

SECTION III

CONTINUED

DR. SIMMONS: This session's speakers will be talking about the use of animal models for dose determination of anti-microbial drugs.

The first speaker is Dr. Donald Campbell, who received his D.V.M. degree from the University of Georgia in 1968. He joined the faculty at the University of Minnesota in 1969 as a research associate. He subsequently joined the faculty of Texas A & M University in 1970 and remained there until 1973 at which time he received his Master's degree. Following that, he joined the faculty at the University of Maryland and remained there until 1979 at which time he left to join the Bureau of Veterinary Medicine. Dr. Campbell is board-certified in theriogenology. He is presently serving as a reviewer in the Food Animal Division, Physiological and Antiparasitic Drugs Product Group. Dr. Campbell...

DR. CAMPBELL: I've been given an opportunity this morning to present to you some data on a model infection that we did at the FDA Research Unit in Beltsville. The other opportunity given me this morning is to wear my only tie, so I thank you for those two opportunities.

The topic of my discussion this morning is a model for the study of bovine pyometra and I'll give you a little background as to why this study originated. We had been looking at drugs for the treatment of bovine pyometra and were having some trouble coming to grips with the fact that we needed untreated controls; there was concern about leaving an animal diagnosed with bovine pyometra untreated for a period of time and that it might be detrimental to the reproductive life of the animal. It was our contention at that time that we did need those controls and we looked at ways of providing untreated controls for this rather infrequent but observed clinical condition. To do that we thought perhaps a model infection would provide us some information as to spontaneous cure rates in untreated controls. The problem with that was that it had been proven difficult to produce the condition of pyometra in the bovine by simply infusing suspensions of microorganisms into the uterus of that animal. So we looked at some of the information that was available in the literature and came up with a means which we thought might do this; which might produce this condition from which we would have a model for treatment and could address spontaneous cure rate.

A MODEL FOR THE STUDY OF BOVINE PYOMETRA

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INTRODUCTION

"True" bovine pyometra is an infrequently encountered clinical entity defined by three criteria: (1) an active corpus luteum on one or both ovaries; (2) a closed (sealed) cervix; (3) a purulent uterine exudate (200 to 20,000 ml). It is usually diagnosed in the post partum or post-breeding periods. In both instances, unknown predisposing factors restrict the animal's natural protective mechanisms from eliminating the uterine bacterial contamination which may result from calving, use of infected semen, or improper breeding technique. Pyometra may also be secondary to mucometra caused by chronic cystic ovaries. (3)

Early diagnosis and treatment of pyometra increases the possibility of its successful correction, with success being judged as pregnancy carried to term. Complete recovery and conception is more likely in cases that have existed for less than 120 days. Cases of longer duration are reported to produce degenerative uterine changes. (3)

Recommended treatments for bovine pyometra include mechanical removal of the mucopurulent material, uterine antibiotic infusion and hormone therapy (oxytocin, estrogen, prostaglandin F₂). (3)

Only limited success has been achieved in earlier attempts to create bovine pyometra under experimental conditions.^(1,2) Uterine infection in the cow has been induced experimentally with Escherichia coli, Pasteurella sp. and Corynebacterium pyogenes during all phases of the estrous cycle.⁽²⁾ It has been demonstrated that the estrous cycle length is shortened by creation of a uterine infection with accompanying inflammation.⁽¹⁾ This probably is effected through uterine prostaglandin release and subsequent luteolysis.

The estrous cycle of the cow can be altered with drug therapy. Administration of a gonadotropin (HCG) during the mid-luteal phase (day 10), or administration of a strong iodine solution (approximately 4%) intrauterine during the late luteal phase (day 14) will lengthen the estrous period. (4,5)

Materials and Methods

Phase I

Seven non-lactating dairy cows between the ages of 3 and 10 years were used. Animals demonstrating a standing estrus were examined by rectal palpation

within 12 hours of the time that estrus was detected. For a complete appraisal of the reproductive tract, the ovaries, uterus and cervix were examined. The anatomical size, shape and consistency of these organs were recorded.

On day 5 following estrus another rectal examination was performed to determine the presence of a corpus luteum (CL).

On day 10 of the cycle, cows were treated with HCG (10,000 IU) intramuscularly.

On day 14 an iodine solution containing 3.3 gm iodine and 6.6 gm potassium iodide in 255 ml water was infused into the uterus.

On day 15-17 animals received a 60 ml intrauterine inoculation of C. pyogenes suspension containing 10^8 CFU/ml. Animals were observed daily for estrus and monitored weekly for 8 weeks or until estrus was detected.

If spontaneous recovery had not occurred by eight weeks, post-inoculation of animals were treated with 25 mg IM prostaglandin F₂ alpha in an attempt to evacuate the uterus. A rectal examination was performed on day 7 post treatment or at the induced estrus to evaluate the condition of the uterus.

Phase II

Fifteen non-lactating dairy cows between the ages of 3 and 9 years were divided into three groups - five cows per group. All groups were handled identically with the exception of the type of inoculum used. Animals were treated as described in Phase I until inoculation. On days 15-17 cows given an intrauterine inoculation were as follows:

Group 1 - Corynebacterium pyogenes - (60 ml - 10^8 CFU/ml)

Group 2 - Fusobacterium necrophorum - (60 ml - 10^8 CFU/ml)

Group 3 - Combination of C. pyogenes (10^8 CFU/ml) and F. necrophorum (10^8 CFU/ml) in a total volume of 60 ml.

Results

Phase I

Five of the seven inoculated cows developed a condition consistent with the definition of pyometra. Two cows did not develop pyometra although their cycles were lengthened by the treatment.

Spontaneous evacuation and return to estrus occurred in four of the five cows with pyometra. The average time from estrus to spontaneous evacuation and return to estrus for these cows was 52 days. The one cow which had not spontaneously evacuated the uterus by 70 days post-inoculation (84 days of post-estrus) was treated intramuscularly (IM) with 25 mg PGF₂ alpha. Uterine evacuation and return to estrus resulted in three days post-treatment.

After HCG treatment on day 10, clinical examinations revealed that a second corpus luteum developed by day 14 in three of the five cows with pyometra. There was no difference in ability of the model to create pyometra relative to the formation of a second corpus luteum although a difference was seen in the length of time to spontaneous cure.

Cows without a second corpus luteum averaged 50 days to spontaneous evacuation and return to estrus. Cows with a second corpus luteum averaged 78 days to return to estrus. (Table 1)

Cows not developing pyometra averaged 29.5 days to return to estrus. (Table 1)

Phase II

Three of 5 cows in Group I developed pyometra. One of these 3 developed a second corpus luteum. One cow in the group without pyometra developed a second corpus luteum. (Table 2)

The average time to return to estrus was 48 days in Group I cows with pyometra and 28.5 days in Group I non-pyometra cows.

Although none of the Group II cows developed pyometra, two cows did develop a second corpus luteum. (Table 2) The time to return to estrus for cows with a second corpus luteum was 39.5 days compared to 25 days for cows without a second corpus luteum.

Average duration of the treatment cycle for all cows in Group II was 30.8 days.

All cows in Group III developed pyometra. Two of the 5 developed a second corpus luteum. (Table 2) Average duration of the treatment cycle was 54 days for cows developing a second corpus luteum and 48 days for cows not developing a second corpus luteum.

Discussion and Conclusions

Previous attempts by investigators to experimentally create bovine pyometra have met with limited success, and shortened estrous cycles have usually resulted. The acute inflammatory response to the bacterial contamination probably causes the release of a luteolytic prostaglandin from the uterine lining and abbreviates the normal luteal phase.⁽³⁾

By using the present model, however, either the sensitivity of the corpus luteum has been altered and/or release of the uterine luteolytic prostaglandin has been eliminated. In either case, the shortening of the estrous cycle was forestalled even in those animals that did not develop pyometra. The proposed mechanism for this phenomenon may be twofold: (1) a diminished capability of normal cyclic release of uterine prostaglandin exists because of damage caused to the endometrium from the infused iodine, or (2) a CL is non-responsive to prostaglandin in response to the HCG, and in some instances is supplemental by a second CL. The combination of treatments presumably alters normal utero-ovarian function in this proposed manner to facilitate the establishment of a pyometra. Once established, the condition is self-perpetuating though and as yet undefined perturbation in the immune response mechanism.

Considering the success rates of the 3 treatments, it seems reasonable to suggest that a synergistic, or possibly symbiotic relationship may exist between the anaerobic and microaerophilic organisms in this model. This would be consistent with the observation that both organisms have been found together in field cases of bovine endometritis.

It should be noted that pyometra has been produced in a minimum of two-thirds of the cattle inoculated with C. pyogenes or a combination of C. pyogenes and F. necrophorum, and that only one cow with pyometra did not experience spontaneous cure within 60 days of inoculation. Thus controlled studies using a model such as the one developed and described in this study could be implemented in order to distinguish between truly effective therapeutic agents, and the temporal coincidence that may occur between a potential therapeutic agent and a spontaneous cure.

TABLE 1

Phase I - Clinical Data

Cow No.	Days to return to estrus	Second Corpus Luteum	Pyometra
2410	30	-	-
22	29	+	-
27	43	-	+
175	57	-	+
235	84	+	+
26	87	+	+
6269	63	+	+

TABLE 2

Phase II - Clinical Data

Organism(s)	Cow No.	Days to return to estrus	Second Corpus Luteum	Pyometra
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Group I				
Coryn	6809	34	+	-
	6694	59	-	+
	6867	41	+	+
	2482	23	-	-
	6091	44	-	+
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Group II				
<u>F. necrophorum</u>	7241	35	+	-
	6669	24	-	-
	6432	29	-	-
	6436	44	+	-
	6628	22	-	-
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Group III				
<u>F. necrophorum</u>	6843	42	+	+
Coryn	6459	48	-	+
	6873	66	+	+
	2423	44	-	+
	6671	53	-	+

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