Endometritis of Dairy Cattle: Diagnosis, Treatment, and Fertility

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Introduction

An important cause of infertility in dairy cattle is uterine contamination, infection and inflammation that occur at the time of calving and persist until the time when the cow should be bred. Most cows have some post-partum uterine contamination and, under normal circumstances, the natural resistance of the uterus eliminates the contamination and inflammation and the uterus recovers. However, if infection remains, it is a cause of inflammation and infertility. The standard treatment for uterine infection is infusion of antibiotics into the uterus. This treatment is widely used in California. The purpose of this research was to determine the incidence of this condition and determine the efficacy of the treatment. In addition, observations were made on the identity of the bacterial contaminants of the uterus, their susceptibility to commonly used antibiotics and the absorption and excretion of antibiotics, including determination of antibiotic residues in milk following infusion.

Survey of Incidence and Efficacy of Treatment

Materials and Methods:

Nine dairies ranging in size from 135 to 800 cows in milk participated in this study. These dairies were located in Southern California, the San Joaquin Valley, and Sonoma-Marin area. They were judged to be well-managed and production was above average. The feeding program on each dairy was examined and found to be adequate and according to recommended standards. All dairies maintained a good record system consisting of cow identification, calving dates, results and date of genital tract examination, breeding date, sire identification and pregnancy examination results. Each dairy had a reporductive herd health program under veterinary supervision consisting of postpartum examination, treatment of uterine disease and pregnancy diagnosis. All dairies except #75 and 82 were on Dairy Herd Improvement Association Program (Table 1).

Table 1

Dairy	Location	Number of Cows	Milk	Fat
74	So. California	360	18,809	693
*75	So. California	710	17,385	610
76	So. California	700	19,593	757
*82	So. California	650	17,385	610
77	San Joaquin	750	19,847	743
78	San Joaquin	800	18,768	707
79	Sonoma-Marin	300	17,800	660
80	Sonoma-Marin	135	19,839	704
81	Sonoma-Marin	180	17,359	674
	Total cows all herds	4585		

* Calculated by average daily production per cow X 305 days

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A major effort in this research involved the measurement of fertility following diagnosis and treatment of postpartum uterine disease. Large numbers were required in order to

have sufficient samples for evaluation. Total number of cows in milk in the participating herds was 4585.

Three practicing veterinarians, one in each district, were involved in the study. Management and veterinary procedures were reasonably similar and typical of California. The veterinary program and management in these herds was not changed for the purpose of this project. During this study, the project veterinarian or an assistant accompanied the practicing veterinarian during **all** fertility examinations and re-examined many of the cows in order to maintain uniformity of records and assure that results were compatible between herds and veterinarians. Records on each cow were obtained by a University staff member accompanying the practicing veterinarian and by abstracting them from the herd breeding records. A total of 3582 cows between 21 and 35 days postpartum (mean 28 days) were examined during the course of the project. Each cow was placed in one of three groups, according to the condition of the uterus, by the practicing veterinarian. This was his normal practice. The veterinarians supervising the fertility programs in herds 74, 75, 76, 79, 80, 81 and 82 determined the condition of the uterus by rectal examination using the following guidelines:

Group A: Normal uterus. Uterine horns 30 mm or less in diameter and less than 5 mm difference between the two uterine horns. There was slight to moderate tone.

Group B: Moderately affected with endometritis. The uterine horns in these cows were between 30 and 40 mm in diameter and there was 5-10 mm difference between the large and the small horns. In some cases there was evidence of a small to moderate uterine discharge. These cows would receive a single intrauterine infusion of antibiotic.

Group C: Cows in this group were considered severely affected. The large uterine horn was greater than 40 mm in diameter. There was more than 5 mm difference between the two horns. The tone of the uterus was abnormal and in some cases fluid could be palpated in the uterus but insufficient to be called pyometra. There was usually a purulent discharge from the vagina and vulva. These cows would receive several uterine infusions of antibiotics.

The cows in herds 77 and 78 were not examined by rectal palpation, but, rather, by speculum examination of the cervix and vagina. In this group, the cows were categorized as follows:

Group A: No discharge. A dry mucosa or clear estrual mucus.

Group B: A small amount of exudate present, usually in the presence of some mucus, and without odor.

Group C: More than 5 ml of exudate present that ranged from thick and creamy to cheesy to thin and red. In some cases there was a fetid odor.

The decision based on speculum examination depended entirely on the contents of the vagina. There is no change in the mucosa of the cervix and vagina associated with endometritis.

For the purposes of this experiment, all cows remained in the original group to which they were assigned.

After Group B cows were identified by the practicing veterinarian, they were assigned to a treatment and control group based on a table of random numbers by the project veterinarian. Those placed in the control group were not treated—those placed in the treatment group received the usual treatment given by that veterinary practitioner. Treatments used were as follows:

Dairies #74, 75, 76 & 82 - 625 mg tetracycline, 25 ml furacin with sufficient water to make 100 ml given as a uterine infusion.

Dairies #77 & 78 - 1,200,000 units procain penicillin and 1.5 gm dihydrostreptomycin in 40 ml of water given as uterine infusion.

Dairies #79, 80 & 81 - 1,800,000 units procaine penicillin and 2.25 gm dihydrostreptomycin in 60 ml water given as uterine infusion.

A computer program designed to store, retrieve and analyze this information was prepared. The information supplied to the computer consisted of cow identification, lactation number, date of most recent parturition, date of postpartum examination, date of treatment, uterine code, breeding date, sire number and pregnancy examination results. Fertility was measured by first service conception rate, by percentage of cows failing to conceive within 150 days of calving, and days open.

Results & Conclusion:

The rate of fertility for the various uterine categories is given in Table II. First service conception rate, percent of cows open after 150 days, and the number of days open are similar for; the normal cows, the total number of cows in Group B and, the treatment and control subdivisions of Group B. There was no difference in fertility between the moderately affected Group B cows and the normal Group A cows, and, furthermore, there was no difference in fertility in those Group B cows that were treated and those Group B cows that were kept as untreated controls. Group C cows which were severely affected had a significant reduction in fertility as indicated by first service conception rate and cows open greater than 150 days ($p < .001$). The mean days open was significantly higher for Group C cows in comparison to Group A ($P < .05$).

Our conclusions from these results are that cows that are severely affected with endometritis have lower fertility. Furthermore, because all of these cows were treated, we must also conclude that the treatment used was not effective

TABLE II

FERTILITY OF COWS IN THE VARIOUS UTERINE GROUPS

Uterine Group	Total cows examined	Total cows used for analysis*	Conceived 1st Service	Percent **	Cows in analysis for days open	Cows open 150 days Postpartum	Percent **	Mean days open
A	1450	1140	498	44 a	937	184	16 a	93.87 c
B	1568	1206	550	46 a	1008	192	16 a	96.37
B_c	631	465	219	47 a	398	63	14 a	95.77
B_t	937	741	331	45 a	610	124	17 a	96.76
C	564	345	111	32 b	246	104	30 b	105.48 d

B - All cows in Group B

B_c - Group B controls

B_t - Group B Treated

* - Total cows examined less those culled during the project

** Statistical comparisons within columns
a - b - $P < .001$
c - d - $P < .05$

TABLE III

DISTRIBUTION OF COWS BY GEOGRAPHIC LOCATION AND DIAGNOSIS (UTERINE GROUP)

Uterine Group	Southern* California		San Joaquin** Valley		Sonoma* Marin		All herds	
A	number of cows	%	number of cows	%	number of cows	%	number of cows	%
A	550 a	26	771 b	74	129 a	30	1450	40
B	1172 a	56	210 b	20	186 a	43	1568	44
C	384 a	18	67 b	6	113 a	26	564	16
Total cows examined	2106		1048		428		8582	

a-b Percentages in a given row with different superscripts indicate statistical significance, P<0.000

* Diagnosis by rectal palpation

** Diagnosis by vaginal speculum

The period of examination was June 16, 1977 to March 1, 1978. There was a total of 4585 cows in the nine participating herds.

in bringing these cows up to normal fertility. Because we did not keep controls in the severely affected group, we cannot tell if the treatment had some beneficial effect.

The numbers of animals falling in a Group A, B and C are given in Table III. The ratios between A, B and C were similar between Southern California and Sonoma-Marin. The ratios between the San Joaquin Valley and Sonoma-Marin and Southern California are distinctly different. This is attributed to the fact that the San Joaquin cows were examined only by vagina speculum, whereas, the other cows were evaluated only by rectal examination and exudate that could be seen in the vulva and on the surface of the vulva. *(See Section on Bacterial and Pathologic Examination of the Uterus.)*

Bacteriologic and Pathologic Examination of the Uterus

Materials and Methods:

During the early stages of the study, 216 cows were selected for bacteriologic and pathologic examination.

These cows were distributed among all of the 9 herds in the study. Selection for biopsy and culture on Group B cows, both treated and control, was based on using all Group B cows that were observed in a single visit to the dairy. This procedure was followed until an excess of 200 cows were examined.

Bacterologic samples were taken with an instrument, similar in design and function to one described earlier.[3] The instrument consisted of two nesting, stainless steel tubes: an outer tube, 6 mm in diameter and 35 cm long, and an inner tube 3 mm in diameter and 45 cm long. A flexible ureteral catheter, 1.65 or 1.98 mm in diameter and 70 cm long was inserted through the inner tube. The tubes were assembled, one inserted inside the other, and the end to be inserted in the uterus was covered with tape. The unit was then sealed in a plastic container and gas-sterilized with ethylene oxide.

The vulva of the cow was wiped clean with paper towels. The vulva was held open and the instrument inserted to the region of the cervix. By rectal manipulation the instrument was passed through the cervix and into the body of the uterus. The inner steel tube was then pushed through the tape and into one horn of the uterus, an additional 3 cm. The catheter was then pushed forward as far as possible until resistance from the uterine mucosa was met and then withdrawn into the inner tube, which was then drawn into the outer tube, at least 6 cm. This protected the inner tube from contamination as the instrument was then withdrawn from the cow.

Blood agar plates were inoculated by first withdrawing the inner steel tube from the posterior end of the outer tube and the urethral catheter from the posterior end of the inner tube. The anterior end of the catheter was then streaked on a blood agar plate. All initial inoculations were done in the field within 45 minutes of sampling and placed in a 37°C incubator with 90 minutes of sampling. A positive control plate was incorporated with each group of field samples.

The culturing technique was validated by sampling series of cows immediately before slaughter and then bringing the genital tract to the laboratory and culturing the uterine contents. In all cases the cervix was plugged prior to slaughter so that the vaginal contamination of the uterine contents did not occur. There was agreement of the antimortem and post-mortem isolates in 18 of 20 cases.

In the laboratory all plates were incubated in a CO_2 enriched aerobic atmosphere. All plates were kept for 10 days. Isolates were identified by bacterial morphology, staining characteristic and appropriate bio-chemical tests. Cultures were classified into two categories: those plates that had one type of isolates (pure culture), and those with more than one isolate (multiple culture). Minimum inhibitory concentrations (MIC) for penicillin, ampicillin, oxytetracycline and nitrofuradantin of the uterine isolates were determined. The method of Erickson[7] was used to determine minimum inhibitory concentration (MIC) for the above antibiotics. The Kirby Baner[8] method was used to determine sensitivity and resistance.

Following cultures, a sample of endometrium was obtained using a human rectal biopsy instrument with a specially constructed, 18 inch shank*. Endometrial tissue was immediately placed in Bouin's solution. The tissue was prepared for histological examination by embedding in parafin, sectioning at 5 microns, and staining with hemotoxilin and eosin.

Results and Conclusions

Bacteria were isolated from 70 fo the 216 samples collected (Table IV). *Corynebacterium pyogenes* was the most frequent isolate. The mixed cultures reported in the Table included *C. pyogenes* in 14 of the 16 cultures. The MIC of antibiotics for bacteria isolated from the uterus in this study are summarized in Table V. All the uterine organisms were susceptible at levels of penicillin and oxytetracycline achieveable in the uterus. *(See levels of Antibiotics Following Uterine Infusion below).* Based on antibiotic susceptibility data, nitrofurazone and dihdrostreptomycin would not be recommended for use as treatment of uterine contaminants.

A total of 207 biopsies were done on cows that were cultured. Fifty-six of these tissues had inflammation of the endometrium (Table IV). The inflammatory infiltrate observed was predominantly mononuclear. Monocytes were observed most frequently and these were associated with a lesser number of plasma cells. Lymphocytes were also present. Most cells were in the zona compacta where they were densely packed in the more severe cases and scattered or localized in foci in milder cases. Many of the tissues had lymphocytic foci which were in varying degrees of formation. The newly formed foci included some immature lymphocytes, which were loosely aggregated. Other foci were well defined and the lymphocytes closely packed. The latter were assumed to be of longer duration. Endometrium with only lymphocytic foci was considered to be in the healing stage.

Neutrophils were present in approximately half of the cases. The heaviest concentration was in the zona compacta just under the epithelium. Some cells were migrating through the epithelium, some were scattered throughout the zona compacta. In the zona glandularis both mononuclear cells and neutrophils were observed. They tended to be peri-glandular and perivascular. A few degenerating glands and fibrous glands were seen. In those sections with heavy concentrations of inflammatory cells in the zona compacta there were many distended glands. In almost every case the epithelium was intact, although the biopsy procedure often partially removes some of the epithelium.

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TABLE IV
RESULTS OF BACTERIOLOGIC EXAMINATION AND ENDOMETRIAL BIOPSY OF 216 COWS IN GROUP B

Organism	Number of Isolations	Number of Biopsies	Number with Lesions	% of cows with bacteria that had Lesions
Corynebacterium pyogenes	16	15	15	100%
Corynebacterium hemolyticum	2	1	1	100%
Mixed culture (included *C. pyogenes*)	16	16	14	87%
Pasturella hemolyticum	7	7	7	100%
Pasturella multocida	2	2	2	100%
Proteus vulgaris	1	1	1	100%
Escherichia coli	5	5	2	40%
Streptococcus sp. and *Zooepidemicus*	11	10	3	30%
Staphylococcus aureus	3	2	1	50%
Isolant died before identification	7	7	4	57%
Sub Total	70	67	50	
No isolation made	146	140	6	4%
TOTAL	216	207	56	

In cases of *Corynebacterium pyogenes, Corynebacterium hemolyticum, Pasturella hemolyticum* and *Pasturella multocida* there was 100% correlation between bacterial isolation and the presence of inflammation in the endometrium. There were six cases in which inflammation was present in the endometrium and no bacterial isolation was made.

The fertility of the cows that were cultured and biopsied is reported in Table VI. Only in the category of "open more than 150 days" was there difference in fertility between those animals that had bacteria in the uterus and those that did not. Conception on first service was the same for those "infected" as those "not infected" and, at the same time, was significantly lower than the total population. This is an indication that the culture and biopsy technique did reduce fertility as determined by first service conception rate. The reason for this was not determined.

A comparison of the percentage of positive cultures found in cows diagnosed by rectal examination and those diagnosed by vaginal examination was made. The results are presented in Table VII. Since there was a high correlation between culture and biopsy results and since a combination of these techniques gives the best definitive

TABLE V
ANTIBIOTIC SENSITIVITIES

Minimum inhibitory concentrations for antibiotics for bacteria isolated from the uterus in this study

Bacteria	µg/ml Penicillin			µg/ml Ampicillin			µg/ml Oxytetracycline			µg/ml Furacin			µg/ml Dihydro-streptomycin
	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range	$\bar{X}$	SD	Range	
Corynebacterium	1.1*	.6	.48 2.0	3.41	7.84	.07 33	20.44	22.28	.25 64	>590			R
Pasteurella	.78	1.15	.19 3.0	.14	.20	.04 .62	25.65	51.27	.46 140	12.83	26.8	31 73	R
Streptococcus	.98	.45	.19 1.75	.43	.38	.04 1.03	27.48	33.18	1.3 75	153.39			R
E. Coli	13.4**	11.51	4.3 30	7.95	14.02	1.03 33	75.54	71.8	6 150	R			R
Staph. aureus	.44	.40	.19 .9	.21	.15	.08 .37	1.42	1.54	.6 3.2	>590			R

* One value >1170 not included.

** One value >580 not included.

R = Resistant

1µ = ∿ 15 International Units

TABLE VI

THE FERTILITY OF THE COWS CULTURED AND BIOPSIED WAS AS FOLLOWS:

	Positive Culture		Negative Culture	
Conceived on 1st service	22/70	31%	44/146	30%
Days Open	82.9		92.5	
Cows Open >150 Days	30/70	43% a	45/146	30% b

a - b, Statistically significant difference, P<0.05

diagnosis, we conclude that the vaginal examination is a more accurate method of determining the presence of endometritis. These results were obtained by different veterinarians in different herds. However, because of the common examination and observation by the University staff veterinarian, it is our judgment that the difference is real.

Levels of Antibiotics Following Uterine Infusion

Materials and Methods:

Samples were assayed for antibiotics by two methods: The Penny cylinder method® (PCM)[9] was used to detect minute amounts of antibotics and the Bennett Plate method® (BPM)[10] was used to detect therapeutic levels of antibiotics. The test organisms for the antibiotic were as follows: *Micrococcus lutea* (ATCC 9341) for penicillin; *Bacillus subtilus* (ATCC 6633) for oxytetracycline; *Bacillus cereus* variety *mycoides* (ATCC 11778) for dihydrostreptomycin. The smallest amount detectable by the PCM assay was .001)g/ml for penicillin, .05)g/ml for oxytetracycline and .1)g/ml for dihydrostreptomycin. Due to the uncertain status of nitrofurones for use in food animals and the lack of a sensitive, microbiological assay, we did not include them in this study. The following dosages for intrauterine infusion of antibiotics and combinations of antibiotics were used in this study:

1. 600 mg oxytetrycycline (Pfizer, Liquimycin 50 mg/cc)
2. 1,000,000 units crystaline potassium penicillin
3. 1,000,000 units procaine penicillin (Pfizer procaine penicillin G)
4. 1,600,000 units procaine penicillin G and 2g dihydrostreptomycin (Pfizer combiotic)
5. 1 g dihdrostreptomycin

Each antibiotic treatment was disolved in 30 cc of sterile, distilled water and infused into the uterus with a plastic inseminating pipette. This volume of fluid was utilized in order to reduce uterine contraction and expulsion of the drug and at the same time to provide sufficient volume to fill the entire lumen of the uterus. Pre-infusion samples were taken. For each antibiotic or combination of antibiotics the following five determinations were made:

A. antibiotics in milk
B. antibiotics in the lumen of the uterus
C. antibiotics in the endometrial tissue
D. antibiotics in urine
E. antibiotics in serum

Non-gravid lactating Holstein-Friesian dairy cows with an involuted uterus and producing greater than 40 pounds of milk per day were used for the Group A determinations. The stage of the estrous cycle was not determined.

TABLE VII

RESULTS OF CULTURE OF THE UTERUS IN RELATION TO METHOD OF CLINICAL EXAMINATION

	Total cows Group B	Number of cows Cultured	Percent of cows cultured	Number of cows Positive	Percent of cows Positive
Rectal Examination	1358	157	11	35	22 a
Vaginal Speculum Examination	210	59	28	35	59 b

a - b, Statistically significance, P 0.05

TABLE VIII

AMOUNT OF ANTIBIOTIC IN MILK FOLLOWING INTRAUTERINE INFUSION

Antiobiotic Treatment and Dose	No. of Samples *	Amount	Milking Post Infusion**
600 mg. Oxytetracycline (Liquamycin Pfizer)	0/70*		
1,600,000 I.U. Penicillin G. 2 g. Dihydrostreptomycin (Combiotic Pfizer)	1/50	.069 µg/ml	1st
1,000,000 I.U. Penicillin G Crystaline Potassium Penicillin G (Squibb)	7/56	.0091µg/ml	1st
		.022 µg/ml	1st
		.053 µg/ml	1st
		.0057µg/ml	1st
		.012 µg/ml	1st
		.0088µg/ml	1st
		trace	
1 g. Dihydrostreptomycin Sulfate (Burns Biotec)	0/56		
1,000,000 I.U. Penicillin G (Procaine Penicillin Pfizer)	0/59		

Penicillin test limit .001 µg/ml
Dihydrostreptomycin test limit .1 µg/ml
Tetracycline test limit .005 µg/ml

* Number positive samples/total cows tested
** All samples collected on the 2nd through the 6th milking were negative

Ten non-lactating, non-gravid dairy cows were used for Group B, C, D and E determinations. These cows had been culled from commercial dairy herds. Their uteri were of normal size and tone. These cows were used repeatedly for these experiments for a six-month period and during this time, no changes of the uteri were observed. The stage of the estrous cycle was not determined.

Determination A — Milk, Table VIII, Table IX

At least 50 cows were infused with each treatment 3-6 hours before milking and samples were taken at 6 subsequent milkings (twice a day milking) (Table VIII). Two animals for each antibiotic treatment had milk samples taken at 10 minutes, 20 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours post-infusion (Table IX). All samples were collected prior to milking and the cow was primed by discarding several streams of milk. Approximately 10 cc of milk was collected for each sample which was then frozen until assayed by the PCM.

Determination B — Antibiotics in the Lumen of the Uterus, Table X

The amount of antibiotic remaining in the lumen of the uterus at various times of post-infusion was determined by

TABLE IX

AMOUNT OF ANTIBIOTIC IN MILK AFTER INTRAUTERINE INFUSION

	Time Post Infusion							
Treatment	0	10"	20"	30"	45"	1 hr	2 hr	3 hr	4 hr
#1	0	0	0	0	0	0	0	0	0
#1	0	0	0	0	0	0	0	0	0
#2	0	0	0	0	0	0	0	0	0
#2	0	0	0	0	0	0	0	0	0
#3	0	0	0	0	0	0	0	0	0
#3	0	0	0	0	0	0	0	0	0
#4	0	.035*	.029	.029	.110	0	0	0	0
#4	0	0	.135	.130	.120	0	.119	0	0

#1 1 g. Dihydrostreptomycin sulfate (Burns Biotec)
#2 600 mg. Oxytetracycline (Liquamycin Pfizer)
#3 1,000,000 IU Penicillin (Procaine Pfizer)
#4 1,000,000 IU Penicillin G (Crystaline Potassium-G (Squibb)

Penicillin test limit .001 µg/ml
Dihydrostreptomycin test limit .1 µg/ml
Oxytetracycline test limit .005 µg/ml

*µg/ml

TABLE X

AMOUNT AND DURATION OF TOTAL ANTIBIOTIC IN THE LUMEN OF THE UTERUS

Infusion in 30 cc water		Total µg in Uterine Lumen						
		6 hours	12 hours	18 hours	24 hours	30 hours	36 hours	42 hours
Dihydro-strepto-mycin	$\bar{X}$	1440	700	500	200	0		
1 gm	±	200	170	100	75	0		
Crystalline Penicillin	$\bar{X}$	2240	920	300	20	0		
1,000,000 units	±	450	250	190	20	0		
Procaine Penicillin	$\bar{X}$	2480	1525	620	370	48	0	
1,000,000 units	±	525	400	325	200	40	0	
Oxytetracycline	$\bar{X}$			60,000	15,000	4000	1000	0
600 mg.	±			16,000	7,000	5000	1000	0

the following method: A Foley catheter was placed in the uterus so that the balloon occupied the body of the uterus in the same manner as used to recover embryos. Approximately 200 ml of sterile saline was allowed to fill the uterus by gravity flow and the exact amount was measured. The solution was then allowed to flow back through the catheter and collected. The amount of antibiotic per ml of the recovered solution was determined and then multiplied by the amount of saline solution infused into the uterus to give the total amount of antibiotic in the uterine lumen.

Determination C — Antibiotic Concentration in Endometrial Tissue, Table XI

Endometrial tissue was taken with a Weck Biopsy instrument following uterine infusion with dosages of antibiotics as indicated above. Samples were frozen and taken to the laboratory, thawed, weighed, washed quickly in physiologic buffered saline so that only the surface was washed. The tissue was then homogenized and centrifuged so that the supernatant could be assayed by antibiotic concentration calculated in)g/mg in the tissue. The procedure was shown to be valid for penicillin by the use of the enzyme penicillinase. We were unable to detect any dihydrostreptomycin and oxytetracycline in the tissue by this procedure.

Determination D — Urine, Table XII

One cow for each antibiotic had a uterine infusion of the dosage indicated above. Using a urine collecting device, the amount of urine voided for each 2-hour period up to 12

TABLE XI

AMOUNT AND DURATION OF PENICILLIN IN THE ENDOMETRIUM

Infusion in 30 cc water		µg per gm of tissue						
		6 hours	12 hours	18 hours	24 hours	30 hours	36 hours	42 hours
Crystalline Penicillin	$\bar{X}$	1000	652	480	300	200		
1,000,000 units	±	251	190	125	125	125		
Procaine Penicillin	$\bar{X}$	900	649	500	320	250	33	
1,000,000 units	±	125	251	188	158	125	63	

TABLE XII

ANTIBIOTIC EXCRETION IN URINE

AFTER INTRAUTERINE INFUSION

Treatment	Hours Post Infusion 2	4	6	8	10	12	24	A	B
#1 mg	120	135	0	0	375	0	0	630	63%
#2 mg	0	0	2.7	5.3	10.7	15.3	120	154	26%
#3 IU	67,000	67,000	57,500	47,900	40,700	31,100	0	311,200	31%
#4 IU	83,700	95,700	71,800	48,000	31,000	24,000	0	354,200	35%

A. Total amount of drug excreted in urine in 24 hours.

B. Percent of drug infused recovered in urine in 24 hours.

#1. 1 g Dihydrostreptomycin sulfate (Burns Biotec)
#2. 600 mg. Oxytetracycline (Liquamycin Pfizer)
#3. 1,000,000 IU Penicillin (Procaine Pfizer)
#4. 1,000,000 IU Penicillin G (Crystallin Potassium Penicillin G).

TABLE XIII

AMOUNT OF ANTIBIOTIC IN SERUM FOLLOWING

INTRAUTERINE INFUSION

Treatment	Time Post Infusion 0	10"	15"	20"	30"	45"	1 hr	2 hr
#1	0	0	0	0	0	0	0	0
#2	0	0	0	0	0	0	0	0
#3	0	0	.10	.09	.11	.01	0	0
#4	0	.11*	.23	.18	.07	.04	0	0

#1 1 g. Dihydrostreptomycin sulfate (Burns Biotec)
#2 600 mg. Oxytetracycline (Liquamycin Pfizer)
#3 1,000,000 IU Penicillin (Procaine Pfizer)
#4 1,000,000 IU Penicillin G (Crystaline Potassium G (Squibb)

Penicillin test limit	.001 µg/ml
Dihydrostreptomycin test limit	.1 µg/ml
Oxytetracycline test limit	.005 µg/ml

*µg/ml

hours was measured. The amount from 12 to 24 hours was measured. Samples for each time period were collected and frozen for subsequent BPM assay.

Determination E — Serum, Table XIII

Two animals were infused into the uterus with each antibiotic treatment and jugular blood samples taken at 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours and 48 hours. The samples were allowed to clot and serum removed and frozen, frozen and assayed by the PCM.

Results and Conclusions:

In milk, only penicillin was detected following the uterine infusion (Table VIII). Using 1,000,000 International Units crystaline potassium penicillin G, 7 cows of a total of 56 had detectable amounts of penicillin in the milk at the first milking following treatment. Using 1,600,000 International Units of procaine penicillin G and 2 g of dihydrostreptomycin, penicillin was detected in one cow of 50 cows sampled on the first milking following infusion. Using 1,000,000 International Units of procaine penicillin G, in 56 of the cows none had detectable amounts of penicillin at the first milking post-infusion. The only antibiotics detected following infusion were those detected at the first milking. No antibiotics were found in any subsequent milkings.

In the trial in which milk was collected a few minutes after infusion (Table IX), only crystaline potassium penicillin G was found following infusion. In one of the cows the penicillin was present 10 minutes after infusion; in both cows, penicillin was present 20 minutes after infusion and remained in one cow until 45 minutes post-infusion and in the other cow until 2 hours post-infusion. No penicillin was found following the use of procaine penicillin G at the dosage stated.

The amounts and duration of antibiotics in the lumen of the uterus is reported in Table X. All antibiotics had disappeared from the uterus by 42 hours post-infusion. The amount and duration of antibiotic in the endometrium is reported in Table XI. Only penicillin could be detected in the endometrium, although attempts were made to detect oxytetracycline and dihydrostreptomycin. Penicillin remained in the tissue at far above accepted blood therapeutic levels for at least 30 hours post-infusion. No penicillin was present 42 hours post-infusion.

The amount of antibiotic in the urine following intrauterine infusion is reported in Table XI. Antibiotics were detected in the urine only during the first 24 hours post-infusion. The total amount of antibiotic recovered is consistent with amounts reported in previous work.

The amount of antibiotic in serum in one cow following intrauterine infusion of antibiotic is reported in Table XIII.

Discussion

This was a field study and, as such, all questions could not be investigated that were relevant to the problem. Also, in every case scientifically acceptable controls were not possible. However, on the other hand, a study of this condition as it occurs naturally can only be done under field conditions. Objective information is reported to the limit possible, however, the authors feel confident in applying some clinical judgment of the interpretation where strict controls were not possible. This is not an apology because this is one of the most extensive studies reported on endometritis in cattle and includes a greater degree of statistical analysis than is usually found in reports regarding this condition.

The severely affected Group C cows were neither biopsied nor cultured and all were treated. Due to the severity of the endometritis in group C cows, it was our clinical judgment that no controls were to be identified and left untreated in this group. Because of the severity of the clinical condition it is almost certain that bacteriologic, histologic evidence of endometritis could have been found in most of these cows. It is interesting that most of these cows were treated at least several times following the initial examination. In spite of this, the fertility was significantly lower. We cannot tell if the treatment did any good or not because no controls were kept. There is clear evidence that endometritis does cause reduced fertility or at least that reduced fertility often follows severe endometritis.

The usual procedure is to treat several times, usually at varying intervals more than 24 hours. From the work done on antibiotic levels in the endometrium and in the uterine lumen following intrauterine infusion, it is clear that if therapeutic levels are to be maintained continuously, treatment must be administered on a 24 hour interval. Perhaps with treatment administered in this manner an increase in fertility could be obtained in the severely affected cows. We hope that this publication will stimulate others to investigate this condition more thoroughly and perhaps conduct this experiment.

The moderately affected cows had fertility almost exactly the same as those diagnosed as normal. Several factors must be considered here. Referring to the bacteriologic and histologic examination of a sample of these cows, we must assume that in fact a substantial number of these cows were normal. It is reasonable to expect, also, that those with endometritis in this group had a milder condition or were closer to spontaneous recovery. A relatively large number of normal animals included in this Group, and the possibility of spontaneous recovery shortly after examination, are probably the major reasons for the normal fertility recorded in this Group.

The examination by vaginal speculum gives a more accurate diagnosis of endometritis than does rectal examination. The participants in this experiment and others that use the vaginal examination as their primary means of

diagnosis in their clinical practice are satisified that they are efficiently and properly examining their cases. They are in the minority, and the usual procedure is rectal examination because it is presumed to be faster. A practitioner not in this experiment examines 5000 cows in a 3 month period by vaginal speculum and claims speed equal to the rectal exam method. We cannot say, from the information available if there is a time factor and if so whether there is a trade-off between the high percentage of normal cows treated by the rectal examination method and the potential time saved by the speculum examination method.

We found that in those cows biopsied, the first service conception rate was significantly reduced. This was based on examination of the total number biopsied, and since approximately two-thirds of these were normal, we feel that there is some decrease in fertility associated with biopsy. The reason is not apparent.

We have confirmed earlier work that the causative organism of endometritis and infertility is primarily *Corynebacterium pyogenes.*[2] *Pasteurella* is seldom reported in cultures of the uterus, however, it was associated with endometritis in this study. The close agreement between culture results and histologic examination suggests that for a survey in which large numbers of animals need to be examined, either method would be satisfactory for diagnosing endometritis.

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