria (Table 4).

3.4. False positive findings by vaginoscopy

In 17.3 and 28.5% of samples both bacteriologically (i.e. *A. pyogenes* or *E. coli*) and cytologically (i.e. >5% and >18% PMN) findings were negative. These cows were regarded as false positive findings of endometritis by vaginoscopy.

Stratified by VDS and considering a threshold of 5% PMN, 27.7, 13.5, and 7.0% of cows with VDS 1, 2, and 3, respectively, were false positive. The relative risk for a false positive diagnosis in cows with VDS 2 and VDS 3 was significantly lower compared to cows with VDS 1 (Table 5). Using a threshold of 18% PMN, the proportion of false positive findings was 41.0, 28.4, and 10.5% for cows with VDS 1, 2, and 3, respectively. The likelihood for a false positive diagnosis was significantly lower in cows with VDS 3 compared to VDS 1.

4. Discussion

The objective of this study was to analyze the relationships between intrauterine bacteriological and cytological findings and vaginal discharge indicative of clinical endometritis. Specifically we wanted to determine the proportion of false positive diagnoses of endometritis by vaginoscopy, using intrauterine findings as a reference standard.

The range of bacterial species isolated from intrauterine samples agrees with previous reports [3,5]. The pathogenicity of different bacteria has been discussed intensively. There is general agreement, that *A. pyogenes* and *E. coli* are relevant uterine pathogens, *A. pyogenes* being the most frequently isolated species [3,29]. Uterine pathogens, however, were also isolated from the uteri of cows that did not show clinical signs of endometritis [30]. In accordance with previous re-

Table 2
Relative risk ratios for intrauterine bacteriological and cytological findings in cows with vaginal discharge score 2 and 3 at 21 to 27 dpp, with score 1 as a reference.

Factor	VDS[a] 2		VDS 3	
	RRR[b]	95% CI[c]	RRR	95% CI
Bacteriologically positive	0.93	0.79–1.10	1.12	0.98–1.29
A. pyogenes	2.02*	1.10–3.71	4.27*	2.50–7.29
in monoculture[d]	2.87	0.93–8.74	7.79*	2.83–21.42
with *E. coli*	0.76	0.13–4.44	0.90	0.15–5.25
with CNS[e] or α-haemolytic *Streptococci*	2.10	0.81–5.40	3.61*	1.49–8.72
E. coli[f]	0.49	0.20–1.21	0.39	0.13–1.12
in monoculture[d]	0.29	0.03–2.50	—	—
with CNS or α-haemolytic *Streptococci*, no *A. pyogenes*[c]	0.49	0.13–1.83	0.39	0.08–1.80
CNS	0.63*	0.44–0.89	0.55*	0.37–0.83
in monoculture[d]	0.57	0.27–1.20	0.38*	0.15–0.96
with *A. pyogenes* or *E. coli*[f]	1.14	0.53–2.49	1.23	0.56–2.72
with α-haemolytic *Streptococci*	0.42*	0.19–0.95	0.36*	0.14–0.90
α-haemolytic *Streptococci*[e]	1.00	0.67–1.50	0.72	0.44–1.18
in monoculture[d]	2.10	0.81–5.40	0.23	0.03–1.83
with *A. pyogenes* or *E. coli*	1.63	0.65–4.08	2.13	0.87–5.19
≥ 5% PMN[g]	1.29*	1.07–1.55	1.38*	1.15–1.65
≥ 18% PMN	1.41*	1.06–1.87	1.64*	1.26–2.15

[a] Vaginal discharge score: VDS 1 = mucus containing flecks of white or off-white pus; VDS 2 = discharge containing less than 50% white or off-white mucopurulent material; VDS 3 = discharge composed of more than 50% white or yellow pus.
[b] RRR: Relative risk ratio.
[c] CI: Confidence interval.
[d] Monoculture or combination with other bacteria than listed in the table.
[e] CNS: coagulase-negative *Staphylococci* (CNS).
[f] Combinations with other bacteria listed before are not shown. Therefore numbers of isolates do not sum up to the total number of positives.
[g] PMN: Polymorphonuclear neutrophils.
* Values differ from the VDS 1 ($P < 0.05$).

ports *A. pyogenes* was the most prevalent bacteria in our study and positively related with vaginal discharge score [5,11,30]. A correlation did not exist between VDS and the prevalence of other bacteria, including *E. coli*. Although *E. coli* has been described to play a key role in the metritis-endometritis complex [5–7], the number of *E. coli* positive samples was surprisingly small. Samples were not cultured for anaerobe uterine pathogens such as *F. necrophorum*. Thus, possible effects of anaerobes can not be discussed.

Table 3
Intrauterine cytological findings in cows with vaginal discharge score 1 to 3 at 21 to 27 dpp.

Cytological findings	VDS[a] 1 n = 83 % (n)	VDS 2 n = 74 % (n)	VDS 3 n = 57 % (n)
≥ 5% PMN[b]	65.1 (54)	83.8 (62)	89.5 (51)
≥ 18% PMN	47.0 (39)	66.2 (49)	77.2 (44)

[a] Vaginal discharge score: VDS 1 = mucus containing flecks of white or off-white pus; VDS 2 = discharge containing less than 50% white or off-white mucopurulent material; VDS 3 = discharge composed of more than 50% white or yellow pus.
[b] PMN: Polymorphonuclear neutrophils.

CNS and α-haemolytic *Streptococci* have been described as non-pathogens [1,5,29]. Williams et al [5] demonstrated that the intrauterine presence of CNS and α-haemolytic *Streptococci* decreased the risk of endometritis. It remains speculative if the higher prevalence of CNS and α-haemolytic *Streptococci* in the present study in cows with VDS 1 compared to VDS 2 and 3 indicates a protective effect of these bacteria against severe endometritis or if these findings resulted from the absence of *A. pyogenes* in cows with VDS 1. Alternatively, a higher prevalence of *A. pyogenes* in cows with VDS 2 and 3 might have reduced the prevalence of non-pathogenic bacteria. In cows positive for CNS or α-haemolytic *Streptococci* the proportion of samples exceeding the threshold for PMN was similar to cows with no bacterial growth, indicating that these bacteria did not have any negative impact on endometrial immune response. Thus, our findings neither support nor contradict the hypothesis of a preventive effect of CNS or α-haemolytic *Streptococci* as proposed by Sheldon et al [31].

In the past, several attempts have been made to define inflammatory conditions of the uterus in dairy cows

Table 4
Intrauterine bacteriological findings in cows with vaginal discharge at 21 to 27 dpp considering two thresholds for polymorphonuclear neutrophils (PMN).

Result from cytological sample		Results from intrauterine bacteriological samples				
		Negative n = 44	*A. pyogenes* n = 71	*E. coli*, negative for *A. pyogenes* n = 16	CNS[a], negative for *A. pyogenes* or *E. coli* n = 58	α-haemolytic *Streptococci*, negative for *A. pyogenes* or *E. coli* n = 45
≥ 5 % PMN[b]	%	68.2[c]	93.0[d]	68.8[c]	69.0[c,*]	75.0[c,*]
≥ 18 % PMN	%	54.5[c]	84.5[d]	37.5[c]	46.6[c,*]	46.7[c,*]

[a] CNS = coagulase-negative *Staphylococci* (CNS).
[b] PMN = Proportion of polymorphonuclear neutrophils in intrauterine cytological samples obtained by cytobrush at 21 to 27 dpp.
[c,d] Values with different superscripts within the same row differ ($P < 0.05$).
* Values in the same column differ ($P < 0.05$).

[14,18,29]. Uterine cytology has been established to distinguish healthy cows from those with subclinical endometritis by an elevated proportion of PMN in endometrial samples [20,24,25]. Barlund et al [25] described intrauterine cytology as the most reliable technique to diagnose clinical endometritis. Complementary to these results, our study demonstrated a correlation between the proportion of PMN and VDS. To our knowledge, there is only limited information available about the relationship of bacterial findings and the proportion of PMN [32]. Therefore, one objective of the present study was to evaluate the relationship between cytological and bacteriological findings. Our results demonstrated a significant correlation between the proportion of PMN and the presence of *A. pyogenes*. Between PMN and other bacteria isolated from uterine samples correlations were not significant. Results indicate that *A. pyogenes* has a more pronounced effect on cellular uterine response than other bacteria. This information is valuable for the interpretation of intrauterine findings of cows with clinical and subclinical endometritis and is in accordance with reports that *A. pyogenes* causes more severe endometrial lesions than *E. coli* [9,10,33].

Table 5
Relative risk ratios for false positive findings by vaginoscopy compared to intrauterine bacteriology and cytology in cows with vaginal discharge score 2 and 3 at 21 to 27 dpp, with score 1 as a reference.

False positive defined as	VDS[a] 2		VDS 3	
	RRR[b]	95% CI[c]	RRR	95% CI
No pathogens[d], < 5 % PMN[e]	0.49*	0.25–0.96	0.25*	0.09–0.69
No pathogens, < 18 % PMN	0.69	0.44–1.08	0.26*	0.12–0.57

[a] Vaginal discharge score: VDS 1 = mucus containing flecks of white or off-white pus; VDS 2 = discharge containing less than 50% white or off-white mucopurulent material; VDS 3 = discharge composed of more than 50% white or yellow pus.
[b] RRR: Relative risk ratio.
[c] CI: Confidence interval.
[d] No pathogens: Intrauterine samples negative for *A. pyogenes* or *E. coli* at 21 to 27 dpp.
[e] PMN: Polymorphonuclear neutrophils.
* Values differ from VDS 1 ($P < 0.05$).

One challenge in the diagnosis of endometritis is to achieve a high accuracy. Recent research described different approaches for vaginal examination with the objective to minimize false negative findings [15,17,19]. Additionally, endometrial cytology has been established with the intent to decrease the number of false negative findings. Results of these studies became the basis for the generally accepted definition of subclinical endometritis [14]. The proportion of false positive findings of clinical endometritis by means of vaginoscopy, however, remains unclear. The approach in our study was to determine false positive findings by intrauterine bacteriology and cytology. As reviewed by Földi et al [3], a variety of bacteria can be isolated from the uterus of cows affected by endometritis, and some of these bacteria act synergistically with others. Furthermore, it is well known, that in some cases of clinical endometritis uterine pathogens were not found, while in others, pathogens were isolated from clinically healthy cows [30]. This is in accordance with results of our study, in which 20% of the samples were bacteriologically negative. It is unknown, if negative findings resulted from the absence of pathogens or from lacking sensitivity of the diagnostic method. It needs to be determined, whether more sophisticated analytic methods, such as PCR would increase the accuracy of the diagnosis.

Despite these considerations, the absence of uterine pathogens might help to identify false positive findings by vaginoscopy. We have additionally performed cy-

tology to describe an inflammatory process within the endometrium with the intent to increase the likelihood of a correct diagnosis through the combination of two independent diagnostic approaches. Since information is not available regarding the proportion of PMN in cows with clinical endometritis, we used two threshold values marking the range that has been used to define subclinical endometritis [20,22,25]. We regarded cows with vaginal discharge but no uterine pathogens in culture and a proportion of PMN below the threshold for subclinical endometritis as false positive for endometritis. This resulted in a proportion of false positive findings of 17.3 and 28.5%, depending on the threshold for PMN. With increasing vaginal discharge score, the likelihood of false positive findings by vaginoscopy decreased. In veterinary practice, the consequence of a false positive diagnosis is an unnecessary or inadequate treatment. In efficacy studies of a given treatment protocol, false positive findings could result in enrollment of animals not meeting the inclusion criteria or in an incorrect assessment of treatment effects.

The diagnosis of endometritis, however, should not only refer to deviations from physiological data, e.g. increased proportion of PMN or bacteria present in the uterus, but also to the impact of these findings on reproductive performance [18]. It has been demonstrated that a vaginal discharge as described as VDS 1 in the present study does not have a negative impact on reproductive performance [4]. This is contradictory to findings that even cows without any vaginal discharge but increased proportion of PMN, i.e. subclinical endometritis, showed a reduced fertility. A retrospective case definition of endometritis by evaluation of fertility traits is helpful to evaluate diagnostic tools, e.g. vaginoscopy. Several cow and management factors, however, have an impact on fertility. In the present study all cows received different treatments for clinical endometritis (unpublished data). Thus, reproductive performance could not be analyzed with regard to the effect of false positive diagnosis on fertility.

In the ongoing discussion on the most accurate and reliable diagnostic technique for clinical endometritis, this study wanted to illustrate the aspect of false positive diagnoses made by vaginoscopy. It was not the authors' intent to present a new definition of endometritis. The definitive diagnosis of endometritis is based on histological examination of endometrial biopsies. This technique, however, is costly, time consuming, not clinically accessible, and may depress fertility [20,34].

5. Conclusions

Using intrauterine bacteriology and cytology as a reference for endometritis 21 to 27 dpp, 17.3 to 28.5% of cows were diagnosed false positive for clinical endometritis by vaginoscopy. False positive diagnoses should be considered into the evaluation of studies on the diagnosis and treatment of clinical endometritis. The proportion of samples positive for *A. pyogenes* or samples exceeding a threshold for PMN of 5 and 18% increased with vaginal discharge score. There was a significant positive correlation between findings of *A. pyogenes* and PMN, but not between other bacteria and PMN.

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