Antibiotic Treatment of Experimental Staphylococcus aureus Infections of the Bovine Mammary Gland

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ABSTRACT

Experimental infections were produced in 78 quarters of 17 cows by the infusion of small numbers of a single strain of Staphylococcus aureus. In each single experiment three quarters in a cow were infected, with the fourth left as a control. At times varying from three to 60 days after the infusion of organisms, a standard intramammary antibiotic treatment was administered on a single occasion. A cure was arbitrarily defined as the absence of the organism in foremilk, from direct plating and replated incubated milk, together with return to normal somatic cell count levels as determined by an electronic counter.

With these standardized conditions the effects of a number of cow associated factors on the outcome of the therapy were determined.

Forty-three of the 78 quarters (55%) were cured by the standard treatment. Significant differences in percentages of quarters cured were found to be associated with the duration of infection before therapy, the lactation age of the cow, the length of time in lactation, somatic cell count in milk at time of treatment, the location of the quarter in the udder and individual cows. No significant effects on the outcome of the standard treatment were found associated with the number of bacteria in the secretion at the time of treatment, previous infection and cure in a quarter nor the season of the year in which treatment was given. Of the 35 quarters in which infection recurred following treatment, organisms were reisolated from 12 within four days, 18 between five and nine days, four between ten and 17 days and one after 28 days.

From these data it is apparent that if, as has been suggested, models such as described are to be used for efficacy trials, standardization of some parameters is essential.

RÉSUMÉ

L'auteur a procédé à des infections expérimentales, dans 78 quartiers, chez 17 vaches, par le truchement d'infusions intra-mammaires d'une légère dose de la souche no. 305 de Staphylococcus aureus. Dans chacune de ses expériences, il n'infecta que trois quartiers par vache, laissant le quatrième comme témoin. A intervalles de trois à 60 jours après l'infection, il administra une seule antibiothérapie intramammaire standard. Il considéra arbitrairement comme indice de guérison l'absence de microbes dans les premiers jets de lait, jointe à un retour à la normale du nombre des cellules somatiques. Il ensemença les échantillons de lait sur gélose, directement ou après une incubation, et il détermina le nombre de cellules somatiques à l'aide d'un appareil électronique.

Selon ces procédés standardisés, il détermina les effets d'un certain nombre de facteurs inhérents aux vaches, sur l'issue du traitement. L'antibiothérapie standard amena la guérison de 43 (55%) des 78 quartiers. Il nota des différences appréciables quant au pourcentage des quartiers guéris, en tenant compte des facteurs suivants: la durée de l'infection avant l'antibiothérapie; le nombre de lactations; la

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période de la lactation; le nombre de cellules somatiques dans le lait lors du traitement; la place du quartier dans le pis et les vaches elles-mêmes. Il n'observa aucun effet appréciable sur l'issue du traitement standard en rapport avec le nombre de bactéries dans le lait au moment du traitement, une infection antérieure, la guérison d'un quartier ou la saison de l'année au cours de laquelle il effectua le traitement. Des 35 quartiers où il y eut récidive, il isola de nouveau des staphylocoques de 12 d'entre eux, en dedans de quatre jours, de 18, entre cinq et neuf jours, de quatre, entre dix et 17 jours, ainsi que d'un autre, après 28 jours.

Il ressort de ces observations que si, comme on l'a déjà suggéré, on se propose d'utiliser un protocole identique à celui que l'auteur vient de décrire, pour effectuer des expériences similaires, la standardisation de certains paramètres s'impose.

INTRODUCTION

Few controlled investigations have been carried out in the cow to determine the efficacy of antibiotic preparations produced and sold for treatment of bovine mastitis. Products have been approved on the basis of sensitivity tests applied to mastitis associated microorganisms in the petri dish (9). Such products have been sold containing six to eight ingredients with little or no controlled efficacy data to show that each drug is necessary (9). Despite favourable petri dish data, intramammary treatment of bovine mastitis has been less effective than is desirable and the time is ripe for the development of mastitis formulations to be removed from the realm of empiricism and to be subjected to a scientific approach (4).

Effectiveness might be determined *in vivo* by the use of experimentally produced infections in herds of cows maintained for the purpose. Such trials would be more easily controlled and conditions better standardized than those in the field. But what conditions should be standardized? What do we know about the factors important to the efficacy of mastitis therapy? It is certain that besides the characteristics of the product itself there are controlling factors related to the bacterial invader (parasite) and the cow (host). These are areas in which little information is available.

In this study a model has been used to elucidate some cow associated factors in which both the bacterial strain and the antibiotic therapy have been kept constant and an arbitrary criterion for a 'cure' adopted. Because *Staphylococcus aureus* infections respond to treatment less readily than those caused by streptococci (3) the former was used as the infective organism.

MATERIALS AND METHODS

Cows

Seventeen Holstein cows were used, some several times, in a total of 27 separate experiments. They were in their first to fifth lactation as shown in Table I. These cows were part of the mastitis research herd at the Ontario Veterinary College and were selected solely on the basis of availability. They were fed a standard diet of corn silage, hay and protein concentrate in winter and mixed pasture with the same protein concentrate as a supplement during the summer. Milking time hygiene consisted of washing udders with individual paper towels and Iosan[1] (1 part in 160 parts of warm water), pasteurizing teat cups between cows at 85°C for seven to eight seconds and dipping teats in Bovadine[2] immediately after milking.

[1]West Agro-Chemical (Canada) Ltd., Toronto, Ontario.
[2]West Agro-Chemical (Canada) Ltd., Toronto, Ontario.

TABLE I. The Lactation Ages of Cows at Time of Infection

Lactation Number	No. of Cows
1	7
2	8
3	8
4	3
5	1

Staph. aureus strain 305 (7) was used throughout for experimental inoculation. Inocula were prepared and infused into the teat cistern as described previously (5). All inocula were prepared from a single batch of freeze dried cultures, a single fresh tube for each experiment. From 40-100 colony forming units of the organism in 0.2 ml of sterile milk was the standard inoculum. Strain 305 is sensitive to all common antibiotics and produces mainly mild subacute infections, although some cows respond with marked clinical signs.

Antibiotic Treatment

A standard commercial product, containing per 10 gm tube 250,000 I.U. of penicillin G potassium and 125 mg streptomycin base (as sulphate) in peanut oil with 2.5% aluminum monostearate, was used as the standard treatment. One 10 gm tube was infused into each infected quarter on the selected day after the afternoon milking.

Plan of Experiments

Three udder quarters of a cow, each free of infection for more than 30 days previously, were randomly selected and, immediately following the afternoon milking, 0.2 ml of inoculum as described above, was infused into each with a tuberculin syringe fitted with a short blunt needle. The fourth quarter in each cow was left as a control to monitor any quarter to quarter spread. The cows were milked normally and foremilk samples taken before each milking for several days, then at the morning milking daily until treatment. Foremilk samples were then taken after the morning milking every second day until 30 days post-treatment. From each sample 0.05 ml was streaked on the surface of a blood agar plate and the somatic cell count determined by an electronic method (6). The plates were incubated at 37°C for 24 hours and any developed colonies counted. Samples, which were bacteriologically negative on direct plating, were incubated at 37°C overnight and approximately .001 ml streaked on a blood agar plate, which was incubated at 37°C overnight and examined for typical colonies.

A quarter was considered to be infected when there was an elevated somatic cell count and organisms were isolated from the foremilk samples continuously, either by direct plating or following preincubation of the sample. No standard increase in cell count was set, as cows differ significantly in their response to a given strain of *Staph. aureus* (8).

The criterion for a cure was a return of the somatic cell count to preinfection or near preinfection levels, together with no isolation of the organisms for a period of 30 days, neither by direct plating nor from preincubated samples.

Initially the times at which infected quarters were treated were selected arbitrarily at three, 15 or 30 days after infusion of the inocula. After eight such experiments the times were changed to 15, 30 or 60 days postinfusion and finally to 15 and 30 days only. The time at which a particular quarter was to be treated was, within each cow, randomly selected so that the three infected quarters of each cow were treated at a different time.

Statistical Analysis

Tabulated results were submitted to the Chi Square Test (2).

RESULTS

Of the 81 udder quarters infused with the standard inocula, 78 became infected as judged by the criteria indicated above. In the other three no organisms were isolated after infusion and no elevated somatic cell

TABLE II. The Effects of Duration of Infection, Lactation Number and Stage of Lactation on the Cure Rate Following Treatment of *Staph. aureus* Infected Quarter

		Number of Quarters	Percent Cured
Days Infected	3	8	87.5
	15	38	47.4
	30	24	54.2
	60	8	62.5
Lactation Number	1	20	80.0
	2	23	60.9
	3	23	47.8
	4	9	26.1
	5	3	0
Months in Lactation	1-4	30	33.3
	5-6	15	53.3
	>7	33	75.8

counts ensued. Of the 78 infected quarters given the standard treatment 43 were cured by the criteria outlined while 35 were not cured, for an overall cure rate of 55.13%.

The variation in cure rate depending on the number of days after initial infusion at which treatment was administered is shown in Table II. Treatment after three days produced a very high cure rate, with little difference between 15, 30 or 60 days. The difference between three and 15 days is significant at $p = >0.05$ and beginning with the earliest experiments treatment at 15 days consistently resulted in the lowest cure rate.

That lactation age had a marked effect is shown by the results in Table II, the difference being significant at $p = >0.05$. There was a marked drop in cure rate with each succeeding lactation, although it should be noted that only one cow accounted for the figures for lactation five. There was a steady increase in the efficacy of treatment as the length of the lactation increased. The differences found are significant at $p = > 0.05$.

The cure rate decreased markedly with increasing somatic cell count at the time of treatment as shown in Table III. These differences are significant at $p = > 0.01$. In contrast to the effect of somatic cell count the *Staph. aureus* count at the time of treatment was without influence. Treatment was unsuccessful in one quarter in which the organisms could be demonstrated only from incubated samples.

Whether or not a quarter had been previously infected had no apparent bearing on the outcome of treatment. Of 28 quarters, which had been infected previously and cured, 57% were cured by the standard treatment, while 54% of 50 quarters not previously infected were cured.

65.8% of front quarters were cured compared to 45% of rear quarters, although this difference is just short of statistical significance. Of some interest is the very great difference in the cure rates of the right front and left rear quarters, as shown in Table III, and this difference is significant at $p = > 0.05$.

There was noticeable variation between cows and even quarters within a cow in response to treatment. Samples of this are shown in Table IV. The three recurrences in cow 80 were all in the left rear quarter.

The seasonal effect on treatment results is shown in Table V. While there appeared to be a lower cure rate during the summer months, the differences shown are not statistically significant.

In 35 quarters the infection recurred and from these the organisms were first reisolated at various times following infusion of the treatment. As seen in Table VI quite a few recurred almost immediately, while in one case the organisms did not reappear for 28 days.

TABLE III. The Effects of Somatic Cell and *Staph. aureus* Counts at Time of Treatment, and Quarter Location on the Cure Rate Following Treatment of *Staph. aureus* Infected Quarters

		Number of Quarters	Percent Cured
Cell Count at Treatment (thousands)	<500	17	82.4
	501-1000	21	66.7
	1001-5000	36	41.7
	>5000	4	0
Staph: count at Treatment per 0.05 ml	<100	21	57.1
	101-500	25	48.0
	>500	32	56.2
Right Front Qtr.		21	76.2
Right Rear Qtr.		21	47.6
Left Front Qtr.		17	52.9
Left Rear Qtr.		19	42.1

TABLE IV. Variation in Cure Rate Between Individual Cows with *Staph. aureus* Infected Quarters

Cow	No. Times Used	Quarters Cured	Quarters Recurred
64	2	0	6
63	3	8	0
49	3	3	6
80	3	6	3

TABLE V. The Effect of Season at Time of Treatment on Cure Rate of *Staph. aureus* Infected Quarters

Months	No. of Quarters	Percent Cured
Jan - Mar	20	60.0
Apr - June	33	57.6
July - Sept	18	33.3
Oct - Dec.	7	85.7

TABLE VI. Time of Recurrence of Infection After Treatment of *Staph. aureus* Infected Quarters

Days after Treatment	No. of Recurrences
4 or less	12
5	1
6	5
7	1
8	7
9	4
10, 13, 16	
17, 28	1 each

DISCUSSION

Since as far as can be determined, no similar studies with experimentally infected cows have been reported, no comparisons are possible. The results reported here, however, do agree in the main with those of Wilson *et al* (10) who, in a large field trial, treated 360 *Staph. aureus* infected quarters during lactation and reported significantly reduced response when antibiotic treatment was administered to 1) older cows, 2) cows with several infected quarters, 3) cows in early lactation and 4) quarters shedding milk with high Whiteside scores. Thus, their data support the idea that, even in field trials, some standardization of cow associated factors may be necessary.

The overall mean cure rate in the present investigation was 55.13%, whereas that reported for staphylococci by Wilson *et al* (10) was 52%. It is difficult to compare these precisely since it is not known how long the infections in the field trial had existed pretreatment and the fact that many strains of staphylococci were involved, in contrast to a single strain in our experimental infections. A further major difference was the use in the field trial of three infusions of antibiotic at 48 hour intervals, as compared to a single infusion here.

As indicated, in the present investigation a single strain of *Staph. aureus* was used throughout. As it is not known what effect other strains would have had on the overall results, it is clear that similar experiments with other strains are needed. At the present time little is known about bacteria associated factors which affect treatment results, other than antibiotic sensitivity. Anderson (1) has reported that while following a field trial it was hypothesized that the difference in treatment results between two herds might have been due to the strains of *Staph. aureus* causing the infections, no evidence could be found by experimental means that differences between strains of staphylococci isolated could account for the failure of one herd to respond to therapy.

Experience from this investigation suggests that there is much variation between cows and even quarters of a cow in response to therapy and that refractory cows might provide a means for assessing therapeutic efficacy. Every quarter in which infection recurred following the standard treatment was finally cured, even though in some cases a number of products and treatment regimens over long periods were needed.

There was marked variation in the length of time after treatment at which recurrence of the infection became evident. That the recurrences encountered were not reinfections is supported by the fact that not a single control quarter became infected in any experiment either within or between cows. Thus there is need to standardize the criterion for a cure which should include a minimum period of three weeks after treatment and preferably a full 30 days.

From the data presented it can be concluded that several of the cow associated parameters examined had a marked effect on the outcome of the standard treatment. While this work indicates the need for a larger investigation and possibly its direction, there are sufficient data to suggest potential usefulness of a model such as described. These results also focus on the need for standardization of factors such as duration of infections before treatment, lactation age of the cows used, the stage in lactation and an acceptable criterion for a cure if such models are to be used in efficacy trials. The inclusion of at least some cows known to be refractory to a standard treatment might also be useful. Further, probably some of these factors should be considered in protocols for field trials.

An experimental model such as is described, in which specific factors are held constant while others are varied, holds promise of providing information which could remove the development of therapeutic products and, possibly of more importance, their application from the realm of empirical medicine.

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Book Review

HISTOLOGY AND COMPARATIVE ORGANOLOGY: A TEXT ATLAS. *William J. Banks. Published by the Williams & Wilkins Company, Baltimore, Maryland. 1974. 285 pages. Price $24.95.*

The author is to be congratulated on tackling the task of providing a comprehensive text atlas on the histology and organology of domestic animals. Such a book was badly needed for veterinary students in English speaking schools. The book was prepared with veterinary students in mind, but the author has tried to broaden its scope for students of comparative vertebrate histology as well.

The illustrations are of good quality and are mostly taken from domestic mammals and avian species. This is the most welcome aspect of this book. However, in many instances the animal species from which a particular photomicrograph was taken are not mentioned in the figure legends. The text material in various chapters is quite useful but the style is cryptic and at times falls short in details of species differences. The author states in the preface that he is endeavouring to correlate structure and function but the relationship presented is inadequately explained for various organs. Terminology used is at times confusing as some out of date terms are retained and new ones introduced without clear explanation. The chapter on cytology is too brief and is good only for a review purpose. Some omissions and confusing statements are also encountered in the chapters on muscular tissue, integumentary system, urinary system, etc.

The value of this book as a standard textbook for students in veterinary medicine would be enhanced by increased emphasis on microanatomical differences between the species and structural and functional correlates. Expansion of the chapter on cell and cell organelles would also help.

In spite of the shortcomings which are inevitable in a first edition, this book is a valuable supplement to human histology textbooks currently used in several veterinary schools in North America. — *M. K. Bhatnagar.*