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The Efficacy of Antibiatic Therapy, with or without Teat Seals, during the Dry Period in the Treatment and Prevention of Mastitis in Irish Dairy Cows

William Joseph Meaney

THE EFFICACY OF ANTIBIOTIC THERAPY, WITH OR WITHOUT TEAT SEALS, DURING THE DRY PERIOD IN THE TREATMENT AND PREVENTION OF MASTITIS IN IRISH DAIRY COWS

by

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A Thesis
submitted for the Degree of
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ABSTRACT

THE EFFICACY OF ANTIBIOTIC THERARY, WITH OR WITHOUT TEAT SEALS, DURING THE DRY PERIOD IN THE TREATMENT AND PREVENTION OF MASTITIS IN IRISH DAIRY HERDS.

W.J. Meaney

This thesis reports on the efficacy of antibiotic and non-antibiotic intramammary formulations for the treatment and prevention of mastitis during the dry period. Survey data on the types of pathogens causing mastitis at drying off and the resposes to treatment are also reported.

The efficacy of seven intramammary antibiotics which were formulated for the treatment of cows at drying off was evaluated. The products were tested primarily on their ability to eliminate *Staph. aureus* infections. The elimination of *Staph. aureus* ranged from 25 *per cent* to 94 *per cent* and in general there was no difference in efficacy between products. There was a significant benefit ($P<0.001$) in treating *Staph. aureus* infections with benzathine cloxacillin when compared with no therapy at drying off. There was no advantage in treating *Staph. aureus* with benzathine cloxacillin on two occasions during the dry period.

The efficacy of a long-acting formulation containing neomycin sulphate in preventing new dry period mastitis was evaluated in uninfected cows at drying off. At the next calving there were fewer new infections in the treated quarters than in the untreated quarters ($P<0.001$).

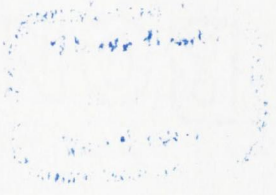
The effectiveness of a bismuth subnitrate teat-sealer was evaluated in a series of eight experiments. The sealer was evaluated primarily on its ability to prevent new infections in cows during the dry period. In two out of three experiments, teat-sealers used in combination with antibiotics were more effective ($P<0.005$; $P<0.05$) in preventing new infections than antibiotics alone. In one experiment, an oil-based antibiotic used in combination with teat-sealer was more effective ($P<0.05$) than the antibiotic alone in eliminating infections at drying off. When sealed and untreated quarters were challenged with cultures of *Staph. aureus* and *Strep. dysgalactiae* during the dry period the sealer was more effective ($P<0.05$) in reducing new infections. The results of a second challenge experiment using *Staph. aureus* showed that there was no difference in the level of new infection during the dry period between quarters which were sealed or infused with benzathine cloxacillin at drying off.

One experiment was undertaken to evaluate the effectiveness of sealing the teats of in-calf heifers at intervals ranging from 9 to 110 days before calving. There was no difference in the level of infection at calving in the sealed teats and untreated teats.

Surveys carried out between 1984-1992 showed that 94 *per cent* of intramammary infections diagnosed at drying off were caused by staphylococci and streptococci. Between 48 and 72 *per cent* of *Staph. aureus* infections were eliminated following dry period antibiotic treatment. Antibiotic therapy was more effective ($P<0.001$) in eliminating *Staph. aureus* infections in first or second lactation animals than in older animals.

DECLARATION

I declare that this thesis has not been submitted as an exercise for a degree at the University of Dublin or any other university and I further declare that the work embodied in it is my own with the exception of the study carried out in Experiment 1 which was carried out in association with Mr. O.H. Langley, Mr. N.P.Cullen and Mr. J.F.Cunningham.



W. J. Meaney

PUBLICATIONS BASED ON SOME OF THIS WORK

Langley, O.H., Meaney, W.J., Cullen, N.P. and Cunningham, J.F. (1971). The control of bovine mastitis.
Veterinary Record **89**: 315 - 316.

Meaney, W.J. (1976). Dry period teat seal. *Veterinary Record* **99**: 30.

Meaney, W.J. (1976). A comparison of two dry cow intramammary treatments.
Veterinary Record **98**: 50.

Meaney, W.J. and Nash, J.M. (1977). Comparison of two dry cow intramammary treatments.
Veterinary Record **100**: 492 - 493.

Meaney,W.J. (1977). Effect of a dry period teat seal on bovine udder infection.
Irish Journal of Agricultural Research **16**: 293 - 299.

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SUMMARY

1. The efficacy of seven intramammary antibiotics which were formulated for the treatment of cows at drying off was evaluated between 1968-1989. The products contained the following active ingredients: benzathine cloxacillin, penicillin and novobiocin, penicillin and dihydrostreptomycin, pencillin and framycetin, penicillin and fradiomycin, neomycin and spiramycin, cefalexin and dihydrostreptomycin, and cephalonium. The products were tested primarily on their ability to eliminate *Staph. aureus* infections. The elimination of *Staph. aureus* ranged from 25 *per cent* to 94 *per cent* and in general there was no difference in efficacy between products tested in specific experiments. There was a significant benefit ($P<0.001$) in treating *Staph. aureus* infections with benzathine cloxacillin when compared with no therapy at drying off. There was no advantage in treating *Staph. aureus* with benzathine cloxacillin on two occasions during the dry period.

2. The efficacy of a long-acting formulation containing neomycin sulphate in preventing new dry period mastitis was compared with an untreated control in uninfected cows at drying off. At the next calving there were fewer new infections in the treated quarters than in the untreated quarters ($P<0.001$). A subset of data for new infections caused by environmental pathogens showed that there were fewer of these present in the treated quarters ($P<0.05$). There was no evidence that the treated quarters were more susceptible to mastitis during the housing period after calving.

3. The effectiveness of a bismuth subnitrate internal teat-sealer was evaluated in a series of eight experiments. The sealer was used alone or in combination with oil-based or water-based antibiotics. The sealer was evaluated primarily on its ability to prevent new infections in cows and heifers during the dry period. Sealers were evaluated under conditions of natural infection and also with artificial bacterial challenge. In two out of three experiments, teat-sealers used in combination with antibiotics were more effective ($P<0.005$; $P<0.05$) in preventing new infections than antibiotics alone under conditions of natural challenge. In one experiment, an oil-based antibiotic used in combination with teat-sealer was more effective ($P<0.05$) than the antibiotic alone in eliminating infections at drying off. This result was not confirmed in other experiments in which there was no difference between the antibiotics in either oil or water bases used alone or in combination with teat-sealers.

   When sealed and untreated quarters were challenged with cultures of *Staph. aureus* and *Strep. dysgalactiae* during the dry period the sealer was more effective ($P<0.05$) in reducing new infections. The results of a second challenge experiment using *Staph. aureus* showed that there was no difference in the level of new infection during the dry period between quarters which were sealed or infused with benzathine cloxacillin at drying off.

   One experiment was undertaken to evaluate the effectiveness of sealing the teats of in-calf heifers at intervals ranging from 9 to 110 days before calving. A comparison was made with untreated teats in each of 32 animals. There was no difference in the level of infection at calving in the sealed teats and untreated teats.

4. The results of surveys carried out between 1984-1992 showed that 94 *per cent* of intramammary infections diagnosed at drying off were caused by staphylococci and streptococci. Sixty-four *per cent* of infections were caused by staphylococci and 27 *per cent* by streptococci in pure culture. A further three *per cent* was caused by staphylococci and streptococci in mixed culture. Between 48 and 72 *per cent* of *Staph. aureus* infections were eliminated following dry period treatment with a variety of antibiotic formulations. Antibiotic therapy was more effective ($P<0.001$) in eliminating *Staph. aureus* infections in first or second lactation animals than in older animals.

CHAPTER 1

GENERAL INTRODUCTION

1.1 EC standards for milk production

The requirements for the production of quality milk are outlined in an EC Directive (Council Directive 92/46/EEC, Anon (1992)). The Directive indicates that milk must be produced in cows that are free from all infectious diseases which may be transferred to humans through milk or milk products. Special attention is given in the Directive to tuberculosis, brucellosis, infections of the genital tract, enteritis with diarrhoea and fever and, finally, mastitis.

1.1.2 EC quality standards for milk and production holdings

Milk must be produced on farms with buildings and facilities which have been built for, or modified to comply with, these new regulations. These standards must be in operation in all member states by January 1st, 1994. From that date, milk produced for the liquid market or for export to another member state must have a somatic cell count (SCC) of ≤400,000 *per ml* and a total bacterial count (TBC) of ≤100,000 *per ml.* Standards have also been set for milk produced for manufacturing : levels of ≤500,000 *per ml* for SCC and ≤400,000 *per ml* for TBC must be achieved after January 1st, 1994. It is envisaged that all milk produced, both for the liquid market and for manufacture, must reach a standard of ≤400,000 *per ml* SCC and ≤100,000 *per ml* TBC after January 1st, 1998.

1.2 Penalties for inferior quality milk

In Ireland, financial penalties have been imposed for many years on milk supplies with TBC levels in excess of 50,000 *per ml.* Severe

penalties, and sometimes milk rejection, are applied to milk supplies contaminated with antibiotics and other chemical substances. Already, many dairy co-operatives have introduced bonus or penalty payment systems, based on the SCC in bulk herd milk. These incentives or penalties vary between co-operatives and may depend, to some degree, on the required specification for milk, used as a raw material for the manufacture of specific dairy products.

1.3 Mastitis control programmes

For many years the control of bovine mastitis has provided a difficult challenge to research workers, veterinarians in practice, dairy advisers and dairy farmers. Major difficulties have been encountered because of the multiplicity of pathogens and environmental conditions associated with the disease and also because of the predominant subclinical nature of the condition.

Booth (1992) stated that:
"Bovine mastitis remains the most widespread disease in dairy cows in Europe despite significant progress made in control over the past twenty years".

This view is further supported by Beretta and Carli (1992). It has been well documented for the last three decades that the subclinical form of the disease is associated with increases and fluctuations in SCC in herd bulk milk. The use of dry period antibiotic therapy has made a major contribution towards reducing the level of subclinical mastitis in herds and, also, in reducing the SCC levels.

1.4 Requirements for manufacture and sale of antibiotics

In most dairying countries, monitoring the effectiveness of antibiotic formulations has been an ongoing process by independent research

institutions and R & D departments within the pharmaceutical industry. Initially this was carried out as a quality control service to the dairy industry and also for product promotional purposes within the chemical industry. Over time this testing and evaluation has been brought under governmental control by organisations like the Food and Drug Administration (F.D.A.) in the U.S.A. and the National Drugs Advisory Board (N.D.A.B.) in Ireland. Efficacy studies now form a major part of a comprehensive dossier which must be presented to the N.D.A.B. for approval before a manufacturing and distribution licence is granted. Manufacturers of intramammary antibiotics must now provide results of scientific studies in support of claims of efficacy made for their products.

1.4.1 Ongoing requirement to evaluate the efficacy of intramammary antibiotics

The present study was undertaken initially to evaluate the effectiveness of formulations designed for use in dry cows and heifers. These data were used to provide a quality control service on antibiotic formulations to the dairy industry. Over time, the project changed direction to provide a service of testing under contract to the pharmaceutical industry in order to satisfy the regulations laid down by the authorities licencing the sale of antibiotics in countries where the products were offered for sale. Coupled with this ongoing requirement, data were extrapolated on the pathogen types associated with mastitis infection in cows at drying off. These data now impact on the effectiveness of mastitis control systems which are targeted at reducing SCC levels in herds attempting to comply with the EC milk quality regulations.

CHAPTER 2

LITERATURE REVIEW

2.1 Bovine mastitis

Bovine mastitis is the most intractable of the major diseases of dairy cattle (Dodd, 1987). It is a multifactorial infectious disease involving microorganisms with differing characteristics and hosts with varying degrees of resistance to disease. The development of infection and mastitis is an interaction between the natural defence mechanism of the mammary gland and the pathogenicity of the microorganisms in contact with the teat orifice and teat canal (International Dairy Federation, 1987).

2.2 Definitions

The International Dairy Federation (I.D.F.) defines mastitis as an inflammatory change of the mammary gland which, along with physical, chemical and microbiological changes, is characterised by an increase in somatic cells in milk and by pathological changes in the mammary tissue (I.D.F., 1971). This definition is further divided into five major subgroups (I.D.F., 1967) as follows:

2.2.1 Normal udders

show no outward signs of a pathological condition and the milk is free from pathogenic organisms and has a normal SCC.

2.2.2 Latent infections

show no outward signs of a pathological condition, pathogens are present in the milk and the SCC is normal.

2.2.3 Subclinical mastitis

shows no outward signs of a pathological condition, pathogens

are present in the milk and the SCC is greater than 500,000 *per ml*. The chemical composition of the milk is altered also.

2.2.4 Clinical mastitis

2.2.4.1 Acute mastitis

shows obvious symptoms of inflammation of the udder such as heat, pain and swelling. The milk is macroscopically abnormal and the animal is febrile.

2.2.4.2 Subacute mastitis

shows no obvious changes in the udder but clots persist, especially in the foremilk.

2.2.4.3 Non-specific or aseptic mastitis

shows no recognisable infection and the symptoms may be subclinical or clinical.

2.2.5 Chronic mastitis

Chronic mastitis occurs when a quarter fails to respond to treatment (usually three complete courses of antibiotic treatment) over a period of time. The quarter may atrophy or show abnormal clinical changes for the rest of the animal's life.

These definitions apply to the examination of quarter milk samples taken at milking time (after discarding the foremilk). In these definitions, the SCC threshold value of normal foremilk is recognised as being equal to or less than 500,000 *per ml*. This threshold value is only suitable in relation to the examination of quarter milk samples (I.D.F., 1967).

2.3 Financial losses associated with mastitis

In Europe, mastitis remains the most widespread disease of dairy cows.

Conservative estimates show that the disease is costing the EC over £2,000 million each year (Booth, 1992). Losses in the United States are estimated to be $2 billion annually (Nickerson, 1990a).

Egan (1989) estimated a cost of IR£60 to IR£70 million annually to the Irish dairy industry. The cost of treating a single clinical case of mastitis was estimated to be in the region of IR£50 (Meaney, 1985). This estimate was based only on the cost of the antibiotics and the cost of discarding milk from the treated animal during the treatment and recommended milk withholding periods.

2.4 Mastitis control programme

Mastitis is a world-wide problem for the dairy industry. While differences exist among nations, the control of this disease remains basically the same (Nickerson, 1990a).

Schemes to control mastitis world-wide are usually based on a five point plan. These have been in operation since the 1970's and the principal elements of the plan were summarized recently by Bramley (1992) as follows:

1. Apply an approved teat disinfectant after every milking.
2. Treat clinical cases of mastitis.
3. Infuse long-acting antibiotics into all quarters at drying-off.
4. Institute an annual milking machine test and appropriate maintenance.
5. Cull cows showing repeated cases of clinical mastitis.

2.5 Dry period intramammary infection

Studies undertaken in Northern Ireland between 1942 and 1945 evaluated the effectiveness of an alum-precipitated toxoid injection and also a commercially produced anaculture in the prevention of summer mastitis (Pearson, 1950). In that study summer mastitis was most

prevalent in non-lactating cows during the months of July, August and September. During that period, *Actinomyces (Corynebacterium) pyogenes* was the principal agent causing summer mastitis and the incidence in the national herd ranged from two to 10 *per cent* of cows infected with this bacterium. Further experiments carried out by Pearson between 1947 and 1949 compared the effectiveness of penicillin (prepared either in an aqueous or oily base) in reducing dry period infection with *A. pyogenes*. The results showed that either procaine penicillin or calcium penicillin incorporated in an oil base gave 50 to 70 *per cent* protection against *A. pyogenes* invasion of the dry udder. The penicillin formulated in the aqueous base, however, offered little protection (Pearson, 1951).

The importance of the non-lactating period in the control of mastitis in winter was recognised by Neave, Dodd and Henriques (1950). In a herd with an average of 45 to 50 cows, large numbers of new infections developed during the dry period. More than 50 *per cent* of these developed during the first three weeks after the last milking of the lactation. These workers also observed that the rate of new infection in the early part of the dry period was significantly higher than during the previous lactation. The authors suggested that the increase in the level of new infection may have been related to either (i) the method of drying off or (ii) the breaking of the natural seal when samples of secretion were collected as part of the experimental procedure. Oliver, Dodd, and Neave (1956a) also suggested that the increase in infections in the early dry period was probably aided by the fact that milk removal had ceased and also the possibility that changes in the dry period udder secretions may favour bacterial growth. Oliver, Dodd, Neave and Lee (1956b) also concluded that if natural sealing of the teat orifice or teat sinus takes place, it is of minor importance in preventing infection in the early dry period.

2.5.1 Method of drying off

A comparison of drying off by an abrupt cessation or by intermittent milking (Oliver, Dodd and Neave, 1956c) demonstrated that there was a significantly higher incidence of new infection in cows that were dried off by abrupt cessation compared to those dried off by intermittent milking. The method of drying off, however, had no effect on the infections that were already established at the time of drying off or on the milk production in the next lactation.

2.5.2 Control strategies for dry period infection

The control of infection in the dry period depends on the elimination of those infections that are present at drying off and in preventing new infections from occurring during the early part of the dry period (Oliver, Neave and Sharpe, 1962). Methods for controlling the rate of new infection during the dry period were evaluated by Oliver *et al.* (1962). At drying off, uninfected teats were infused with 200,000 units of both penicillin G and dihydrostreptomycin. All teats were challenged by dipping in cultures of *Staph. aureus* and *Strep. uberis* during the dry period. Irrespective of the pathogen type, the infusion of the antibiotics at the end of lactation appeared to give the best protection against new infection of the dry udder. As a result of this experiment, it was also concluded that pathogens that contaminate the teats at the end of lactation are an important source of udder infection in the dry period. This may be a particular problem in herds where routine post-milking teat skin disinfection is not practiced during the lactation. In herds where teat disinfectants are not used, as many as 50 to 70 *per cent* of teat orifices may be colonised with *Staph. aureus* (Neave, 1971; Meaney, 1974). As a result, teat canal colonisation could be a prelude to intramammary infection (du Preez, 1985).

2.5.3 Bacteria isolated from infected quarters at drying off

Mastitis can be caused by any one or more than 100 microorganisms but 95 *per cent* of all cases are caused by *Staph. aureus*, *Strep.agalactiae*, *Strep. dysgalactiae* and *Strep. uberis*. The remaining five *per cent* of cases are caused by coliforms and other organisms (Nickerson, 1990b). *Staph. aureus* is gradually replacing streptococcal spp. as the dominant isolate (Sandholm, Kaartinen and Pyorala, 1990).

The majority of intramammary infections detected at drying off are caused by Gram-positive bacteria. The major pathogen types isolated are *Staph. aureus* and a variety of streptococcal spp. This infection pattern has not changed during the last 25 years. Similar trends have been reported in most dairying countries (Smith, Westgarth, Jones, Neave, Dodd and Brander, 1967; Pearson and Wright, 1969; Langley, Meaney, Cullen and Cunningham, 1971; Wilson, 1973; Schultze and Mercer, 1976; Poutrel, 1977; Storper and Ziv, 1985; Buddle, Herceg, Ralston and Pulford, 1987; Batra, 1988; Browning, Mein, Barton, Nicholls and Brightling, 1990).

2.5.3.1 Staphylococcus aureus

Staph. aureus causes a contagious form of mastitis that is difficult to control (Nickerson, 1990a; Beretta and Carli, 1992). This bacterium also causes pustular dermatitis and teat skin lesions (Neave, 1971) which expose the teats to infections. The poor rate of bacteriological cure of *Staph. aureus* mastitis is largely due to physiological and physical barriers to effective drug/organism interaction (Francis, 1989). *Staph. aureus* infection may also become chronic and refractory to antibiotic therapy because of the organism's ability to survive within the mammary gland, in macrophages and polymorphonuclear neutrophils (Sandholm *et al.*, 1990). Very few antibiotics can achieve the effective intracellular concentrations required to eliminate *Staph. aureus* (Sandholm *et al.*, 1990).

2.5.4 Dry period antibiotic therapy

The use of antibiotic therapy at drying off has been advocated as an effective method of reducing the level of mastitis during the dry period (Pearson, 1951; Smith *et al.*, 1967; Dodd and Neave, 1970; Bramley and Dodd, 1984; Eberhart, 1986; Batra, 1988; Francis, 1989).

As a result of efficacy studies carried out on a variety of antibiotic formulations (Smith *et al.*, 1967; Pugh, Harris, Marshall and Evans, 1973; Meaney, 1976a; Meaney and Nash, 1977; Curtis, Hendy, Watson, Harris, Davies and Marshall, 1977; Ziv, Storper and Saran, 1981) dry period therapy has been an integral component in mastitis control programmes. Dry period treatment is targeted at eliminating existing subclinical mastitis and also reducing the incidence of new infection during the dry period (Smith *et al.*, 1967).

2.5.4.1 Use of multiple infusions of antibiotics during the dry period

All infections, particularly those caused by *Staph. aureus,* are not necessarily eliminated following antibiotic treatment during the dry period. Because of this, some workers have experimented with the use of additional antibiotic infusions during the dry period. Multiple infusions have been used, therefore, to maintain elevated concentrations of antibiotic in non-lactating udders for longer periods. A study by Smith, Rautenbach, Dodd and Brander (1975) showed that two infusions of 500 mg benzathine cloxacillin (one at drying off and a second 21 days after drying off) eliminated almost 90 *per cent* of *Staph. aureus* infections which had been diagnosed at drying off. In Israel, a study by Storper and Ziv (1985) compared the efficacy of single and double infusions (two infusions administered simultaneously at drying off) using three antibiotic formulations. The formulations used in this study were : (i) 100 mg nafcillin, 250,000 iu procaine penicillin and 250 mg dihydrostreptomycin; (ii) 500 mg benzathine cloxacillin and (iii) 500 mg benzathine cloxacillin and 500 mg neomycin. The results

showed that double infusions with either of these products did not improve efficacy. Cummins and McCaskey (1987) also concluded that multiple dry cow treatments using 500 mg benzathine cloxacillin did not offer any advantage over single treatments.

2.5.4.2 Selective antibiotic therapy at drying off

Some researchers have suggested that dry cow antibiotic therapy should be used only in cows with infected udder quarters at drying off (Edwards and Smith, 1967).

Bratlie (1973) was not in favour of the routine use of dry cow therapy in all cows (infected and uninfected) since approximately 70 *per cent* of the cows in his study were free from pathogens at drying off. This view was also supported by Serieys and Roguinsky (1975), Morse (1975) and Funke (1975).

Under Australian conditions of seasonal calving and pasture feeding, relatively few new infections developed during the dry period in cows that were uninfected at drying off. In an experiment involving 700 cows, it was concluded that dry cow therapy was not required as a means of preventing new infections during the dry period in uninfected cows (Browning *et al.*, 1990).

Buddle *et al.* (1987) showed that cows with three or four quarters infected prior to treatment with dry cow therapy were more susceptible to infection in the following lactation. Susceptibility to re-infection or new infection was lower in cows that had either one or two quarters infected at drying off and this trend was also shown in heifers (Buddle *et al.*, 1987). The results of an experiment carried out by Cagienard (1983), however, indicated that in some circumstances the use of dry cow therapy may be conducive to the establishment of new infections. This author advocated a reduction in the widespread use of dry period therapy.

2.5.5 Use of non-antibiotic teat-sealers in the dry period

The level of new infection in herds practising drying-off therapy is still very variable. Langley *et al.* (1971) showed new infection levels to vary from one to four *per cent.* Serieys and Roguinsky (1975) and Morse (1975) reported new infection levels of 24 *per cent* and 34 *per cent,* respectively. An experiment carried out by Browning *et al.* (1990) in Australia showed new infection levels to vary between 3.9 and 8.9 *per cent.* When the rate of new infection is low, some workers have suggested that dry cow antibiotic therapy should not be used to protect the uninfected quarters at drying off (Cagienard, 1983; Browning *et al.*, 1990).

Sealing the teats of uninfected cows at drying-off may provide an acceptable alternative. External applications of sealers reported by Oliver, Dodd and Neave (1956d) failed to achieve satisfactory control, 48 hours after application. Farnsworth, Wyman and Hawkinson (1980) also reported on the use of an acrylic latex teat-sealer. The sealer was applied externally by teat dipping after milking. This sealer was effective in reducing the incidence of subclinical mastitis. The authors also recommended its use particularly for the control of coliform infections.

Pearson (1949) sealed teats internally with a bismuth iodoform paraffin paste. However, the product was either absorbed or neutralised within 14 days of infusion. Various propamidine cream and jelly compounds were also evaluated and all either lacked persistence or caused irritation to the udder (Pearson, 1949).

The use of a bismuth subnitrate and acriflavine internal teat-sealer was also considered as an alternative to antibiotic therapy in uninfected cows (Meaney 1976b; Meaney, 1977).

2.5.6 Project objectives

The primary objective of the studies reported in this thesis was to evaluate the efficacy of a variety of intramammary products which were recommended for the control of mastitis during the non-lactating period.

The specific aims were:

1. to evaluate the efficacy of a variety of intramammary antibiotics;

2. to evaluate the efficacy of non-antibiotic teat-sealers; and

3. to survey the pathogens which were responsible for mastitis infections at drying off.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 General experimental protocol

A series of 21 experiments was carried out over a 25-year period (1968 to 1992) to evaluate the effectiveness of a variety of long-acting (dry cow) antibiotic formulations, non-antibiotic internal teat-sealers and different treatment regimes. In general, the milk sampling, drying off and infusion techniques were similar for all the experiments. The laboratory methods used to identify the causative organisms associated with infection and the cytological examinations of milk were also similar for all the experiments. The definitions of subclinical and clinical mastitis were standardised. A general protocol, therefore, is described for all the experiments and any changes for individual experiments are identified and described.

3.2 Selection of cows for dry period experiments

All experiments were carried out in Friesian cows and heifers. Infected and uninfected cows and udder quarters, as required for individual experiments, were selected following analyses of milk samples collected from individual udder quarters at seven to ten days before drying off. These samples were always collected before milking and in an aseptic manner. The in-calf heifers used in Experiment 17 were selected as infection-free based on analyses of samples of secretion collected at selected intervals before calving.

3.3 Aseptic collection of milk samples before drying off

A milk sample was collected from each udder quarter before the last milking of the lactation. The teats were disinfected with cotton wool swabs impregnated with methylated spirits before the milk samples were taken. If the teats were very dirty, several swabs were used.

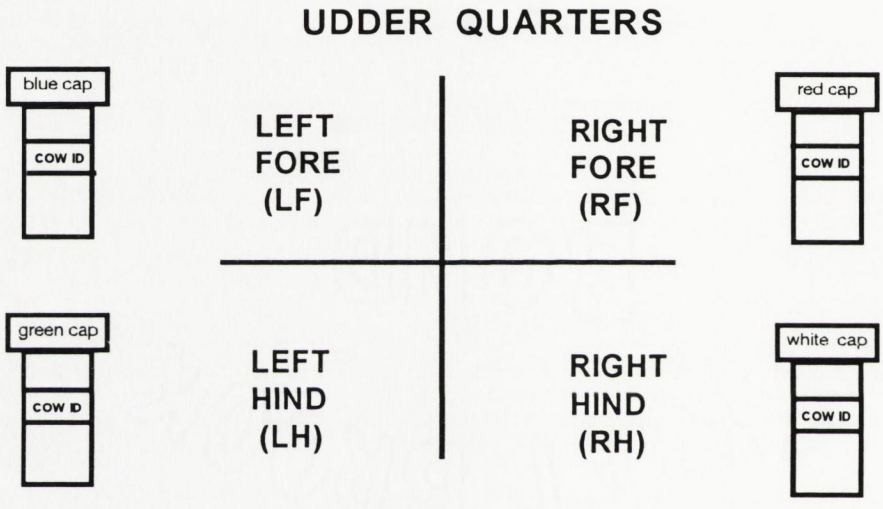


Figure 1: Identification of milk sample from each udder quarter

As a general rule, dirty teats were not washed with water before sampling. Three squirts of milk were discarded before the milk samples were collected into each of four sterile sample bottles. The coding used to identify the teats and the sample bottles is illustrated in Figure 1. All samples were refrigerated at approximately $4^{o}C$ before analysis.

3.3.1 Milk sampling procedure during the dry period

If a quarter developed clinical signs of mastitis during the dry period, a sample of secretion was collected in an aseptic manner before therapy was administered. These samples were usually collected by the herdsman.

3.3.2 Milk sampling procedure after calving

Foremilk or colostrum samples were collected by the herdsmen from all cows at calving (before the first machine milking) using the sampling procedures already described for cows at drying off. A second set of foremilk samples was collected by the technical staff seven to 14 days after calving. If a quarter developed clinical signs of mastitis in the interval between the first and second post-calving samplings then a further milk sample was collected before applying medication.

3.4 Drying off procedure

In all experiments (with the exception of the commercial herds in Experiment 1, where the drying off procedure was not recorded) the cows were dried off abruptly at the end of lactation. The average level of milk production at drying off was usually ≤5 kg per day. Again, this was not recorded for the cows in the commercial herds in Experiment 1. A higher average drying off level of milk production was used in Experiment 7 (≤8.6 kg per day).

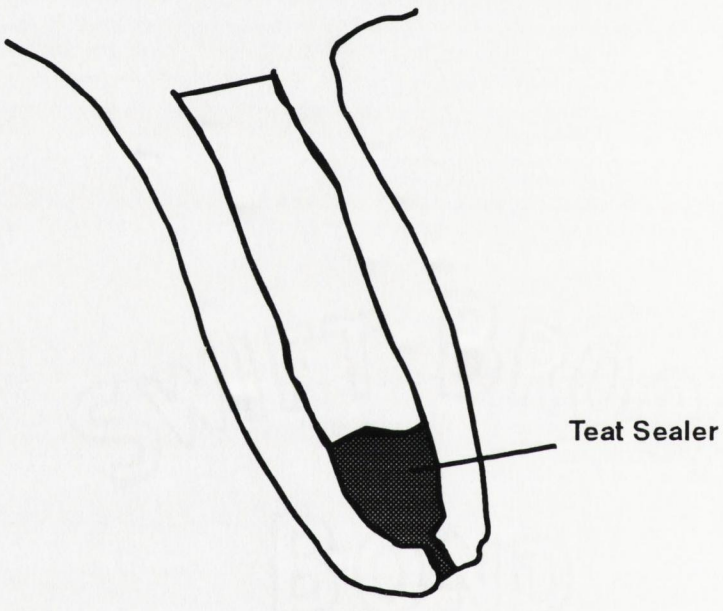


Figure 2: Location of the sealer in bovine teat as described by manufacturer

3.5 Antibiotic infusion technique

All the antibiotic infusions at drying off were administered by trained technicians. After the last milking of the lactation, the tip of each teat was disinfected with a cotton wool swab impregnated with methylated spirits. The formulations were infused as recommended by the manufacturer. The complete nozzle tip of either the aluminum tube (used in the earlier experiments) or the plastic syringe was inserted into the teat prior to infusing its contents into the mammary gland. After infusion, the tip of the teat was held between the thumb and forefinger of one hand and the infusion was massaged upwards into the gland using the thumb and forefinger of the other hand. After infusion, the teats were either dipped or sprayed with a teat skin disinfectant.

3.5.1 Teat-sealer infusion technique

The teat-sealers were infused at drying off from plastic syringes of a similar type to those used for intramammary antibiotics. The sealer was not manipulated in the teat after infusion. This was to allow the seal to form a plug in the teat sinus and duct (Figure 2). Where the sealer was used without an antibiotic, it was infused after the last milking of the lactation. In the in-calf heifer study described in Experiment 17, however, the sealer was infused at intervals ranging from 9 to 110 days before calving.

In the experiments where the sealer was used in conjunction with an antibiotic, the antibiotic and sealer were usually presented in separate syringes (Experiments 14, 15, 18 and 20). In Experiment 19, however, the antibiotic and teat-sealer were presented in a single syringe from which the antibiotic was delivered first and the sealer later. The sealers were always infused by trained technicians.

Table 1 The composition of intramammary antibiotic and teat-sealing formulations used in 21 experiments

Antibiotic reference code	Active ingredients	Concentrations	
A - A9	Benzathine cloxacillin	500	mg
B	Penicillin	300	mg
	Sodium novobiocin	250	mg
C	Procaine benzylpenicillin	300	mg
	Dihydrostreptomycin sulphate	250	mg
D - D1	Penethmate hydriodide	100	mg
	Procaine penicillin	300	mg
	Framycetin sulphate	100	mg
E	Procaine penicillin	1,000	mg
	Dihydrostreptomycin sulphate	1,000	mg
F	Procaine penicillin	1,000	mg
	Dihydrostreptomycin sulphate	500	mg
G-G2	Spiramycin	1,200	mg
	Neomycin sulphate	1,000	mg
H-H3	Procaine benzylpenicillin	400	mg
	Diethylaminoethyl penicillin hydriodide	100	mg
	Fradiomycin sulphate	100	mg
I	Cefalexin	500	mg
	Dihydrostreptomycin sulphate	500	mg
J	Cephalonium	250	mg
K	Neomycin sulphate	500	mg
	Hydrocortisone acetate	20	mg
	Hydrocortisone sodium succinate	12.5	mg
L-L7	Bismuth subnitrate*	25	% w/w
	Acriflavine	0.075	% w/w
M-M2	Procaine penicillin **	300	mg
	Dihydrostreptomycin sulphate	300	mg
N	Procaine pencillin	200	mg
	Novobiocin	400	mg

All the formulations with the exception of L-L7 and M-M2 contained antibiotics and were presented in long-acting oily bases.

* L-L7 were non-antibiotic teat seal formulations.

** M-M2 contained antibiotics in an aqueous base.

All the products were recommended for use in non-lactating dairy cows.

3.6 Composition of antibiotic and teat-sealer formulations

The composition of the antibiotic and sealer formulations used in the 21 experiments is presented in Table 1. A summary of the experiment numbers, year in which the experiment was carried out and the type of product evaluated is presented in Table 2. The treatment randomisation system used in the experiments is illustrated in Figure 3.

Table 2 Summary of experiment numbers, year of experiment, and animals and products tested in each experiment (1968-1992)

Chapter	Expt. No.	Year	Animal type	Infection type: Natural	Infection type: Artificial Challenge	Products evaluated
4	1	1968-69	Cows	*		A; B; C
	2	1969-70	Cows	*		A1; Untreated Control
	3	1970-71	Cows	*		A2; D
	4	1973-74	Cows	*		A3; E
	5	1975-76	Cows	*		A4; F
	6	1979-80	Cows	*		A5
	7	1987-88	Cows	*		A6; G
	8	1988-89	Cows	*		A7; G1
	9	1989-90	Cows	*		A8; G2
	10	1989-90	Cows	*		D1 ; H
	11	1989-90	Cows	*		I ; J
5	12	1981-82	Cows	*		K ; Untreated Control
6	13	1972-73	Cows		*	L ; Untreated Control
	14	1972-73	Cows		*	M/L1; Untreated Control
	15	1972-73	Cows	*		M1/L2; Untreated Control
	16	1974-75	Cows		*	A9; L3
	17	1975-76	In-calf heifers	*		L4 ; Untreated Control
	18	1975-76	Cows	*		H1 ; L5
	19	1976-77	Cows	*		H2 ; L6
	20	1978-79	Cows	*		H3 ; L7 ; M2
7	21	1984-89	Cows	*		A; D; G; I; J
	21	1990-92	Cows	*		A; D; H; I; J; N

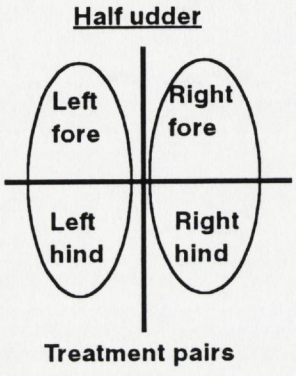
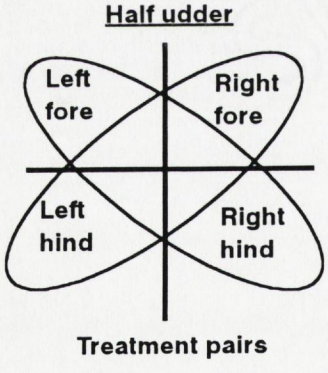
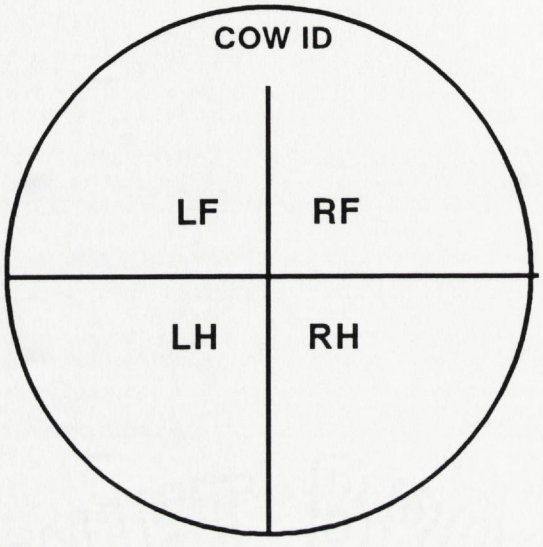
Experiment Type	Experiment Numbers
Complete udder	1; 7; 8; 9; 10; 11; 18; 21
Three quarters infused in each cow	15
	2; 3; 13; 14; 16; 17; 19; 20
	4; 5; 12
Individual infected quarters	6
Experiments using untreated controls	2; 12; 13; 14; 15

Figure 3: Treatment randomisation system used in all experiments



Aesculin blood agar plate

Figure 4: Method of culturing quarter foremilk samples on blood agar plate

3.7 Coding system for antibiotic and teat-sealer formulations

The antibiotic and teat-sealer formulations were allocated an experiment code. Benzathine cloxacillin, for example, was coded A in Experiment 1. When that product was used again in additional experiments it was coded A1, A2 ..... A9. A similar coding system was used for other formulations (Table 1).

3.8 Laboratory Analyses

3.8.1 Microbiological examination of milk

Milk samples (0.025 ml) from the individual udder quarters were streaked on the surface of aesculin blood agar plates (ABA) and incubated aerobically at 37°C. The samples from the four quarters of each cow were streaked on separate quadrants of an ABA plate (Figure 4). The plates were examined after 24 and 48 hours incubation. The ABA was prepared from blood agar base No. 2 (Oxoid[1] Code CM 271) to which 0.1 *per cent* aesculin and 7 *per cent* citrated whole calf blood were added.

3.8.1.1 Identification of Staphylococcus aureus

The major pathogens were identified morphologically. *Staph. aureus* isolates were classified using the slide coagulase test. Doubtful slide test readings were confirmed using the tube coagulase test (IDF, 1971). The modified Gram stain (Preston and Morell, 1962) was also used to categorise the bacteria into Gram-positive or Gram-negative groups.

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[1] Unipath Ltd., Wade Road, Basingstoke, Hampshire, RG24, OPW, England.

3.8.1.2 Identification of streptococcal spp.

In the earlier experiments (1968-1980) the streptococci were classified initially on their appearance on the ABA plate and also on their reaction to the Gram stain. *Strep. agalactiae* was identified by its bluish colour on the aesculin plate. *Strep. dysgalactiae* was identified by its green appearance on the ABA plate. Finally, aesculin-hydrolysing colonies were identified by their brown appearance on the ABA plate (IDF, 1971). In the more recent experiments (1981-1992), all presumptive streptococcal isolates were subjected to a series of additional carbohydrate fermentation tests (IDF, 1981).

3.8.1.3 Identification of Actinomyces pyogenes

A. pyogenes was identified primarily on cultural characteristics on ABA and also on its reaction to the Gram stain.

3.8.1.4 Identification of Gram-negative spp.

Between 1968-1982 coliform spp. were identified on their appearance on aesculin blood agar and McConkey Agar (Oxoid Code CM 7) and on their reaction to the Gram stain. In experiments carried out after 1982, the API 20E classification system for Enterobacteriaceae (La Balme Les Grottes, France) was used in addition, to identify the coliform spp.

3.8.1.5 Identification of fungi

Fungi were identified on cultural characteristics on ABA. No attempt was made to identify the isolates further.

3.9 Preparation of bacterial challenge cultures

Four strains of *Staph. aureus*, which were previously isolated from cows with clinical mastitis, and one strain of *Strep. dysgalactiae*, CE127,

were used in the challenge experiments 13 and 14. *Staph. aureus* cultures alone were used in Experiment 16. Two colonies from each strain were sub-cultured from a 16h culture on ABA to 10 ml of brain heart infusion broth (BHI, Oxoid Code CM 225). The staphylococci were grown in mixed culture in one container and the streptococcus was grown separately. After overnight incubation (approximately 16h) each culture was added to a 400 ml mixture of antibiotic-free skim milk and BHI (50:50 concentration) in two separate containers. After six hours incubation at 37°C, the number of colony forming units (cfu *per ml)* of each species was assessed by colony counts on blood agar. Both stock cultures were mixed and stored at 4°C before use. Fresh stock cultures were prepared each week. The concentrations of the cultures used in individual experiments (13, 14 and 16) are expressed as the mean number of colony forming units *per ml* for all cultures prepared during each experiment.

3.10 Cytological examination of milk

The milk samples collected from each udder quarter were also tested to evaluate the somatic cell concentration using the qualitative California Mastitis Test (CMT) (Schalm and Norlander, 1957). The reading of the reaction was standardised for all of the experiments (Table 3).

3.10.1 Somatic cell count analysis

Somatic cell count analysis was introduced into the laboratory in 1977. All milk samples in experiments carried out after 1977, therefore, were also analysed for SCC. The Fossomatic 180 somatic cell counter (Foss Electric, Hillerød, Denmark) was used after it had been calibrated and validated using the microscopic method of Prescott and Breed (1910). Later it was standardised following the procedures set out in the IDF standard methods (IDF, 1984).

Table 3 : California Mastitis Test (CMT) codes and reactions

Reaction	Code	Definition of Reaction
Negative	-	Mixture remains liquid with no evidence of formation of precipitate
Trace	+/-	A slight precipitate, which tends to disappear with a continued movement of the paddle
Weak positive	+	A distinct precipitate, but no tendency towards gel formation
Distinct positive	++	The mixture thickens immediately with definite gel formation. As the mixture swirls it tends to coagulate towards the centre of the paddle
Strong positive	+++	A distinct gel forms which tends to adhere to the bottom of the paddle and during swirling, a distinct central peak forms on the gel

Table 4 : Classification of clinical mastitis

Macroscopic estimation of abnormalities in the milk from clinical cases of mastitis

Code	Description of milk
C1	Milk appears normal in colour, but contains very small flaky clots
C2	Milk appears normal in colour but contains very definite clots
C3	Milk appears slightly abnormal in colour and/or contains definite clots
C4	Milk appears grossly abnormal in colour and/or contains very large clots

3.11 Definitions of mastitis and infection

3.11.1 Subclinical mastitis:

(a) A CMT reading of ++ or +++ and the isolation of pathogens in pure or mixed culture.

or

(b) A somatic cell count equal to or greater than 500,000 *per ml* and the isolation of pathogens in pure or mixed culture.

3.11.2 Clinical mastitis:

A CMT reading of +++ in macroscopically abnormal milk containing pathogens in pure or mixed culture.

3.11.3 Clinical non-specific mastitis:

A CMT reading of +++ in macroscopically abnormal milk from which pathogenic bacteria were not isolated.

3.11.4 Classification of clinical mastitis

Abnormalities observed in the milk (clotting, discolouration etc.) were also recorded when performing the CMT test. An arbitrary score of C1, C2, C3 and C4 (Table 4) was used to indicate the severity of clotting and discolouration in the milk. The method used to classify clinical mastitis was based on a method used by Neave 1971 (personal communication).

The CMT test was used in all the experiments. After 1977, the SCC was also carried out in conjunction with the CMT. The SCC assay, however, was never performed on colostrum samples or on abnormal milk samples. The CMT was carried out on colostrum, normal milk and abnormal milk samples.

3.11.5 Environmental mastitis

The definition "environmental mastitis" is a general term used in this thesis to describe infections associated with *Strep. dysgalactiae*, aesculin hydrolysing streptococcal spp., *A. pyogenes* and all Gram-negative spp.

3.11.6 Definition of mastitis at drying off

An udder quarter was defined as mastitic at drying off, if a subclinical, clinical or non-specific case of mastitis was identified.

3.11.6.1 Definition of mastitis during the dry period

A quarter was defined as mastitic during the dry period if:

(a) signs of clinical mastitis were evident on palpation and pathogens were isolated from the secretion

(b) if pathogens were isolated from the secretion but there were no obvious signs of mastitis.

The CMT or SCC analyses were not used to support the diagnosis when assessing infection or mastitis during the dry period.

3.11.7 Definition of mastitis after calving

The definition of mastitis after calving was based on the same criteria as described for mastitis at drying off.

3.12 Elimination of mastitis during the dry period

3.12.1 Infection elimination

An infection was considered to be eliminated during the dry period if the pathogens isolated at drying off were not present at the next calving or at the second sampling after calving. Elimination of infection was also based on a reduction in CMT to + or less and/or a reduction in SCC to less than 500,000 *per ml.*

3.12.2 Elimination of non-specific mastitis

Non-specific mastitis was considered to be eliminated during the dry period if the milk from the affected quarter was normal (free from clots and discolouration) at the two samplings after calving.

3.13 Continuation of infection (treatment failure)

Infection was considered to have continued if the pathogens isolated at the samplings after calving were similar to those isolated at drying off. Treatment failure was also recorded in quarters that had eliminated the bacterium that was present at drying off and had been subsequently infected with a different pathogen during the dry period.

3.13.1 Non-specific mastitis

Non-specific mastitis was considered to have continued during the dry period if the milk from the affected quarter continued to show signs of clinical mastitis after calving.

3.14 New infection during the dry period

A new infection: When an udder quarter that was free of mastitis at drying off developed an infection or non-specific mastitis either during the dry period or at the next calving. A new infection was also recorded

in infected quarters that had eliminated the bacterium that was present at drying off and had been subsequently infected with a different pathogen during the dry period.

3.15 Statistical analyses

3.15.1 Chi-square test

A chi-square test for independent samples was used to test the homogeneity of response across the treatments under investigation (Siegel, 1956). This test was used in most of the experiments. Where a different test was used, it is indicated in the results for the particular experiment.

3.15.2 Fisher's exact probability test

In the case of independent samples for a two-by-two table where the frequencies were small, Fisher's exact probability test was used to compare treatment responses (Siegel, 1956). This test was used on the data generated in Experiment 7.

3.15.3 Cochran's Q test

The Cochran's Q test was used to compare effects with related samples (Siegel, 1956). This test was used in Experiment 12 (Tables 18 and 19).

CHAPTER 4

EVALUATION OF DRY COW ANTIBIOTIC PRODUCTS IN ELIMINATING INFECTIONS AT DRYING OFF

4.1 Introduction

Mastitis in dairy cows frequently occurs during the dry period and in early lactation (Batra, 1988; Nickerson, 1990a). These high risk infection periods were first identified by Neave *et al.* (1950).

The infusion of long-acting antibiotics at drying off is an effective method of reducing mastitis in the dry period (Smith *et al.*, 1967; Francis, 1989). For this purpose, a variety of antibiotic formulations have been developed and manufactured by the pharmaceutical industry in co-operation with research institutions. These formulations are presented for intramammary infusion using single antibiotics or a combination of different antibiotics. As a result of efficacy studies carried out on a variety of these formulations, dry cow therapy is now incorporated as part of mastitis control programmes in all the major dairying countries.

A series of 11 experiments are reported in this chapter. These were carried out between 1968 and 1990. The efficacy of a variety of antibiotic products was evaluated, primarily on their ability to eliminate *Staph. aureus* infections which had been diagnosed at drying off.

The products contained the following major active ingredients : benzathine cloxacillin; penicillin/novobiocin; penicillin/framycetin; penicillin/dihydrostreptomycin; neomycin/spiramycin; penicillin/fradiomycin; cefalexin/dihydrostreptomycin; and cephalonium.

In Experiment 2, the effectiveness of benzathine cloxacillin was compared with an untreated control. An evaluation was also made on the effectiveness of a single or double (one infusion at drying off and a second infusion 28 days later) infusion of benzathine cloxacillin in eliminating *Staph. aureus* during the dry period (Experiment 6).

Summary data were only available for Experiments 1 to 6 (1968 to 1980) when this thesis was written. Therefore the results of the more recent experiments (7 to 11) are presented in greater detail (Tables 12 to 16).

4.2 Experiment 1 (1968-1969)

The effectiveness of benzathine cloxacillin, penicillin and novobiocin, or penicillin and dihydrostreptomycin in eliminating Staph. aureus infections at drying off

Twenty-one dairy herds, each having 25 cows or more, were selected. Three were research herds attached to Moorepark Research Centre. Eighteen were selected commercial herds located within an 80 km radius of Moorepark. In total, 630 cows, all machine milked, were included in the study. The cows in the herds attached to Moorepark were milked twice daily at 16/8h (night/day) intervals between milking. The cows in the commercial herds were milked twice daily but between-milking intervals were not recorded. The effectiveness of three antibiotic formulations was evaluated on their ability to eliminate existing intramammary infection and to reduce the level of new infection during the dry period. The formulations contained the following active ingredients: benzathine cloxacillin (Product A), penicillin and novobiocin (Product B), and penicillin and dihydrostreptomycin (Product C). The formulation and concentration of each active ingredient in each product is presented in Table 1. The treatments were assigned to cows at random within herds and selected cows were infused in all quarters with either product A, B or C.

4.2.1 Results of Experiment 1

There was no difference in efficacy between the cloxacillin and penicillin/novobiocin or between the cloxacillin and penicillin/dihydrostreptomycin products. The penicillin/novobiocin was more effective ($P<0.01$) than the penicillin/dihydrostreptomycin product (Table 5).

New *Staph. aureus* infections developed in 13, 12 and 21 udder quarters of cows treated with products A, B and C, respectively.

All three formulations eliminated over 90 *per cent* of a total of 87 streptococcal infections that were present at drying off.

Table 5 : The effectiveness of benzathine cloxacillin, penicillin and novobiocin, or penicillin and dihydrostreptomycin in eliminating *Staph. aureus* infections at drying off (1968-1969)

Antibiotic formulation	Number of infected quarters	Number of infections eliminated (%)
Benzathine cloxacillin (A)	124	90 (73)
Penicillin/ novobiocin (B)	135	106[a] (79)
Penicillin/ dihydrostreptomycin (C)	121	75[b] (62)

from Langley, O.H., Meaney, W. J., Cullen, N. P. and Cunningham, J. F. (1971)

[ab]Values with different superscripts differ significantly ($P<0.01$)

4.3 Experiment 2 (1969-1970)

The effectiveness of benzathine cloxacillin in the treatment of Staph. aureus infections when compared with an untreated control

In the farm trial described in Experiment 1, there were no untreated controls. In Experiment 2, therefore, the efficacy of a cloxacillin product (A1) was compared with an untreated control. A within-cow experiment was set up in one research herd attached to Moorepark. The two right side udder quarters (right fore and right hind) of each cow were infused with the product A1. The two left side udder quarters (left fore and left hind) were selected as untreated controls (Figure 3). Ninety-one cows were treated. There was one inactive quarter at the time of treatment in the udder quarters selected for infusion, which resulted in 181 udder quarters infused with product A1 and 182 quarters used as untreated controls. All cows were machine-milked twice daily and were managed under similar conditions to those already described for the research herds in Experiment 1. All cows were treated regardless of lactation age.

4.3.1 Results of Experiment 2

Fifteen (79 *per cent*) of the 19 *Staph. aureus* infections treated at drying off were eliminated. Five (17 *per cent*) of the 30 *Staph. aureus* infections which were not treated during the dry period recovered spontaneously. This result was statistically significant ($P<0.001$). Four and seven new *Staph. aureus* infections developed in the treated and control quarters, respectively. The infection level with *Staph. aureus* in the treated udder quarters was reduced by 58 *per cent* and there was an overall increase (of 6 *per cent*) in infections associated with *Staph. aureus* in the untreated udder quarters.

Table 6 : The effectiveness of benzathine cloxacillin in the treatment of *Staph. aureus* infections when compared with an untreated control (1969-1970)

Treatment	Number of infected quarters	Number of infections eliminated (%)
Benzathine cloxacillin (A1)	19	15[a] (79)
Control (untreated)	30	5[b] (17)

[ab]Values with different superscripts differ significantly ($P<0.001$)

4.4 Experiment 3 (1970-1971)

The effectiveness of benzathine cloxacillin or penicillin and framycetin in eliminating Staph. aureus infections at drying off

Product C, tested in Experiment 1, was removed from the market. A new product containing penicillin and framycetin (Product D, Table 1) was compared with the benzathine cloxacillin product (coded A2) used in Experiment 1.

A within-cow experiment was set up in one research herd attached to Moorepark in the 1970 drying-off season. Again, this was designed as a half-udder experiment which was similar to Experiment 2; 98 cows were treated. The right fore and right hind udder quarters were infused with product A2 and the left fore and left hind udder quarters were infused with Product D (Figure 3). There was one inactive udder quarter in each

treatment set which resulted in 195 quarters infused with product A2 and 195 with Product D. The drying-off procedures and management practices were similar to those already described for Experiment 2. All cows were treated, regardless of lactation age.

4.4.1 Results of Experiment 3

Forty *Staph. aureus* infections were treated with benzathine cloxacillin and 39 were treated with the penicillin and framycetin formulation. Thirty-three (83 *per cent*) of the infections treated with benzathine cloxacillin were eliminated. Twenty-nine (74 *per cent*) of the 39 *Staph. aureus* infections treated with the penicillin and framycetin were also eliminated (Table 7). These differences were not statistically significant. New *Staph. aureus* infections developed in six quarters treated with benzathine cloxacillin and in two quarters treated with penicillin/framycetin.

The benzathine cloxacillin eliminated all of the five *Strep. agalactiae* infections and all of the three non-specific clinical cases of mastitis present at drying off. All the cows in this experiment had been quarter sampled individually at monthly intervals during the previous lactation. These samples were examined microbiologically and SCC determined by CMT analysis so that it was possible to identify 13 *Staph. aureus* infections which had persistently failed to respond to therapy. These were classified as chronic. Seven of these cases were included in the 40 cases that were treated with benzathine cloxacillin; in three cases the *Staph. aureus* was eliminated. The remaining six were included in the 39 cases treated with penicillin and framycetin; the infection persisted in each of them.

Table 7 : The effectiveness of benzathine cloxacillin or penicillin and framycetin in eliminating *Staph. aureus* infections at drying off (1970-1971)

Antibiotic formulation	Number of infected quarters	Number of infections eliminated (%)
Benzathine cloxacillin (A2)	40	33 (83)
Penicillin/ framycetin (D)	39	29 (74)

4.5 Experiment 4 (1973-1974)

The effectiveness of benzathine cloxacillin or penicillin and dihydrostreptomycin in reducing infections during the dry period

The effectiveness of a product containing a mixture of penicillin and dihydrostreptomycin (Product E, Table 1) was compared with that of a benzathine cloxacillin product (coded A3). Product A3 was used as a control because efficacy trends had been established for this product over the three previous experiments. In total, 181 cows were selected in two research herds attached to Moorepark. Both products were used in all cows. Each product was assigned in a random manner to either the right fore and left hind quarters or to the left fore and right hind quarters (Figure 3). There were four inactive quarters in the group treated with benzathine cloxacillin and three in the group treated with penicillin and dihydrostreptomycin. The drying-off procedures and management were similar to those already described for Experiment 3. All cows were treated, regardless of lactation age.

4.5.1 Results of Experiment 4

Product A3 was infused into 55 infected quarters and Product E was infused into 47 infected quarters. The number of infections eliminated during the dry period is shown in Table 8. The types of infections present at drying off and the subsequent response to therapy for both products are shown in Table 9. Thirty-three (72 *per cent*) of the 46 *Staph. aureus* infections treated with benzathine cloxacillin were eliminated. Thirty-one (72 *per cent*) of the 43 *Staph. aureus* infections treated with penicillin and dihydrostreptomycin were also eliminated. In addition to the bacterial infections (Table 9), 17 non-specific clinical mastitis cases were treated. Seven of these were infused with Product A3 and 10 with Product E. All 17 were cured.

Twenty-three new infections developed during the dry period in quarters that were free of infection at drying off. Twelve of these infections developed in quarters that had been infused with benzathine cloxacillin and 11 in quarters infused with penicillin/dihydrostreptomycin. Six quarters that were infected at drying off had the original infection eliminated and were subsequently infected with different pathogen types. Two of these infections developed in quarters treated with benzathine cloxacillin and four in quarters treated with penicillin/dihydrostreptomycin.

The two products were equally effective in eliminating infections at drying off.

Table 8 : The effectiveness of benzathine cloxacillin or penicillin and dihydrostreptomycin in reducing infections during the dry period (1973-1974)

Antibiotic formulation	Number of quarters	Number of quarters infected at drying off	Number of infections eliminated (%)
Benzathine cloxacillin (A3)	358	55	49 (89)
Penicillin/ dihydrostreptomycin (E)	359	47	44 (94)

Table 9 : Bacteria isolated from infected quarters at drying off and the subsequent response to antibiotic treatment during the dry period (1973-1974)

	Antibiotic treatment			
	Cloxacillin (A3)		Penicillin/dihydrostreptomycin (E)	
Pathogen	No. of infections treated	No. of infections eliminated (%)	No. of infections treated	No. of infections eliminated (%)
Staph. aureus	46	33 (72)	43	31 (72)
Streptococcal spp.	7	7 (100)	3	3 (100)
Staph. aureus plus streptococcal spp.	2	1 (50)	1	1 (100)
No bacteria isolated	7	7 (100)	10	10 (100)

4.6 Experiment 5 (1975-1976)

The effectiveness of benzathine cloxacillin or penicillin and dihydrostreptomycin in eliminating Staph. aureus infections during the dry period

The effectiveness of Product F (Table 1) containing penicillin and dihydrostreptomycin was compared with a benzathine cloxacillin antibiotic (A4). Product A4 was used as a control because previous efficacy data had been generated with this formulation. A total of 140 cows were selected in two research herds attached to Moorepark. Seventy-five *per cent* of these cows were less than sixth lactation at the time of infusion. Each antibiotic was infused either into the right fore and left hind or the left fore and right hind quarters (Figure 3). Drying off was achieved by an abrupt cessation of milking. The two products were used in all treated cows. A total of 280 udder quarters were infused with Product A4 and 277 quarters were infused with Product F. There were three inactive udder quarters in the group treated with Product F.

4.6.1 Results of Experiment 5

The numbers of quarters infected with *Staph. aureus* at drying off and at the next calving are shown in Table 10. This bacterium was eliminated from 94 *per cent* of infected quarters treated with Product A4 and from 86 *per cent* of infected quarters treated with Product F. These differences were not statistically significant. In addition to the bacterial infections, 24 non-specific clinical cases were treated. Twelve (92 *per cent*) cases returned to normal after treatment with cloxacillin and 11 (100 *per cent*) after treatment with penicillin and dihydrostreptomycin.

Five new *Staph. aureus* infections developed during the dry period (three in the quarters treated with Product A4 and two in the quarters

treated with Product F). One of these occurred in a quarter that had been infected at drying off. A new infection was confirmed in that quarter when the pathogens isolated at calving differed from those at drying off. The two antibiotic formulations were equally effective in eliminating infections during the dry period.

Table 10 : The effectiveness of benzathine cloxacillin or penicillin and dihydrostreptomycin in eliminating *Staph. aureus* infections during the dry period (1975-1976)

Antibiotic formulation	No. of infected quarters	No. of infections eliminated (%)
Benzathine cloxacillin (A4)	35	33 (94)
Penicillin/ dihydrostreptomycin (F)	36	31 (86)

4.7 Experiment 6 (1979-1980)

The effectiveness of a single or a double (one infusion at drying off and a second infusion 28 days later) infusion of benzathine cloxacillin in eliminating Staph. aureus infections during the dry period

A comparison was made between a single and a double infusion of benzathine cloxacillin (coded A5, Table 1) in infected udder quarters at drying off and during the dry period. A total of 171 quarters infected with *Staph. aureus* was selected in cows in three dairy herds. Drying off was achieved by an abrupt cessation of milking.

At drying off, all 171 infected quarters were infused with Product A5. Twenty-eight days after the first infusion, 129 udder quarters were re-infused with the contents of a second syringe of Product A5. The lactation number of the cows treated was not taken into account in the analysis.

4.7.1 Results of Experiment 6

Thirty-four (81 *per cent*) of the 42 infections treated at drying off were eliminated. Ninety-seven (75 *per cent*) of the 129 infections treated on two occasions were eliminated (Table 11). The difference was not statistically significant.

Table 11 : The effectiveness of a single or double infusion of benzathine cloxacillin in eliminating *Staph. aureus* infections during the dry period (1979-1980)

	No. of antibiotic infusions	
	1	2
No. of infected quarters	42	129
No. of infections eliminated (%)	34 (81)	97 (75)

4.8 Experiment 7 (1987-1988)

The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period

The effectiveness of benzathine cloxacillin (Product A6) was compared with spiramycin in combination with neomycin (Product G, Table 1). The cloxacillin product was used as a control because of data generated in six previous experiments. Product G was a formulation not used previously in Ireland.

In total, 227 cows were selected in five research herds attached to Moorepark. One hundred and twelve cows were infused in all udder quarters at drying off with Product A6 and 115 cows were infused in all udder quarters with Product G. The cows ranged from first to twelfth lactation at the time of infusion. All cows were dried off by an abrupt cessation of milking when the average milk production was less than 8.6 kg per day.

4.8.1 Results of Experiment 7

At drying off, there were 19 cows infected in each group (Table 12). Twenty-five infected quarters were treated with benzathine cloxacillin and 30 with spiramycin and neomycin. Seventeen (68 *per cent*) responded to cloxacillin and twelve (40 *per cent*) responded to the spiramycin/neomycin mixture. This difference was significant ($P<0.058$). *Staph. aureus* and streptococcal spp. were isolated in pure culture from 24 of the infected quarters treated with A6 and from 27 of the infected quarters treated with G. The number of each bacterial type treated and the responses to therapy are presented in Table 12. Of the 17 *Staph. aureus* cases treated with A6, 10 (59 *per cent*) were eliminated and five (25 *per cent*) of the *Staph. aureus* infections treated with G were eliminated. This difference was significant ($P<0.05$).

4.8.2 New infections during the dry period

Of the 112 cows infused with benzathine cloxacillin (Product A6) 19 were infected and 93 were uninfected at drying-off. Ten of the uninfected cows had incomplete infection data at the next calving and these were excluded from the results. Amongst the remaining 83 non-infected cows treated with Product A6 at drying off, 11 developed new infections.

Of the 114 cows infused with spiramycin and neomycin (Product G) 19 were infected and 96 were uninfected at drying-off. Sixteen of the

uninfected cows had incomplete infection data after calving and these were excluded from the results. The remaining 80 non-infected cows treated at drying off with Product G developed six new quarter infections during the dry period. The difference in the new infection levels in the two treatment groups was not significant.

Table 12 : The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period (1987-1988)

	Antibiotic formulation			
	Benzathine cloxacillin (A6)		Spiramycin/ neomycin (G)	
No. of cows infected	19		19	
No. of quarters infected	25		30	
No. of quarter infections eliminated (%)	17	(68)	12	(40)
No. of *Staph. aureus* infections	17		20	
No. of *Staph. aureus* infections eliminated (%)	10	(59)	5	(25)
No. of streptococcal spp. treated	7		7	
No. of streptococcal spp. eliminated (%)	7	(100)	7	(100)
No. of *Staph. aureus* and streptococcal spp.* treated	0		3	
No. of *Staph. aureus* and streptococcal spp.* eliminated (%)			3	(100)
No. of coliform spp. treated	1		0	
No. of coliform spp. eliminated (%)	1	(100)		

* Mixed infections

4.9 Experiment 8 (1988-1989)

The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period

Because of the differences in efficacy between the products tested in the 1987-1988 season (Experiment 7), particularly in the treatment of *Staph. aureus*, it was decided to undertake a smaller controlled experiment to re-evaluate the efficacy of the products. The experiment was undertaken in two commercial herds. All animals were less than sixth lactation at the time of drying off. This selection of cows for Experiment 8 was made in order to reduce the probability of including chronic *Staph. aureus* infections in the treatments. Only infected cows were selected and preferably cows with *Staph. aureus* infections.

Six cows were infused with benzathine cloxacillin (A7) and ten with the spiramycin and neomycin combination (Product G1). All quarters in each cow were treated with the selected products. In these herds, the cows were dried off when the milk yield was less than 1 kg per day.

4.9.1 Results of Experiment 8

In total, six infected cows were treated with cloxacillin and 10 with the spiramycin/neomycin. *Staph. aureus* was isolated from seven of the quarters treated with A7 and from eight of the quarters treated with G1. Two (29 *per cent*) of the *Staph. aureus* infections treated with A7 were eliminated and six (75 *per cent*) of the infections treated with G1 were eliminated (Table 13). Seven streptococcal spp. were also treated with G1 and all of these were eliminated. While these percentage differences appear large they were not significant. This was principally due to the small number of cases in the study.

Table 13 : The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period (1988-1989)

	Antibiotic formulation	
	Benzathine cloxacillin (A7)	Spiramycin/ neomycin (G1)
No. of cows infected	6	10
No. of quarters infected	7	15
No. of quarter infections eliminated (%)	2 (28.6)	13 (87)
No. of *Staph. aureus* infections	7	8
No. of *Staph. aureus* infections eliminated (%)	2 (28.6)	6 (75)
No. of streptococcal spp. infections	0	7
No. of streptococcal spp. infections eliminated (%)		7 (100)

4.10 Experiment 9 (1989-1990)

The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period

A third experiment was undertaken to validate the efficacy of the spiramycin and neomycin combination (Product G2). Again, the comparison was made against the benzathine cloxacillin (Product A8).

In total, 30 infected cows were selected at drying off in four research herds attached to Moorepark. All animals were less than sixth lactation at drying off. Fifteen cows were infused in all udder quarters with Product A8 and a further 15 with Product G2. All cows were dried off by an abrupt cessation of milking when the average daily yield was less than 5 kg per day.

4.10.1 Results of Experiment 9

Staph. aureus was isolated from 17 of the quarters treated with A8 and from 16 of the quarters treated with G2. Ten (59 *per cent*) of the *Staph. aureus* infections treated with A8 and nine (56 *per cent*) of the *Staph. aureus* infections treated with G2 were eliminated (Table 14). Four (67 *per cent*) of six streptococcal spp. treated with A8 were eliminated. The three streptococcal spp. treated with G2 were also eliminated. The difference in efficacy between the two products was not significant.

4.10.2 Combined results of Experiments 8 and 9

It was considered valid to combine the data from experiments 8 and 9 since the selection criteria for both were similar (cows less than sixth lactation). These data were combined in Table 15. In total, 31 infections were treated with the A7/A8 product and 17 (55 *per cent*) were

eliminated. Thirty-six infections were treated with the G1/G2 product and 27 (75 *per cent*) were eliminated (Table 15). There were 24 *Staph. aureus* infections treated with each product and 12 (50 *per cent*) were eliminated with the cloxacillin and 15 (63 *per cent*) with the spiramycin and neomycin combination. The difference in efficacy between the products was not statistically significant.

Table 14 : The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period (1989-1990)

	Antibiotic formulation	
	Benzathine cloxacillin (A8)	Spiramycin/ neomycin (G2)
No. of cows infected	15	15
No. of quarters infected	24	21
No. of quarter infections eliminated (%)	15 (63)	14 (67)
No. of *Staph. aureus* infections	17	16
No. of *Staph. aureus* infections eliminated (%)	10* (59)	9 (56)
No. of streptococcal spp. infections	6	3
No. of streptococcal spp. infections eliminated (%)	4 (67)	3 (100)

* One *Staph. aureus* infection was eliminated but the quarter developed an infection with *A. pyogenes* during the dry period.

Table 15 : The effectiveness of benzathine cloxacillin or spiramycin and neomycin in eliminating infections during the dry period (combined data for experiments 8 and 9)

	Antibiotic formulation	
	Benzathine cloxacillin (A7/A8)	Spiramycin/ neomycin (G1/G2)
No. of cows infected	21	25
No. of quarters infected	31	36
No. of quarter infections eliminated (%)	17 (55)	27 (75)
No. of *Staph. aureus* infections	24	24
No. of *Staph. aureus* infections eliminated (%)	12* (50)	15 (63)
No. of streptococcal spp. infections	6	10
No. of streptococcal spp. infections eliminated (%)	4 (67)	10 (100)

* One *Staph. aureus* infection was eliminated but the quarter developed an infection with *A. pyogenes* during the dry period.

4.11 Experiment 10 (1989-1990)

The effectiveness of penicillin and framycetin or penicillin and fradiomycin in eliminating infections during the dry period

A total of 40 infected and 44 uninfected cows was selected at drying off in four research herds attached to Moorepark and also in four commercial herds. The cows were dried off abruptly, when the average daily milk yield was less than 5 kg per day. All cows were less than sixth lactation at drying off. Two groups of 20 infected and 22 uninfected cows were selected and infused with the dry cow antibiotics using the following regime:

Treatment group 1 : All quarters were infused at drying off with a combination of penicillin and framycetin (Product D1, Table 1).

Treatment group 2 : All quarters were infused at drying off with a combination of penicillin and fradiomycin (Product H, Table 1).

4.11.1 Results of Experiment 10

At drying off, there was a total of 31 infected quarters treated with Product D1 and 28 treated with Product H. Fifteen (48 *per cent*) of the infections treated with D1 were eliminated, and fifteen (54 *per cent*) of the infections treated with H were eliminated (Table 16).

Staph. aureus was isolated from 24 quarters treated with D1 and from 23 quarters treated with H. Nine (38 *per cent*) of the *Staph. aureus* infections treated with D1 and 10 (44 *per cent*) of infections treated with H were eliminated (Table 16). The difference was not statistically significant.

There were five streptococcal infections treated in each group and all were eliminated (Table 16).

4.11.2 New infections during the dry period

Three new infections developed in the 22 uninfected cows treated with Product H. Two of these (one clinical *Staph. aureus* and one *Proteus*) were present at calving and a further clinical (*Strep. faecalis*) infection developed within a five-day period after calving.

A new clinical (*Staph. aureus*) infection developed in one of the 22 uninfected cows treated with D1.

There was no difference in the incidence of new infection (three *per cent* of quarters treated with Product H and one *per cent* of quarters treated with Product D1) amongst the animals treated with the two formulations.

Table 16 : The effectiveness of penicillin and framycetin or penicillin and fradiomycin in eliminating infections during the dry period (1989-1990)

	Antibiotic formulation			
	Penicillin/ framycetin (D1)		Penicillin/ fradiomycin (H)	
No. of cows infected	20		20	
No. of quarters infected	31		28	
No. of quarter infections eliminated (%)	15	(48)	15	(54)
No. of *Staph. aureus* infections	24		23	
No. of *Staph. aureus* infections eliminated (%)	9	(38)	10	(44)
No. of streptococcal spp. infections	5		5	
No. of streptococcal spp. infections eliminated (%)	5	(100)	5	(100)
No. of *Staph. aureus* and *Strep. uberis* infections*	2		0	
No. of *Staph. aureus* and *Strep. uberis* infections* eliminated (%)	1	(50)		

* Mixed infections

4.12 Experiment 11 (1989-1990)

The effectiveness of cefalexin and dihydrostreptomycin, or cephalonium in eliminating infections during the dry period

A total of 33 infected cows and 22 uninfected cows was selected at drying off in four commercial herds. All cows were less than sixth lactation at drying off. One treatment group of 16 infected and 12 uninfected cows was selected and infused with a combination of cefalexin and dihydrostreptomycin (Product I, Table 1). The remaining 17 infected and 10 uninfected cows were infused with cephalonium (Product J, Table 1). All the cows were dried off abruptly when the milk yield was less than 5 kg per day.

4.12.1 Results of Experiment 11

At drying off there was a total of 34 quarter infections treated with Product I and 31 treated with Product J. Twenty-three (68 *per cent*) of the infections treated with Product I and 20 (65 *per cent*) of the infections treated with Product J were eliminated.

Staph. aureus had been isolated from 28 quarters that were treated with Product I and from 23 quarters treated with product J. Seventeen (61 *per cent*) of the *Staph. aureus* infections treated with Product I were eliminated. Twelve (52 *per cent*) of the *Staph. aureus* infections treated with Product J were also eliminated (Table 17). This difference was not statistically significant.

There were three streptococcal infections in the quarters treated with Product I and one in the quarters treated with Product J and all were eliminated (Table 17).

4.12.2 New infections during the dry period

There were no new infections at calving in the 12 uninfected cows infused with Product I. Two new subclinical *Staph. aureus* infections developed in one of the 10 uninfected cows infused with Product J. There was no difference in the level of new infections (none in the quarters treated with Product I and two in quarters treated with Product J) amongst the cows treated with the two formulations.

Table 17 : The effectiveness of cefalexin and dihydrostreptomycin, or cephalonium in eliminating infections during the dry period (1989-1990)

	Antibiotic formulation			
	Cefalexin/ dihydrostreptomycin (I)		Cephalonium (J)	
No. of cows infected	16		17	
No. of quarters infected	34		31	
No. of quarter infections eliminated (%)	23	(68)	20	(65)
No. of staphylococcal spp.*	31		29	
No. of staphylococcal spp. eliminated (%)	20	(65)	17	(59)
No. of *Staph. aureus*** infections	28		23	
No. of *Staph. aureus* infections eliminated (%)	17	(61)	12	(52)
No. of streptococcal spp. infections	3		1	
No. of streptococcal spp. eliminated (%)	3	(100)	1	(100)
No. of *Proteus* infections	0		1	
No. of *Proteus* infections eliminated (%)			1	(100)

* Includes all staphylococci, haemolytic and non-haemolytic types

** Subset of *Staph. aureus* types only.

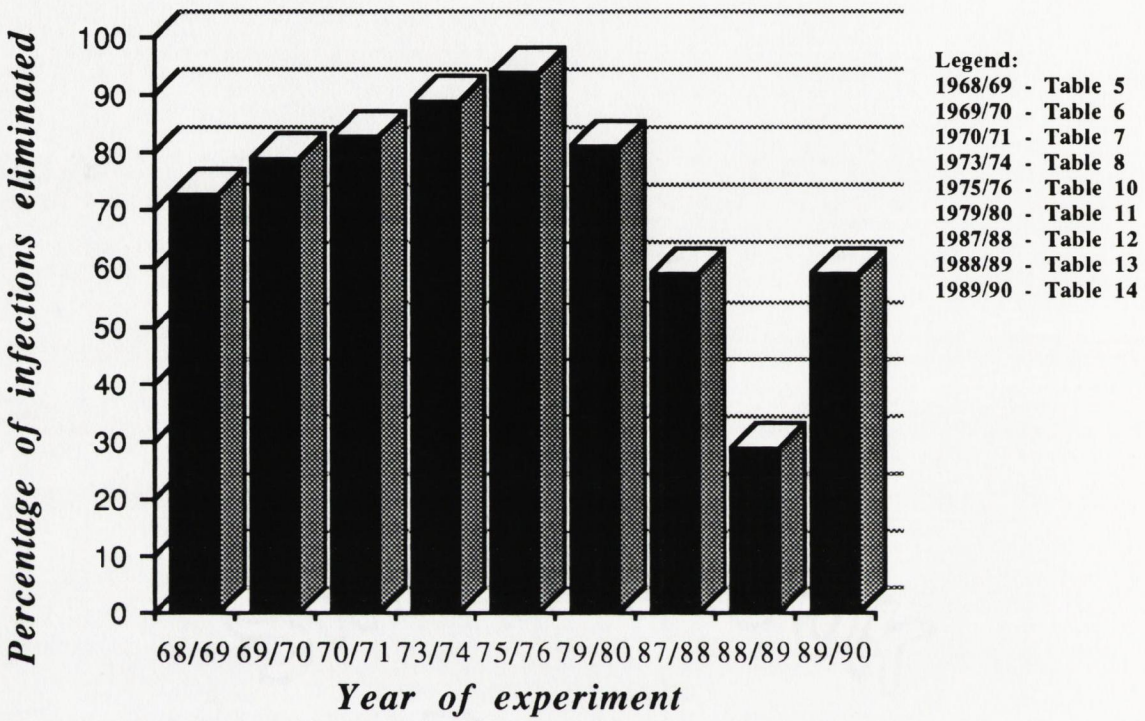

Figure 5 : **Efficacy of benzathine cloxacillin in eliminating *Staph. aureus* infections during the dry period in nine experiments between 1968 and 1990**

4.13 General Results and Discussion

The treatment of cows with long-acting antibiotics at drying off (dry cow therapy) is now an integral component in mastitis control programmes (Smith *et al.*, 1967; Dodd and Neave, 1970). Dry period antibiotic therapy provides both a therapeutic and prophylactic effect when applied to infected and uninfected udder quarters at drying off (Ziv *et al.*, 1981). The rationale for using dry cow therapy is based on the infusion of antibiotics which are formulated in slow release paraffin oils. These formulations provide minimum inhibitory concentrations (MIC's) of antibiotic in the udder secretions of dry cows ranging from three weeks for benzathine cloxacillin (Smith *et al.*, 1967) to 10 weeks for cephalonium (Curtis *et al.*, 1977). A variety of these formulations have been used to control mastitis during the past 25 years. The efficacy of some of these formulations has been evaluated and reported in this chapter.

4.13.1 Benzathine cloxacillin

The efficacy of benzathine cloxacillin (500 mg) was evaluated in nine experiments. This formulation eliminated between 29 and 94 *per cent* of *Staph. aureus* infections (Figure 5). The 29 *per cent* response for cloxacillin (Table 13) was exceptionally low but there were only seven infections treated in that experiment. However, there was no difference in efficacy between the cloxacillin and a combination of neomycin and spiramycin which was evaluated in the same experiment (Table 13). Other studies on the efficacy of 500 mg benzathine cloxacillin in the treatment of *Staph. aureus* at drying off have shown infection elimination rates ranging from 10 to 100 *per cent* (Rosenzuaig and Mayer, 1970; Dodd and Griffin, 1975; Ziv *et al.*, 1981). Results from a large field trial carried out by Dodd and Griffin (1975) showed that there were major differences in efficacy between herds in the treatment of *Staph. aureus* with 500 mg benzathine cloxacillin.

In the experiments reported in this chapter, the percentage of *Staph. aureus* infections eliminated with benzathine cloxacillin seems to have declined in recent years. In the earlier experiments, 72 to 94 *per cent* of *Staph. aureus* infections were eliminated, whereas in more recent years (1987 to 1989), 29 to 63 *per cent* of infections were eliminated (Figure 5). Whether this result is real or more a reflection of sample size is unknown.

4.13.1.1 Comparison of benzathine cloxacillin and untreated controls

In one experiment *Staph. aureus* infections were treated with benzathine cloxacillin and a comparison was made with untreated controls (Table 6). The treatment was more effective ($P<0.001$). Seventeen *per cent* of the *Staph. aureus* infections in the untreated quarters were eliminated spontaneously during the dry period. Storper and Ziv (1985) reported a 9.6 *per cent* spontaneous recovery in untreated quarters infected with *Staph. aureus*. In contrast, Morse (1975) and Serieys and Roguinsky (1975) reported spontaneous recoveries of 31 *per cent* and 42 *per cent*, respectively, in untreated quarters. In the latter studies, however, the types of bacteria associated with the infections at drying off were not described. It is possible, therefore, that many of these infections may have been caused by streptococcal spp. which are usually more transient than *Staph. aureus* infections.

4.13.1.2 Repeated infusions of benzathine cloxacillin

In one experiment a comparison was made between a single infusion of 500 mg benzathine cloxacillin in each udder quarter at drying off and a double infusion (one at drying off and a second infusion 28 days after drying off). Only *Staph. aureus* infections were selected for treatment. There was no difference in efficacy between the two treatment regimes. This result is in agreement with other studies (Storper and Ziv, 1985; Cummins and McCaskey, 1987). In contrast, Smith *et al.* (1975) showed

that there was a beneficial effect from infusing 500 mg benzathine cloxacillin at drying off and again 21 days after drying off.

4.13.2 Penicillin and novobiocin

A formulation containing a combination of 300 mg penicillin and 250 mg sodium novobiocin was evaluated in one experiment. This product eliminated 106 (79 *per cent*) of 135 *Staph. aureus* infections (Table 5). This was similar to the results reported by Uvarov, Evans, Marshall and Sizer (1967) and Daniel and Steffert (1969) for a similar product.

4.13.3 Penicillin and dihydrostreptomycin

The effectiveness of combinations of penicillin and dihydrostreptomycin formulations was evaluated in three experiments. A combination of 300 mg penicillin and 200 mg dihydrostreptomycin gave similar results to a product containing 500 mg benzathine cloxacillin (Table 5). A penicillin and novobiocin combination evaluated in the same experiment was more effective ($P<0.001$) than this combination of penicillin and dihydrostreptomycin (Table 5). Formulations containing either 1,000 mg penicillin and 1,000 mg dihydrostreptomycin or 1,000 mg penicillin and 500 mg dihydrostreptomycin were compared with 500 mg benzathine cloxacillin in two separate experiments. The difference in efficacy between each of the penicillin plus dihydrostreptomycin formulations and the cloxacillin control was not statistically significant. Similar response levels with 1,000 mg penicillin and 1,000 mg dihydrostreptomycin were reported by Roberts, Meek, Natzke, Guthrie, Field, Merrill, Schmidt and Everett (1969) and Serieys and Roguinsky (1975). The response levels to 1,000 mg penicillin and 500 mg dihydrostreptomycin were also similar to those reported by Pugh *et al.* (1973).

4.13.4 Comparison of penicillin/framycetin and penicillin/fradiomycin

The efficacy of a product containing 100 mg penethamate hydroiodide, 300 mg procaine penicillin and 100 mg framycetin (Product D1) was compared with a formulation containing 400 mg procaine benzylpenicillin, 100 mg diethylaminoethyl penicillin hydriodide and 100 mg fradiomycin sulphate (Product H, Table 16) in Experiment 10. There was no difference in efficacy between the products. The penicillin/framycetin product had been evaluated previously in a comparison with 500 mg benzathine cloxacillin (Experiment 3, Table 7). In that experiment, also, there was no difference in efficacy between the products (Langley *et al.*, 1971). The penicillin/framycetin eliminated 74 *per cent* of 39 *Staph. aureus* infections in cows in all lactation age categories (Table 7). A similar product eliminated only 38 *per cent* of 24 *Staph. aureus* infections in cows which were selected as less than sixth lactation at the time of infusion (Experiment 10, Table 16). However, the results of both Experiments 3 and 10 showed that there was no difference in efficacy between the penicillin/framycetin combination and the control antibiotics.

4.13.5 Spiramycin and Neomycin

The efficacy of a product containing 1,200 mg of spiramycin and 1,000 mg of neomycin sulphate was compared with 500 mg of benzathine cloxacillin in three experiments (Experiments 7, 8 and 9). In Experiment 7 (Table 12), the cloxacillin was more effective than the spiramycin/neomycin ($P<0.05$). This difference was shown primarily in the treatment of *Staph. aureus* infections. In order to validate the result of Experiment 7, two further comparisons of the products (G and A6) were carried out in cows infected with *Staph. aureus*. Cows with less than six lactations were selected for these experiments in order to reduce the risk of treating chronic *Staph. aureus* infections which are more prevalent in older animals. There was no difference in efficacy

between the spiramycin/neomycin and the cloxacillin in these experiments (Tables 13 and 14). By combining the data from Experiments 8 and 9, 63 *per cent* of *Staph. aureus* infections were eliminated with spiramycin and neomycin and 50 *per cent* were eliminated with the cloxacillin (Table 15). This amalgamation of data from Experiments 8 and 9 was considered to be justified since the cow selection criteria used in the two experiments was similar.

4.13.6 Cephalosporins

The efficacy of two cephalosporin-type products was evaluated in one experiment. A combination of 500 mg cefalexin and 500 mg dihydrostreptomycin was compared with a product containing 250 mg of cephalonium. There was no difference in efficacy between the formulations (Table 17). The cephalonium formulation eliminated 52 *per cent* of *Staph. aureus* infections and this response was comparable to the 60 *per cent* response reported by Curtis *et al.* (1977) on a similar product. A study by Ziv *et al.* (1981) demonstrated a 77.5 *per cent* response to cephalonium in the treatment of *Staph. aureus*. However, there was considerable variation between the herds (43.8 *per cent* to 100 *per cent*) in the study reported by Ziv *et al.* (1981).

4.13.7 Efficacy of antibiotics in young cows

In order to reduce the risk of treating chronic *Staph. aureus* infections at drying off, experiments undertaken after 1986 (Experiments 7-11) were carried out in cows of less than sixth lactation. In 1989, six products were evaluated and the percentage of *Staph. aureus* infections eliminated varied from 38 to 61 (Tables 14, 16 and 17). While there was no difference in efficacy between the products evaluated in each experiment in 1989, the response rate was lower than the response shown in the earlier experiments. This result was unusual since in the experiments conducted before 1987 cows were treated in all lactation age categories and with a greater risk of treating chronic infections.

The variation in efficacy between herds, however, may be the most significant factor (Dodd and Griffin, 1975; Ziv *et al.*, 1981).

In all the experiments, *Staph. aureus* was the principal bacterium isolated from infected quarters at drying off. When streptococcal spp. were isolated and treated with dry cow therapy, between 90 and 100 *per cent* were eliminated. In general, the number of streptococcal spp. treated was too small to warrant additional comment.

Where new infection was monitored, the incidence in treated quarters was at five to six *per cent.*

4.13.8 Conclusions

With the exception of Experiment 1, there was no significant difference in efficacy between the products in the treatment of *Staph. aureus* infections. There was a significant benefit ($P<0.001$) in treating *Staph. aureus* with benzathine cloxacillin when compared with no therapy at drying off. There was no added advantage in treating *Staph. aureus* with 500 mg benzathine cloxacillin on two occasions during the dry period. There was an indication that the effectiveness of antibiotics in the treatment of *Staph. aureus* had reduced in recent years in herds which consistently used dry period antibiotic therapy.

CHAPTER 5

THE EFFECT OF A DRY COW ANTIBIOTIC PRODUCT IN PREVENTING NEW INFECTIONS DURING THE DRY PERIOD

5.1 Introduction

The experiments reported in Chapter 4 were designed primarily to evaluate the efficacy of a variety of antibiotics in eliminating infections which were diagnosed at drying off. In general, the number of uninfected cows treated was too small to evaluate the effectiveness of the products in reducing the incidence of new infection during the dry period.

The ongoing debate on the merits or problems associated with the use of dry period therapy has already been cited in the literature review. Because of this debate an experiment was set up to evaluate the effectiveness of dry cow therapy in preventing new infections in uninfected cows at drying off. One experiment on 226 uninfected cows is reported in this chapter.

5.2 Experiment 12 (1981-1982)

The incidence of new infections at calving in cows selected as infection-free at drying off and infused in two quarters with neomycin at drying off

A total of 226 uninfected cows was selected in three research farms. At drying-off, two quarters (right fore and left hind, Figure 3) were infused with neomycin sulphate (Product K, Table 1). The remaining two quarters (left fore and right hind) were not treated (Figure 3). Cows were dried off using an abrupt cessation of milking. The lactation numbers of the cows were not recorded. In this experiment, new infections were monitored at calving and also in the interval between calving and turnout to pasture.

5.2.1 Results of Experiment 12

5.2.1.1 The incidence of new infection during the dry period

Forty-seven new infections developed in the untreated quarters and 20 in the treated quarters. This difference was significant ($P<0.001$). The new infections in the untreated quarters consisted of 32 subclinical and 15 clinical cases. In the treated quarters, ten of the infections were subclinical and ten were clinical (Table 18).

Seventeen new infections in the untreated quarters and six in the treated quarters were caused by *Staph. aureus*. Environmental infections accounted for 28 and 11 new infections in the untreated and treated quarters, respectively (Table 18). This difference was also statistically significant ($P<0.05$).

5.2.1.2 New infection in the interval between calving and turnout to grass

New infection was also monitored in the interval between calving and turnout to grass. These infections were additional to those already detected at calving. Thirteen new cases were recorded in the untreated quarters and 15 in the treated quarters. This difference was not significant (Table 19). *Staph. aureus* was isolated from five of the quarters that were not treated at drying off and from three of the treated quarters. Environmental pathogens were isolated from seven control quarters and from 12 treated quarters (Table 19).

Table 18 : New infections at calving in 226 cows selected as infection-free at drying off and infused in two quarters with neomycin sulphate (1981-1982)

	No. of new infections			
	Untreated quarters		Quarters treated with Neomycin sulphate (K)	
Pathogen	Subclinical	Clinical	Subclinical	Clinical
Staph. aureus	14	3	4	2
Strep. dysgalactiae	5	3	2	0
Streptococcal spp. (aesculin-positive)	2	1	2	0
Staph. aureus and *Strep. dysgalactiae*	0	1	0	0
Coliform spp.	11	5	2	3
A. pyogenes	0	0	0	2
No bacteria isolated	0	2	0	3
Subtotals	32	15	10	10
Totals	47[a]		20[b]	
Subset of environmental infections	28[c]		11[d]	

[ab] Values with different superscripts on the same line differ significantly ($P<0.001$)

[cd] Values with different superscripts on the same line differ significantly ($P<0.05$)

Table 19 : New infections in the interval between calving and turnout to pasture in 226 cows selected as infection-free at drying off and infused in two quarters with neomycin sulphate (1981-1982)

	No. of new infections			
	Untreated quarters		Quarters treated with Neomycin sulphate (K)	
Pathogen	Subclinical	Clinical	Subclinical	Clinical
Staph. aureus	3	2	1	2
Strep. dysgalactiae	0	1	1	1
Streptococcal spp. (aesculin-positive)	1	1	0	2
Staph. aureus and *Strep. dysgalactiae*	0	1	0	2
Coliform spp.	0	3	1	4
A. pyogenes	0	0	0	1
No bacteria isolated	0	1	0	0
Subtotals	4	9	3	12
Totals	13		15	

5.3 Results and Discussion

The effectiveness of dry period therapy is usually evaluated on the ability of a product to eliminate existing infections and to reduce the incidence of new infection (Oliver *et al.*, 1962). The infusion of intramammary antibiotics into all quarters at drying off is a recommended method for reducing mastitis during the dry period (Smith *et al.*, 1967; Booth, 1988; Francis, 1989). However, this approach is not supported by all workers and some have suggested that only the infected quarters should be treated with antibiotics at drying off (Bratlie, 1973; Morse, 1975). Others (Rindsig, Rodewald, Smith and Spahr, 1978) favour both complete or selective therapy depending on the requirements in specific herds.

In the present study on 226 cows there were more new infections ($P<0.001$) in the untreated quarters than in the quarters treated with neomycin sulphate at drying off. This result is in agreement with other studies (Pankey, Barker, Twomey and Duirs, 1982; Bramley and Dodd, 1984; Eberhart, 1986; Batra, 1988).

Four *per cent* of the quarters treated with neomycin developed new infections. Similar new infection levels were recorded in Experiments 10 and 11 (**Chapter 4**) with different antibiotic formulations and also in studies reported by Langley *et al.* (1971) and Rindsig *et al.* (1978).

Ten *per cent* of the untreated quarters developed new infections during the dry period. This was higher than the 4.4 *per cent* level reported by Langley *et al.* (1971) and lower than the levels reported by Morse (1975), Serieys and Roguinsky (1975) and Storper and Ziv (1985).

Oliver *et al.* (1956c) reported that there was a higher incidence of new infection during the dry period in untreated cows which were dried off abruptly than in cows that were dried off by a gradual cessation of milking. However, studies by Natzke, Everett and Bray (1975) showed

that the method used to dry off cows did not influence new infection levels when cows were infused with long-acting antibiotics at drying off. Based on the results of these studies it could be argued that the half udder design used in Experiment 12 was not the most suitable to evaluate the effect of the untreated control. It may have been more appropriate to compare untreated cows dried off by an intermittent milking technique with treated cows dried off by abrupt cessation of milking. However, Morse (1975) favoured within-cow comparisons in order to reduce "between cow" variations in natural resistance to udder infection, environmental conditions and nutritional and management practices.

Some workers have also suggested that the use of dry period antibiotics in uninfected quarters may increase susceptibility to coliform mastitis (Howell, 1972; Armstrong, 1977; Eberhart, 1986). The results (Experiment 12) indicated that the incidence of new infections caused by environmental pathogens including coliform spp. was significantly higher ($P<0.05$) in the untreated quarters (Table 18). Additionally, when new infection was monitored in the interval between calving and turn-out to pasture there was no evidence that the treated cows were more susceptible to new infection (Table 19).

5.3.1 Conclusions

The treatment of uninfected cows at drying off with a long-acting formulation containing neomycin sulphate significantly reduced the incidence of new infection during the dry period. The level of environmental mastitis in the treated quarters was also reduced during the dry period. There was no indication that the treated quarters were more susceptible to coliform mastitis during the indoor period after the next calving.

CHAPTER 6

EVALUATION OF TEAT-SEALERS FOR THE PREVENTION OF NEW INFECTION DURING THE DRY PERIOD

6.1 Introduction

Dry cow antibiotic therapy is accepted worldwide as being an effective method of eliminating subclinical mastitis. There is disagreement, however, as to the most effective method of using dry cow products. While some workers advocate treating both infected and uninfected cows at drying off (Bramley and Dodd, 1984; Pankey, 1984), others favour the treatment of infected cows only (Browning *et al.*, 1990). In situations where only infected cows are treated at drying off, the uninfected cows are not protected against new infection during the dry period.

The development of a non-antibiotic teat-sealer for infusion at drying off may provide either an acceptable alternative or an addition to existing antibiotic formulations. A series of experiments is presented in this chapter on the effectiveness of teat-sealers on their own or in combination with antibiotics in reducing infections during the dry period.

Eight experiments (Experiment numbers 13-20) were set up to evaluate the efficacy of a non-antibiotic teat-sealer. At drying off, the sealer was infused into infected and uninfected quarters. In some experiments the sealer was infused in combination with intramammary antibiotics.

In Experiments 13, 14 and 16 both sealers and antibiotics were infused into uninfected cows at drying off. The teats of these cows were challenged by teat dipping with bacterial cultures during the dry period. The teats of in-calf heifers were also sealed before calving as a method of preventing infection after calving (Experiment 17).

Experiments 15, 18, 19 and 20 were carried out either in uninfected animals or in animals that had spontaneous infections at drying off.

6.2 Experiments 13 and 14 (1972-1973)

The incidence of new infection in sealed and untreated (control) quarters when challenged with cultures of bacteria during the dry period

Forty-five Friesian cows were selected at drying-off. Thirty-one were uninfected and 14 had infections in one or more udder quarters. Before drying off, all animals were machine milked twice daily using a 16/8 h (night/day) interval between milkings. The cows ranged in lactation age from first to seventh with a mean lactation age of 3.4.

6.2.1 Experiment 13 (1972-1973)

Each of fourteen uninfected cows was dried off when the milk yield on three successive days reached a mean of 1.9 kg/day (range 0.6 to 5 kg/day). Two teats in each of the cows (right fore and right hind or left fore and left hind, Figure 3) were alternately assigned at drying-off to a teat-sealer (Product L, Table 1) or to untreated control. The treatment was a non-antibiotic sealing formulation. The teats of these cows were dipped in cultures of bacteria during the dry period.

6.2.2 Experiment 14 (1972-1973)

Each of the remaining infection-free cows was dried off when the milk yield on three successive days reached a mean of 1.9 kg/day (range 0.6 to 5 kg/day). Two teats in each of the 17 cows were infused at drying off with a penicillin and dihydrostreptomycin product (M). These quarters were re-infused with Product L1 immediately after infusing Product M. The treatment randomisation used in this experiment was similar to that used in Experiment 13. The composition of Product M is shown in Table

1. Again, all teats were challenged by dipping in a culture of bacteria during the dry period.

The seal and antibiotic formulations were presented in a pack of eight intramammary syringes for each cow. The first four contained the antibiotic in an aqueous base. The second set of four syringes contained the teat-sealing material. The sealing component, without the antibiotic, was used in Experiment 13. In Experiment 14, the antibiotic was infused after the last milking of the lactation. After the antibiotic was infused and massaged into the teats, the sealer was then infused without further manipulation; this was to ensure that the seal remained intact in the teat sinus and duct.

6.2.3 Bacterial challenge and method of contaminating teats

All the teats in the cows in Experiments 13 and 14 were challenged, by dipping in a bacterial culture, once each week during the dry period. Dirty teats were washed with cold running water and dried with individual paper towels before being dipped in the culture. The minimum of handling was used to avoid displacement of the seal. The bacterial challenge contained a mixture of *Staph. aureus* (885 x 10^7 cfu/ml) and *Strep. dysgalactiae* (103 x 10^7 cfu/ml) .

6.2.4 Specification of teat-sealer

The seal consisted of a heavy inorganic salt in a paraffin wax base and was antibiotic free. It was available commercially and recommended for use in the prevention of summer mastitis in dairy cows. The product was presented in a disposable plastic syringe for intramammary use and contained 7.5 grams of sealing material together with acriflavine.

The manufacturer's specification stated that the seal formed a physical barrier in the teat duct and sinus to invasion by potential mastitis causing pathogens. The seal was also described as being non-toxic and

easily removed by the stockman or by the suckling calf.

6.2.5 X-ray techniques used in Experiments 13 and 14

X-rays were used to monitor the location of the seal within each teat. The X-ray unit was a portable Microx Model HX-77[2]. X-rays were taken on Dentus T2 plates 5.6 cm x 7.5 cm and developed and fixed in G150 developer[3] and G334 fixer. The X-ray plates were positioned on the teats with rubber bands and exposed for 1.25 to 1.5 seconds using a 6-kilovolt current supply. The seal was sufficiently radioopaque to produce X-ray pictures without the inclusion of additional marker material. The sealed teats in Experiment 13 and 14 were X-rayed once each week during the dry period. X-ray monitoring commenced one week after infusing the teat seals. When seals were absent in two consecutive weeks, monitoring was discontinued.

6.2.6 Examination of milk after calving

Foremilk from each sealed quarter was examined for particles of sealer at calving and daily for the first 21 days of the lactation.

6.2.7 Post-mortem examinations in culled cows

An additional three cows (two pregnant and one non-pregnant) that were selected for culling were infused with teat-sealer in all udder quarters at drying off. Twenty-four days later these cows were slaughtered. The udders were removed at the abattoir and returned to the laboratory for post-mortem examination.

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2 Microx Model HX-77, X-ray Power Unit, Zeniter, Heiwa Electronic Industrial Co. Ltd., Osaka, Japan

3 Agfa-Gevaert Ltd., Naas Road, Dublin 12

Figure 6a: X-ray plate illustrates the appearance and position of the sealer in the teat sinus, canal and orifice

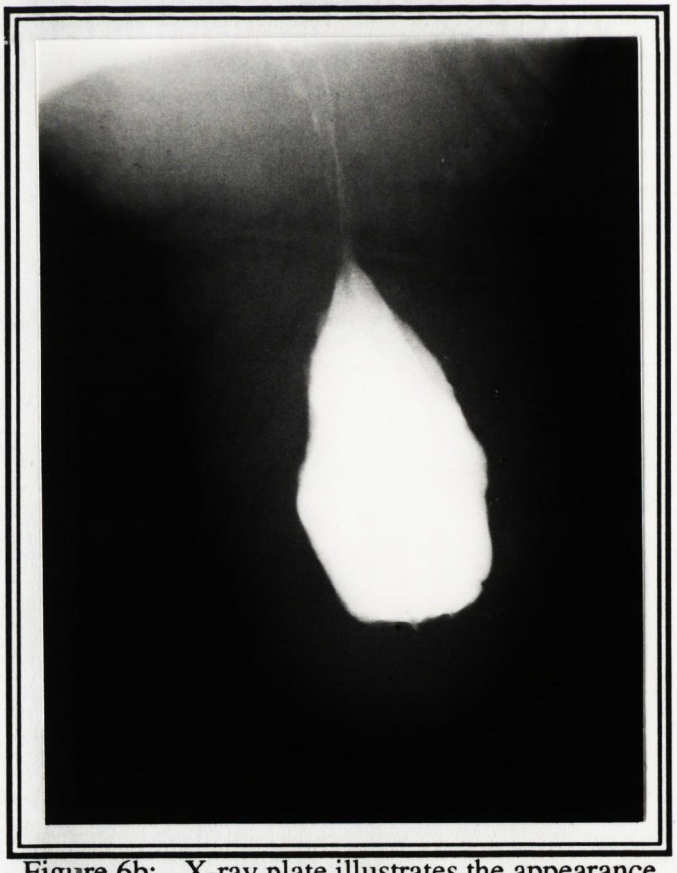

Figure 6b: X-ray plate illustrates the appearance and position of the sealer in the teat sinus

A visual inspection on the udders of these slaughtered animals was made in order to locate the position of the sealer within the gland.

6.2.8 Results of Experiments 13 and 14

The results showed that the teat seals infused at drying off significantly reduced the levels of new infection associated with the bacterial challenge during the dry period (Table 20). The differences in the levels of new infection between control and treated quarters in Experiments 13 and 14 were statistically significant ($P<0.05$). In Experiment 13, two udder quarters became infected at 5 and 26 days, respectively, after drying off. Both infections developed, however, in the untreated quarters. The bacteria isolated from new cases of mastitis in sealed and non-sealed teats in Experiments 13 and 14 are listed in Table 21.

6.2.8.1 X-ray observations

Fourteen sealed teats in seven cows were x-rayed once each week during the dry period. The sealer remained in position for a full dry period (10-25 weeks) in 13 (93 *per cent*) teats. The remaining teat retained the seal for 11 weeks of a 14-week dry period. These 14 teats remained free of infection. New infections developed in two of the 14 teats that had been infused with the antibiotics.

Generally, the seal remained as a complete unit and in position in the teat duct and sinus for 3 to 4 weeks after infusion. There was limited evidence of seal losses via the orifice in some teats during the dry period.

A total of 38 teats (including the 14 teats already described) were X-rayed an average of 2.6 days before calving and 32 teats (84 *per cent*) had seals in position in the teat sinus. One new infection developed in these 32 teats. Photographs of two X-ray plates illustrate the appearance and position of the sealer within the teats (Figures 6a and 6b).

6.2.8.2 Post-mortem examinations in culled cows

A visual inspection on the udders of the pregnant cows showed that one teat had lost the seal completely. The remaining seven teats contained fragmented particles of sealer distributed throughout the alveolar tissue. Seals were not present in the teat ducts or sinuses of these animals. The udders of these animals contained an appreciable amount of dry period fluids. The seals were intact in the teat ducts and sinuses of the third cow. This cow was diagnosed as being nonpregnant and the udder was totally free of dry period secretions.

Table 20 : The numbers of new infections in sealed and untreated (control) quarters when challenged with *Staph. aureus* and *Strep. dysgalactiae* during the dry period (1972-1973)

	Experiment number			
	13		14	
	Sealer (L)	Untreated control	Pencillin/ dihydrostreptomycin and sealer (M + L1)	Untreated control
Number of udder quarters	28	28	34	34
Number of new infections during the dry period	1	9	2	11
Percentage of new infections during the dry period	3.6	32.0	5.9	32.4

Table 21 : Bacteria isolated from new infections in sealed and untreated (control) quarters when challenged with *Staph. aureus* and *Strep. dysgalactiae* during the dry period (1972-1973)

	Experiment number			
	13		14	
	Sealer (L)	Untreated control	Pencillin/ dihydrostreptomycin and sealer (M + L1)	Untreated control
Staph. aureus	0	3	2	8
Strep. dysgalactiae	0	3	0	1
Strep. uberis	0	2	0	0
Coliform spp.	1	1	0	2
Total	1	9	2	11

6.2.8.3 Visual examinations of milk samples after calving

After calving, milk from a total of 104 sealed teats in Experiments 13 and 14 was examined for traces of seal. Fragments were found in milk from nine quarters over a 21-day period after calving. Seal particles persisted in the milk from one quarter for seven days, from six quarters for seven to 14 days, and from two quarters for 14 to 21 days.

6.3 Experiment 15 (1972-1973)

The response of infected quarters to treatment with an antibiotic and teat-sealer at drying off

Fourteen cows with infections in one or more udder quarters were selected for this experiment. They were dried-off when the daily milk yield on three successive days reached a mean of 1.8 kg/day (range 0.86 to 3.0 kg/day). One uninfected quarter in each cow was pre-selected as a control (untreated). The remaining three quarters (infected and uninfected) in each cow were infused with Product M1. These quarters were re-infused with Product L2 immediately after infusing Product M1. These cows had spontaneous infections at drying off and they were not challenged with bacteria during the dry period.

6.3.1 Results of Experiment 15

A total of 14 infected udder quarters were treated. These were made up of nine *Staph. aureus* infections, one *Strep. dysgalactiae* and four *Strep. uberis* infections. Thirteen were infected subclinically and one staphylococcal infection displayed clinical abnormalities at drying off. Eleven (78 *per cent*) of these infections were eliminated and three of the *Staph. aureus* infections continued. Three new infections developed in the treated quarters and two in the control quarters. Dry period infection was reduced by 57 *per cent* in the infected treated quarters. New infections developed in two (14 *per cent*) of the 14 control quarters and in three (11 *per cent*) of the 28 uninfected quarters.

6.4 Experiment 16 (1974-1975)

The incidence of new infections in sealed quarters and in quarters infused with benzathine cloxacillin when challenged with cultures of bacteria during the dry period

The effectiveness of a product containing benzathine cloxacillin (A9) was compared with that of a non-antibiotic teat sealing product (L3). Twenty-five uninfected Friesian cows were selected at the end of lactation. Quarters were paired (right fore and right hind or left fore and left hind) and selected pairs were assigned, at random, to either antibiotic or teat-sealer. All teats were challenged by dipping in culture of *Staph. aureus* each week during the dry period. The concentration of the *Staph. aureus* culture was not standardised at each propagation and this resulted in a variation in concentration from 0.18 x 10^6 to 1,220 x 10^6 cfu/ml (mean 299 x 10^6 cfu/ml) over the duration of the experiment. The strains of *Staph. aureus* used and the method of preparing the cultures was similar to those outlined in Experiments 13 and 14. The animals remained dry from 75 to 233 days (mean ± SD: 145.5 ± 45.7). Infections were monitored as they developed during the dry period and at calving.

6.4.1 X-ray recording

Teats were X-rayed as described in Experiment 13.

6.4.2 Results of Experiment 16

Two animals aborted and were culled; data were available for 23 of the 25 cows on the experiment. Six new infections were recorded in the sealed teats at the time of calving. Two of these developed in quarters that had a history of infection in the previous lactation. A further three developed in quarters that had lost the seals during the dry period. Only one subclinical streptococcal infection developed in a

quarter that had a seal in position in the teat sinus at the last X-ray observation four days before calving. A total of seven new infections developed in the quarters treated with antibiotics (Table 22).

Table 22 : The numbers of new infections in sealed quarters and in quarters infused with benzathine cloxacillin when challenged with *Staph. aureus* during the dry period (1974-1975)

	Treatment	
Pathogen	Sealer (L3)	Benzathine cloxacillin (A9)
Staph. aureus	3	5
Streptococcal spp.	2	1
No bacteria isolated	1	1
Total	6	7

6.5 Experiment 17 (1975-1976)

The incidence of new infections at calving in heifers that were teat-sealed at intervals ranging from 9 to 110 days before calving

Thirty-two in-calf heifers were selected for the experiment over two calving seasons. Sixteen were treated before calving in 1975 and a further 16 heifers were infused before calving in 1976. Two udder quarters in each of the heifers (right fore and right hind or the left fore and left hind) were randomly assigned to treatment with sealer (Product L4) or were untreated (control). In this instance a smaller

quantity of sealer (3.0g) was infused. During the 1975 season the animals were infused from 9 to 52 days before calving (mean 26 days). During the 1976 season the animals were infused from 14 to 110 days before calving (mean 59 days). It was possible to infuse the sealer into 63 of the 64 quarters selected for treatment.

6.5.1 Sampling and infusion procedures

Samples of secretion were collected in an aseptic manner (using sampling methods similar to those used for cows at drying off) from the heifers, at the time of infusion. After collecting the samples, the teats were cleaned with cotton wool swabs soaked in methylated spirits and the seals were infused in the selected quarters. Both the sealed and control teats were dipped in an iodophor teat skin disinfectant after treatment. Samples of foremilk or of colostrum were collected in an aseptic manner from all heifers at calving (before the first machine milking) using the sampling procedures already described for cows.

6.5.2 Results of Experiment 17

All samples of secretion collected at the time of the infusions were free from pathogenic bacteria and from macroscopic signs of mastitis. The incidence of new infection was low in both the treated and control quarters (Table 23). Three new infections developed in sealed quarters and four in untreated quarters. Thus, there was no evidence that breaking the natural seal one to two months before calving rendered the heifers more vulnerable to new infection.

Table 23 : The numbers of infections at calving in heifer quarters that were sealed at intervals ranging from 9 to 110 days before calving or used as untreated controls (1975-1976)

	Sealer (L4)		Untreated	
	Experiment number			
Pathogen	1	2	1	2
Strep. dysgalactiae	1	0	0	0
Strep. uberis	0	0	0	2
E.coli	1	0	0	0
Proteus	0	0	1	0
No bacteria isolated	0	1	0	1
Subtotal	2	1	1	3
Total	3		4	

6.6 Experiment 18 (1975-1976)

The effectiveness of three antibiotic and teat-sealer regimes in reducing infection during the dry period

In total, 235 cows were selected, regardless of infection status, in three research herds attached to Moorepark. The cows in all treatment groups had an average lactation number of 3.8 to 4.1 (range for individual cows

was 1-12). These cows were randomly allocated within each herd to three treatment groups as follows :

6.6.1 Treatment 1 : Seventy cows were infused in all quarters at drying off with a product containing penicillin and fradiomycin (H1).

6.6.2 Treatment 2 : Eighty-six cows were infused in all quarters at drying off with Product H1 followed by an infusion of teat-sealer (L5) immediately after H1.

6.6.3 Treatment 3 : Seventy-nine cows were infused in all quarters at drying off with Product H1 and infused with a teat-sealer (L5) 28 days after drying off.

6.6.4 Teat-sealer product presentation

The teat-sealer product was similar to the formulation used in Experiment 17.

6.6.5 Results of Experiment 18

The percentage of cows infected at drying off was similar for the three groups and ranged from 26 *per cent* to 28 *per cent*. The percentage of infected quarters at drying off was also similar and ranged from 9 *per cent* to 10 *per cent* (Table 24). The percentage of infections eliminated was similar for treatments 2 and 3 (70 *per cent* and 73 *per cent*, respectively) but was lower in treatment 1 (60 *per cent*) (Table 24). The difference, however, was not statistically significant.

At drying off, *Staph. aureus* was the predominant bacterium isolated from infected quarters and accounted for 20, 28 and 23 infected

quarters in treatment groups 1, 2 and 3, respectively. The difference in the response to the treatment of *Staph. aureus* was not significant. The remaining cases of mastitis were caused by streptococcus spp. (dysgalactiae or uberis) or non-specific clinical cases.

New infections developed during the dry period in three quarters in group 1, in six quarters in group 2 and in 17 quarters in group 3 (Table 25). The pathogens isolated from each new case of mastitis are presented in Table 26. There was no difference in the incidence of new infections between the quarters infused at drying off with either antibiotic or antibiotic and teat-sealer. There was more new infections ($P<0.01$) in the quarters treated with sealer 28 days after drying off than in the quarters infused with antibiotic only at drying off. There was also more new infections in the quarters that were sealed 28 days after drying off than in the quarters sealed at drying off ($P<0.02$). The highest number of new infections associated with environmental bacteria was present in Treatment 3.

The data were further categorised to select only the new infections that developed in cows which were not infected at drying off. Two new infections were detected in group 1, four in group 2 and nine in group 3 (Table 25). These values were not statistically different. The number of cows that developed new infections was one in group 1, two in group 2 and 7 in group 3.

Table 24 : Infection levels at drying off and the subsequent response to treatment during the dry period in cows treated with three different antibiotic and teat-sealing regimes (1975-1976)

	Treatment						
	1 Penicillin/ fradiomycin (H1)		2 Penicillin/fradiomycin and sealer at drying off (H1 + L5)		3 Penicillin/fradiomycin at drying off and sealer 28 days later (H1 + L5)		Statisical Significance
No. of cows assigned	70		86		79		
No. of cows infected at drying off (%)	18	(26)	24	(28)	22	(28)	
No. of quarters infected at drying off (%)	25	(9)	33	(10)	30	(10)	
No. of quarter infections eliminated (%)	15	(60)	23	(70)	22	(73)	NS
No. of *Staph. aureus* infections treated at drying off	20*		28*		23*		
No. of *Staph. aureus* infections eliminated (%)	10	(50)	19	(68)	17	(74)	NS

* These *Staph. aureus* infections are already included in the number of quarters infected at drying off.

NS = Not significant

Table 25 : The numbers of new infections during the dry period in quarters treated with three different antibiotic and teat-sealing regimes (1975-1976)

	Treatment		
	1 Penicillin/ fradiomycin (H1)	2 Penicillin/fradiomycin and sealer at drying off (H1 + L5)	3 Penicillin/fradiomycin at drying off and sealer 28 days later (H1 + L5)
No. of cows with new infections (% of total cows)	2 (3)	6 (7)	14 (18)
No. of quarters with new infections (% of total quarters)	3 (1)	6 (2)	17 (5)
No. of new infections in previously uninfected cows (%)	2[1] (0.8)	4[1] (1.3)	9[1] (3.0)

[1] These infections are already included in the number of quarters with new infections.

Table 26 : Bacteria isolated from new infections in quarters treated with three different antibiotic and teat-sealing regimes (1975-1976)

Pathogen	Treatment 1 Penicillin/fradiomycin (H1)	Treatment 2 Penicillin/fradiomycin and sealer at drying off (H1 + L5)	Treatment 3 Penicillin/fradiomycin at drying off and sealer 28 days later (H1 + L5)
Staph. aureus	1	2*	6
Streptococcal spp.	0	0	4
Staph. aureus plus streptococcal spp.	0	0	1
E.coli	2	1*	1*
Proteus	0	0	1
Fungi	0	1	1
No bacteria isolated	0	2	3
Total	3	6	17

* One quarter had a history of mastitis in the previous lactation

6.7 Experiment 19 (1976-1977)

The effectiveness of treating cows with an antibiotic or an antibiotic and teat-sealer in reducing mastitis during the dry period

Two research herds of spring-calving Friesian cows were used. Each herd was self contained and the system of management for each herd was similar. One herd was situated on a low-land farm (BDR) and the other was situated on a hill-land farm (CNK).

6.7.1 Animals

There was a total of 80 cows on experiment in the BDR herd and 88 in the CNK herd. The animals were managed as a single herd in each location. Animals were dried off when the average daily milk yield dropped below 3 kg per day. The lactation number of the animals ranged between two and 12 (mean 4.8) and two and nine (mean 2.8) for the BDR and CNK herds, respectively. The length of the dry period in the BDR herd ranged between 68 and 208 days (mean 119.4 ± 30.8) and the dry period ranged between 84 and 226 days (mean 130.3 ± 34.34) in the CNK herd. Cows were selected regardless of infection status and half udders (right fore and right hind or left fore and left hind, Figure 3) were assigned at random to each of the two treatments as follows :

6.7.1.1 Treatment 1 : Two quarters in each cow were infused with a penicillin and fradiomycin formulation (Product H2).

6.7.1.2 Treatment 2 : The remaining two quarters in each cow were infused with a combination of Product H2 and a teat-sealer (L6).

At drying off, there was one inactive (non-functional) quarter in the group of cows at the BDR farm and five in the CNK farm.

The infection status of each quarter was assessed, at drying off, at calving, and seven to 10 days after calving.

6.7.2 Teat-sealer presentation

The antibiotic and seal formulation in this experiment was presented in the same intramammary syringe. When the plunger in the syringe was pressed, the antibiotic was infused first and the sealer later.

6.7.3 X-ray examinations

Fifty sealed teats in 26 cows in the BDR herd were x-rayed once during the dry period to establish the location of the seals. The location and the amount of seal in the teat was observed in the radiographs and classified into four categories as follows : Large seal plug in teat; small plug; traces of seal; or no sealer present.

6.7.4 Results of Experiment 19

At drying off, the number of infections in each treatment group was similar and ranged from nine *per cent* to 12 *per cent* of quarters infected (Table 27). The response to therapy was variable, but the most effective response in both herds was shown in the quarters treated with the antibiotic and teat-sealer combination. The difference in efficacy between the treatments was significant ($P<0.05$) in the BDR herd only. When the data were combined for both herds, 47 *per cent* of infections were eliminated in Treatment 1. In Treatment 2, 78 *per cent* of infections were eliminated. This difference was statistically significant ($P<0.05$).

The types of pathogens isolated from the infected quarters at drying off and the numbers of these that were eliminated during the dry period are presented in Table 28. The number of non-specific mastitis cases that returned to normal during the dry period is also presented in Table 28. *Staph. aureus* was the predominant pathogen isolated from infected quarters in the two treatment groups at drying off (Table 28). In Treatment 1, 36 *per cent* of the *Staph. aureus* infections in the BDR herd and 50 *per cent* in the CNK herd were eliminated. This difference was statistically significantly ($P<0.05$). In Treatment 2, 72 *per cent* of the *Staph. aureus* infections in the BDR herd and 75 *per cent* in the CNK herd were eliminated (Table 29).

Table 27 : Infection levels at drying off and the subsequent response to treatment with penicillin and fradiomycin or penicillin/fradiomycin and teat-sealer at drying off (1976-1977)

	Treatment			
	Penicillin/fradiomycin (H2)		Penicillin/fradiomycin and sealer (H2 + L6)	
herd:	BDR	CNK	BDR	CNK
No. of quarters assigned	160	173[1]	159[2]	174[3]
No. of quarters infected at drying off (%)	15 (9)	15 (9)	19 (12)	17 (10)
No.of infections eliminated during the dry period (%)	5 (33)	9 (60)	14 (74)	14 (82)
No.of infections eliminated during the dry period in both herds (%)	14 (47)		28 (78)	

[1]3 inactive quarters [2]1 inactive quarter [3]2 inactive quarters

Table 28 : Bacteria isolated from infected quarters at drying off and the subsequent response to treatment with penicillin and fradiomycin or penicillin/fradiomycin and teat-sealer at drying off (1976-1977)

	Treatment			
	Penicillin/fradiomycin (H2) (H2)		Penicillin/fradiomycin and sealer(H2 + L6)	
Pathogen	No. of infected qrs treated	No. of infections eliminated (%)	No. of infected qrs treated	No. of infections eliminated (%)
Staph. aureus	26	11[a] (42)	30	22[b] (73)
Strep. dysgalactiae	1	1	1	1
Strep. uberis	2	1	1	1
No bacteria isolated	1	1	4	4
Total	30	14 (47)	36	28 (78)

a,b Values with different superscripts on the same line differ significantly ($P<0.05$)

6.7.4.1 Previous mastitis history in the treated infected quarters

During the previous lactation, all the cows on the experiment had been monitored for mastitis (in all udder quarters), once each month. An analysis of these data was undertaken in an attempt to explain the difference in efficacy between Treatments 1 and 2. From these data a calculation was made on the number of days an individual udder quarter was infected with *Staph. aureus* before drying off. This was used as a measure of the persistence of infection and as an indicator of chronic mastitis. The average number of days that quarters were infected with *Staph. aureus* was calculated for each treatment within each herd. These data were further subdivided into the average number of days infected for: (a) quarters where the infection continued after the next calving and (b) quarters where the infection was eliminated during the dry period.

In general, there was no difference in the average duration of infection within herds in quarters from which the infections were eliminated. The average duration of infection before drying off in Treatment 1 was 23 to 33 days longer in the infected quarters that continued after calving than in the quarters from which the infections were eliminated (Table 29). In Treatment 2, the average duration of infection before drying off was 62 to 70 days longer in quarters from which the infections continued than in the quarters from which the infections were eliminated (Table 29). Even though the efficacy of Treatment 2 was superior to Treatment 1 (Table 27) the average duration of infection before drying off in the quarters that remained infected after calving in Treatment 2 was 43 to 54 days longer than for Treatment 1.

In a within-herd comparison of the effectiveness of Treatments 1 and 2 in the treatment of *Staph. aureus* there was no difference in efficacy between the treatments in either the BDR or CNK herds (Table 29).

6.7.4.1.1 *Mastitis history in quarters with new infections*

Of the 23 new infections in Treatment 1, seven had been infected in the previous lactation. A summary of the number of days that each of these quarters was free of infection before drying off is presented in Table 30. Three udder quarters in Treatment 1 were free of mastitis for an average of 28 days (range 14 to 44) before drying off, the remaining four quarters were free of mastitis for an average of 153 days (range 86 - 227) before drying off. The quarter which was free of infection for only 14 days before drying off had a *Staph. aureus* infection during the lactation and developed an *A. pyogenes* infection during the dry period. Only one quarter which developed a new infection in Treatment 2 had an infection in the previous lactation. That infection was eliminated, however, 122 days before drying off (Table 30).

Table 29 : Duration of *Staph. aureus* infections during the lactating period prior to treatment with either penicillin and fradiomycin or penicillin/fradiomycin and teat-sealer at drying off (1976-1977)

Treatment	Farm	No. of infections	No. eliminated (%)	Duration of infections before drying off (mean days)	No. of infections continued	Duration of infections before drying off (mean days)
1	BDR	14	5 (36)	105.4	9	128.5
	CNK	12	6 (50)	86.8	6	120.3
2	BDR	18	13 (72)	121.3	5	182.8
	CNK	12	9 (75)	92.5	3	163.3

Table 30 : Previous infection history in quarters that developed new dry period infections and had been treated with either penicillin and fradiomycin or penicillin/fradiomycin and teat-sealer at drying off (1976-1977)

Treatment	Farm	No. of quarters with new infections	No. of these infected in previous lactation	No. of days free of infection before drying off
1	BDR	11	5	14*, 27, 44, 86, 107
	CNK	12	2	191, 227
2	BDR	3	0	
	CNK	4	1	122

* *Staph. aureus* was the causative organism 14 days before drying off. This infection was eliminated but the quarter became infected with *A. pyogenes* during the dry period.

6.7.4.2 New infections in the dry period

A breakdown of the new infection types for each treatment and each herd is shown in Table 31. There was a total of 23 new infections in Treatment 1 and seven in Treatment 2. Twelve and seven of the new infections in Treatments 1 and 2, respectively, were caused by *Staph. aureus* or streptococcal spp. Of the additional 11 infections in Treatment 1, three had clinical non-specific mastitis (Table 31). *A. pyogenes* was isolated from three infected quarters, *E. coli* from four quarters and a fungus from one quarter. The antibiotic and teat-sealer treatment was more effective ($P<0.005$) in eliminating new infections than the antibiotic alone.

6.7.4.3 Results of X-ray examinations

A total of 50 teats were X-rayed in the 26 cows. The teats were X-rayed 92 ± 26 days after drying off and 30 ± 21 days before calving. Based on the results of these X-ray recordings, a subjective assessment was made on the quantity of sealer in each of the X-rayed teats. The results showed that 48 *per cent* of teats contained a large plug of sealer, 10 *per cent* had a small plug of seal and 18 *per cent* of teats contained small traces of seal. Twenty-four *per cent* of teats had no visible evidence of sealer.

In this experiment the presence or absence of teat sealer was not correlated with the incidence of new infection at calving.

Table 31 : Bacteria isolated from new infections in quarters treated with either penicillin and fradiomycin or penicillin/fradiomycin and teat-sealer at drying off (1976-1977)

	Treatment			
	Pencillin/fradiomycin (H2)		Pencillin/fradiomycin and Sealer(H2 + L6)	
Pathogen	BDR	CNK	BDR	CNK
Staph. aureus	4	2	2	2
Streptococcal spp.	3	3	1	2
A.pyogenes	1	2	0	0
E.coli	1	3	0	0
Fungi	0	1	0	0
No bacteria isolated	2	1	0	0
Subtotal	11	12	3	4
Total	23		7	

6.8 Experiment 20 (1978-1979)

The effectiveness of penicillin and fradiomycin or penicillin/fradiomycin in combination with a teat-sealer in eliminating infections during the dry period

The effectiveness of two treatment regimes was evaluated in 507 cows at drying off. Two hundred and thirty-four cows were allocated to Treatment 1 and 273 to Treatment 2 as follows:

6.8.1 Treatment 1 : All quarters in each of the 234 cows were infused at drying off with a mixture of penicillin and fradiomycin in an oily base (Product H3). Immediately after infusing the antibiotic, selected pairs of teats (either the right fore and right hind or the left fore and left hind: Figure 3) in each cow were infused with teat sealer (L7).

6.8.2 Treatment 2 : Two quarters in each of the 273 cows were selected in pairs (similar to Treatment 1) and infused with Product H3. The remaining pairs were infused with a penicillin and dihydrostreptomycin product in an aqueous base (M2). Immediately after infusing M2 these teats were also infused with teat-sealer (L7).

The treatment regimes may be summarized as follows:

(i) Two quarters were infused with Product H3
and
Two quarters were infused with Product H3 plus teat-sealer L7.

(ii) Two quarters were infused with H3

and

Two quarters were infused with Product M2 plus L7.

The objectives of the experiment were: (i) to quantify the effectiveness of the antibiotic (H3) used as a standard drying off treatment (control) and also used in combination with a teat-sealer and (ii) to compare the efficacy of the antibiotic (H3) and teat-sealer with that of the antibiotic (M2) and teat-sealer.

The effectiveness of the three treatment regimes was evaluated on the basis of elimination of infection at drying off and in the prevention of new infections during the dry period.

6.8.3 Results of Experiment 20

At drying off, the percentage of quarters infected in each treatment group ranged from five to 13 in the fore-quarters and from nine to 15 in hind quarters. When these data were combined for fore and hind quarters within each treatment group the percentage of infected quarters ranged from seven to 14 (Table 32).

There was a total of 102 infected quarters treated with H3 (control) and 48 of these infections (47 *per cent*) were eliminated. Seventy-three infected quarters were treated with H3 and L7 and 44 (60 *per cent*) of these infections were eliminated. Forty infected quarters were treated with M2 and L7 and 22 of these infections (55 *per cent*) were eliminated (Table 32). The difference in the elimination of infections between the three treatment regimes was not statistically significant.

6.8.3.1 New infection levels in quarters that were uninfected at drying off

Three-hundred and forty-seven cows were selected (from the 507 cows on the experiment) as uninfected at drying off. Six-hundred and ninty-four quarters were treated with H3 and 694 were treated with either H3 or M2 followed by teat-sealer (L7).

New infections developed in 32 (5 *per cent*) of the quarters treated with H3 and in 17 (2 *per cent*) of the quarters treated with H3 or M2 and teat -sealer (Table 33). The difference in new infection between the unsealed and sealed quarters was statistically significant ($P<0.05$).

Table 32 : The effectiveness of penicillin and fradiomycin or penicillin/fradiomycin in combination with teat-sealer or penicillin and dihydrostreptomycin in combination with teat-sealer in eliminating infections during the dry period (1978-1979)

Treatment	Udder quarters	No. of qrs. assigned	No. of qrs. infected (%)	No. of quarter infections eliminated (%)
Pencillin/ fradiomycin (H3) (Control)	fore	268	27 (10)	16 (59)
	hind	273	42 (15)	18 (43)
	Total	541	69 (13)	34 (49)
Pencillin/ fradiomycin (H3) and sealer (L7)	fore	267	35 (13)	25 (71)
	hind	271	38 (14)	19 (50)
	Total	538	73 (14)	44 (60)
Pencillin/ fradiomycin (H3) (Control)	fore	234	11 (5)	6 (55)
	hind	232	22 (9)	8 (36)
	Total	466	33 (7)	14 (42)
Pencillin/ dihydrostreptomycin (M2) and sealer (L7)	fore	231	13 (6)	8 (62)
	hind	234	27 (12)	14 (52)
	Total	465	40 (9)	22 (55)

Table 33 : The effectiveness of three antibiotic and teat-sealing regimes in reducing new infections in cows selected* as uninfected at drying off (1978-1979)

Drying off Treatment	Udder quarters	No. of qrs. assigned	No. of new infections (%)
Penicillin/fradiomycin (H3)	fore	347	19 (5)
(Control)	hind	347	13 (4)
	Total	694	32 (5)
Pencillin/fradiomycin (H3)	fore	347	4 (1)
or			
Penicillin/dihydrostreptomycin (M2)	hind	347	13 (4)
and sealer (L7)			
	Total	694	17 (2)

* Selected as uninfected from the original group of 507 cows

6.9 Results and Discussion

6.9.1 Artificial challenge experiments

The results of Experiments 13 and 14 showed that teat seals infused either alone or in combination with antibiotics at drying off significantly reduced ($P<0.05$) the level of new infection during the dry period (Table 20). In general the incidence of new infection following the bacterial challenge used in the untreated quarters in these experiments was similar to levels in cows with spontaneous natural infection (Serieys and Roguinsky, 1975). It is possible that a bacterial challenge technique using direct inoculation of bacteria into the teat duct rather than dipping the teats in culture may have provided a more successful challenge. While these experiments showed a difference in new infection levels between the sealed and the untreated quarters they did not show the effect of a standard dry period antibiotic formulation under similar conditions. In Experiment 16, a comparison was made between a teat-sealer and a standard antibiotic formulation containing 500 mg benzathine cloxacillin under conditions of artificial bacterial challenge. The results indicated that there was no difference in efficacy between the treatments. There was evidence, however, from the X-ray observations which were carried out four days before calving that the sealer was not present in three out of the six quarters that had developed new infections during the dry period. A further two infections in the sealed quarters had a history of mastitis during the previous lactation. When these factors were taken into account there was only one new infection in the teats where the sealers remained intact.

6.9.2 X-Ray observations

In Experiments 13 and 14, the seals remained in position in the teat sinuses and ducts for the complete dry period (10 to 25 weeks) in 93 *per cent* of the teats that were X-rayed. There was evidence of the loss of

some sealer through the teat orifice. There was also an indication that the sealer was being displaced into the teat cistern in some animals. This was confirmed by the disappearance and re-emergence of sealers as shown in the X-ray plates taken over consecutive weeks. This sealer was more persistent than the bismuth iodoform paraffin paste used by Pearson (1949) which was absorbed or neutralised 14 days after infusion.

6.9.3 Teat sealers in heifers

Teat-sealers were used also to prevent new infection in in-calf heifers. There was no difference in the level of new infection at calving between the sealed and untreated quarters; the number of animals selected for the experiment was probably too small and the new infection level too low (5 *per cent* to 6 *per cent*) to reveal significant differences. In comparison, 19 *per cent* of quarters developed new infections at calving in 60 heifers wintered in similar environmental conditions (Meaney, 1981). Recently, other workers found that antibiotic therapy administered prepartum was effective in reducing the level of new infection in heifers at calving; however, the authors expressed concern regarding the potential risk of antibiotic residues in milk following the use of this technique (Oliver, Lewis, Gillespie and Dowlen, 1992). Effective teat sealing would eliminate the risk.

There was no indication in Experiment 17 that breaking the natural seal in the teats of heifers during sampling before calving increased the risk of new infection. This is in agreement with a similar study in cows (Oliver *et al.*, 1956b).

6.9.4 Spontaneous infection experiments

The effectiveness of a variety of antibiotics in aqueous and oil bases was evaluated in conjunction with teat-sealers in cows with spontaneous infections in Experiments 15, 18, 19 and 20.

In Experiment 15, sixty-seven *per cent* of nine *Staph. aureus* infections were eliminated after treatment with an aqueous based penicillin and dihydrostreptomycin formulation in combination with a teat-sealer at drying off. This response was similar to responses obtained with oil based products evaluated in Chapter 4. Because the number of infections treated was small, the product was tested again in a larger population in Experiment 20.

In Experiment 18, there was no difference in efficacy between either of the antibiotic or teat-sealer treatments in eliminating infections at drying off (Table 24). The percentage of *Staph. aureus* infections eliminated was similar to the results obtained with a variety of antibiotic formulations as described in **Chapter 4**. The variation in the efficacy between the treatments (50 *per cent* to 74 *per cent*) was also observed in other experiments (Dodd and Griffin, 1975; Ziv *et al.*, 1981). The treatments using antibiotic or antibiotic and sealer at drying off were more effective ($P<0.01$; $P<0.05$) than the treatment using antibiotic at drying off and sealer 28 days later. When an adjustment was made in the data to include only the new infections in quarters that had no history of mastitis in the previous lactation, then there was no difference between the treatments (Table 25). The results of this experiment emphasise the need to maintain a historical data base on the infection status of experimental animals in order to reduce the risk of misinterpreting infection data.

Experiment 19 was undertaken in order to re-evaluate two of the treatments (antibiotic infused at drying off or antibiotic and teat-sealer infused at drying off). The seal treatment in this experiment, however, differed from Experiment 18 in that the antibiotic and teat-sealer were presented in the same intramammary syringe. When the data were combined for the two herds on experiment the antibiotic and teat seal formulation was more effective ($P<0.05$) in eliminating infection at drying off than the antibiotic alone (Table 27). This difference was primarily in the treatment of *Staph. aureus* infections ($P<0.005$), (Table

28). The result was unusual since it was anticipated that the primary function of the teat-sealer was to reduce the incidence of new infection. A possible explanation for the higher success with the antibiotic/seal combination was that there were fewer chronic infections treated with that product. When the infection history in the *Staph. aureus* infected quarters was studied, using duration of infection as an indicator of chronic status, there was no evidence to support this theory (Table 29). Variation in the response of *Staph. aureus* infections to antibiotic therapy, however, is not unusual, and has been reported by others (Dodd and Griffin, 1975; Ziv *et al.*, 1985).

Another possible explanation for the variation in efficacy was that the sealer may have prolonged the period of activity of the antibiotic. This was possible since the antibiotic and seal were presented in the same syringe. Further experimentation would be required to obtain data to support or reject this theory. The combination of antibiotic and teat-sealer was also more effective ($P<0.005$) in reducing new infections than the antibiotic treatment alone. When an examination of the mastitis history was undertaken for the quarters that developed new infections, only one quarter was infected 14 days before drying off (Table 30). When the data for this quarter were removed from the analysis the difference between the treatments was still significant ($P<0.005$).

In Experiment 20, a standard dry period antibiotic containing penicillin and fradiomycin (used in Experiments 18 and 19) was used as a control. This product was also used in combination with a teat-sealer. An aqueous based penicillin and dihydrostreptomycin product (already used in Experiment 15) was also used in combination with a teat-sealer. There was no difference in the elimination of dry period infection between the three treatments (Table 32). The uninfected quarters that were infused with the antibiotics (both oil based and aqueous based) and teat-sealer had fewer new infections ($P<0.05$) than the quarters that were treated with the antibiotic alone.

CHAPTER 7

A SURVEY OF THE EFFICACY OF VARIOUS ANTIBIOTIC FORMULATIONS IN ELIMINATING SPECIFIC PATHOGENS ISOLATED AT DRYING OFF

7.1 Introduction

It was apparent from the literature review that most studies indicate that *Staph. aureus* and a variety of streptococcal spp. are responsible for the majority of mastitis infections at drying off (Daniel and Steffert, 1969; Wilson, 1973; Batra, 1988; Phillips, 1979). A similar trend has been shown in all the experiments reported in this thesis.

In a survey presented in Experiment 21 in this Chapter, infection data assembled at drying off and after calving were extracted from some of the experiments reported already. Data were added from an additional herd to complete the data set for the 1984 to 1989 survey period. A more recent data set was assembled for a period 1990 to 1992. These data were assembled in order to present up-to-date information on the pathogen types isolated from cows at drying off under Irish conditions.

Analyses were carried out on a total data set of 666 infected cows and 979 infected quarters. This relatively large data set was used to (i) identify the major pathogen types isolated from cows at drying off, (ii) determine the efficacy of a variety of antibiotic products in eliminating these pathogens, (iii) determine the effect of lactation number on the efficacy of treatment of *Staph. aureus* infection, and (iv) determine the effect of single or multiple quarter infections in individual cows on the efficacy of treatment of *Staph. aureus* infections.

7.2 Experiment 21 (1984-1989; 1990-1992)

7.2.1 Infection survey between 1984 and 1989

At drying off, the types of bacteria causing mastitic infections were classified (from Experiments 7 to 11 inclusive) in 246 infected quarters in 157 cows. Similar data were collected in one commercial herd (between 1984 and 1986) from a further 179 infected quarters in 137 cows; these data were not reported in any of the previous 20 experiments. The combined data set on 294 cows and 425 quarters was collected in 11 dairy farms between 1984 and 1989. The infections were treated at drying off with a total of six antibiotic formulations (product types A; D; G; H; I and J) and the composition of these is shown in Table 1.

7.2.2 Infection survey between 1990 and 1992

The incidence of infection at drying off was surveyed in five research herds attached to Moorepark in 1990, 1991 and 1992. The incidence of mastitis was also monitored after calving in 1991 and 1992 in the same animals. Three hundred and seventy-two infected cows and 554 quarters were surveyed in the five herds in the three-year period. The infections were treated at drying off with a total of six antibiotic formulations (products A; D; H; I; J and N) and the composition of these is shown in Table 1. These data were not reported in any of the previous 20 experiments.

The method of selecting animals for drying off, infusion routines, drying off procedures, milk sampling procedures and definitions of mastitis were similar to those described for all the other experiments.

7.3 Results of Experiment 21

7.3.1 Mastitis pathogens isolated at drying off

7.3.1.1 Infection survey 1984 - 1989

Infection data were collected from the 425 treated quarters at the next calving but analyses were confined to 416 quarters that had been infected with staphylococci or streptococci at drying off.

The major pathogens isolated were *Staph. aureus* and streptococcal spp. At drying off, 259 (61 *per cent*) of the infections were caused by *Staph. aureus*, 118 (28 *per cent*) were caused by streptococcal spp. and 27 (6 *per cent*) were caused by non-haemolytic staphylococcal spp. (Table 34).

Table 34 : Bacteria isolated from 979 mastitic quarters in 666 cows at drying off (1984-1989; 1990-1992)

Pathogen	1984/1989		1990/1992		Combined Data 1984-1992	
	No. of qrs infected	% of total infections at drying off	No of qrs infected	% of total infections at drying off	No. of qrs infected	% of total infections at drying off
Staph. aureus	259	61	293	53	552	56
Staphylococcal spp. (non-haemolytic types)	27	6	55	10	82	8
Streptococcal spp.	118	28	142	26	260	27
Staph. aureus and streptococcal spp ·	12	3	13	2	25	3
Coliform spp.	5	1	13	2	18	2
No bacteria isolated	4	1	38	7	42	4
Total	425	100	554	100	979	100

7.3.1.2 Infection survey (1990 - 1992)

Five hundred and fifty-four infected quarters in 372 cows were identified in the five herds during three drying off seasons. Data are presented for all 554 quarters at drying off. After calving, however, data were available for only 388 of the 554 quarters that were infected at drying off. The losses in data was generally due to culling or transferring cows to different farms. Some drying off infection data were also collected during 1992 and the post-calving (1993) data were not available when the information was assembled. Analyses in the post-calving period were confined to 350 quarters that were infected with either staphylococci or streptococci at drying off.

The major pathogens isolated at drying off were *Staph. aureus* (53 *per cent*) and streptococcal spp. (26 *per cent*). Non-haemolytic staphylococcal spp. accounted for 10 *per cent* of the total infections (Table 34).

7.3.1.3 Combined infection survey data (1984-1989; 1990-1992)

When the data were combined for the two survey periods, 979 infections were identified in 666 cows. The bacteria isolated in each survey were similar. Overall, 919 infections (94 *per cent*) were caused by Gram-positive bacteria and two *per cent* by Gram-negative bacteria. Four *per cent* had non-specific mastitis at drying off (Table 34).

7.3.2 Effectiveness of long-acting antibiotics in eliminating infections caused by staphylococci and streptococci (1984-1989)

The data presented in Table 34 showed that 94 *per cent* of mastitis at drying off was caused by either staphylococcal spp. or streptococcal spp. A subset of data containing 416 of the most prevalent bacteria isolated from infected quarters was selected of which 243 (58 *per cent*) were eliminated during the dry period (Table 35). Forty-eight *per cent* of 259 *Staph. aureus*

infections and 78 *per cent* of 118 infections caused by streptococcal spp. were eliminated (Table 35).

Table 35 : The effectiveness of long-acting antibiotics in eliminating intramammary infections caused by staphylococci and streptococci during the dry period (1984-1989; 1990-1992)

Pathogen (Gram-positive)	No. of quarter infections treated					
	1984/89		1990/92		All (1984 - 1992)	
	NT[a]	NE[b] (%)	NT	NE (%)	NT	NE (%)
Staph. aureus	259	125 (48)	205	148 (72)	464	274 (59)
Staphylococcal spp. (non-haemolytic types)	27	21 (78)	41	39 (95)	68	56 (82)
Streptococcal spp.	118	92 (78)	95	67 (71)	213	159 (75)
Staph. aureus and streptococcal spp.	12	5 (42)	9	5 (56)	21	11 (52)
Total	416	243 (58)	350	259 (74)	766	500 (65)

[a]NT Number treated

[b]NE Number eliminated

7.3.2.1 Effectiveness of long-acting antibiotics in eliminating infections caused by staphylococci and streptococci (1990-1992)

A data set containing 350 of the most prevalent bacteria isolated from infected quarters was selected of which 259 (74 *per cent*) were eliminated during the dry period (Table 35). Seventy-two *per cent* of 205 *Staph. aureus* infections and 71 *per cent* of 95 infections caused by streptococcal spp. were eliminated (Table 35).

7.3.2.2 *Effectiveness of long-acting antibiotics in eliminating infections caused by staphylococci and streptococci (combined data 1984-1989; 1990-1992)*

When the data were combined for the two surveys, 766 infections caused by either staphylococci or streptococci were treated of which 500 (65 *per cent*) were eliminated (Table 35). Fifty-nine *per cent* of 464 *Staph. aureus* and 75 *per cent* of 213 streptococcal infections were eliminated (Table 35). Eighty-two *per cent* of the 68 infections with non-haemolytic staphylococcal spp. were eliminated.

7.3.3 *The effect of lactation number on the treatment of Staph. aureus with long-acting antibiotics*

7.3.3.1 *Survey 1984-1989*

In the 1984-1989 survey, a total of 191 cows and 259 quarters were infected with *Staph. aureus* at drying off. These data were subdivided into three lactation age categories as follows: (i) first and second lactation (category 1); (ii) third to fifth lactation (category 2) and (iii) greater than fifth lactation (category 3). The effectiveness of the antibiotics in treating *Staph. aureus* was quantified for each lactation category.

The results for cows and quarters are presented in Tables 36 and 37. Sixty-eight *per cent* of 68 infected quarters were eliminated in category 1, 46 *per cent* of 138 infections were eliminated in category 2 and 30 *per cent* of 53 infections were eliminated in category 3 (Table 37). The antibiotic treatment was more effective ($P<0.01$) in eliminating *Staph. aureus* infection in cows in category 1 than in category 2 (Table 36). The treatment was also more effective ($P<0.01$) in eliminating *Staph. aureus* in cows in category 1 than in category 3. A similar trend was shown in quarters (Table 37). The treatment was more effective in eliminating *Staph. aureus* infections in quarters in category 1 than in category 2 ($P<0.01$). The treatment was also more effective in eliminating *Staph. aureus* in quarters in category 1 than in category 3 ($P<0.001$).

7.3.3.2 Survey 1990-1992

In the 1990-1992 survey, a total of 164 cows and 205 quarters were infected with *Staph. aureus* at drying off. The data were divided into the same lactation categories as described for the 1984-1989 survey. The results for cows and quarters are presented in Tables 36 and 37. Eighty-five *per cent* of 67 quarter infections were eliminated in category 1, 69 *per cent* of 59 infections were eliminated in category 2 and 63 *per cent* of 79 infections were eliminated in category 3 (Table 37). The antibiotic treatment was more effective in eliminating *Staph. aureus* infections in cows in category 1 than in category 3 ($P<0.01$) (Table 36). A similar trend was shown in quarters (Table 37). The antibiotic was more effective in eliminating *Staph. aureus* infections in quarters in category 1 than in category 3 ($P<0.05$). There was no difference in efficacy between categories 1 and 2 or categories 2 and 3 for either cows or quarters.

7.3.3.3 Combined survey data (1984-1989; 1990-1992)

When the data from the two surveys were combined, 54 *per cent* of 355 treated cows and 59 *per cent* of 464 quarters were free of *Staph. aureus* infections after calving (Tables 36 and 37). Seventy-six *per cent* of 135 infections in category 1 were eliminated, 53 *per cent* of 197 infections in category 2 were eliminated and 50 *per cent* of 132 infections in category 3 were eliminated (Table 37). The antibiotic treatment was more effective in eliminating *Staph. aureus* infections in cows in category 1 ($P<0.001$) than in either categories 2 or 3 (Table 36). There was a similar trend ($P<0.001$) in quarters (Table 37). There was no difference in efficacy in quarters between categories 2 and 3 either in the 1984-1989 or the 1990-1992 or in the combined data.

Table 36 : The effectiveness of long-acting antibiotics in eliminating *Staph. aureus* infections in cows in different lactation age categories (1984-1989; 1990-1992)

Lactation Range	1984 -1989 No. of cows infected	1984 -1989 No. of cow infections eliminated (%)	1990 - 1992 No of cows infected	1990 - 1992 No of cow infections eliminated (%)	Combined data 1984-1992 No. of cows infected	Combined data 1984-1992 No. of cow infections eliminated (%)
1 -2 (Category 1)	52	33 (63)	56	45 (80)	108	78 (72)
3 - 5 (Category 2)	99	37 (37)	72	46 (64)	171	83 (49)
>5 (Category 3)	40	13 (33)	36	17 (47)	76	30 (39)
All lactations	191	83 (43)	164	108 (66)	355	191 (54)

Values in the same column connected by a line are significantly different *(P<0.05); **(P<0.01); ***(P<0.001).

Table 37 : The effectiveness of long-acting antibiotics in eliminating *Staph. aureus* infections in quarters of cows in different lactation age categories (1984-1989; 1990-1992)

Lactation Range	1984 -1989 No. of quarter infections treated	1984 -1989 eliminated (%)	1990 - 1992 No. of quarter infections treated	1990 - 1992 eliminated (%)	Combined data 1984-1992 No. of quarter infections treated	Combined data 1984-1992 eliminated (%)
1 - 2 (Category 1)	68	46 (68)	67	57 (85)	135	103 (76)
3 - 5 (Category 2)	138	63 (46)	59	41 (69)	197	104 (53)
>5 (Category 3)	53	16 (30)	79	50 (63)	132	66 (50)
All lactations	259	125 (48)	205	148 (72)	464	273 (59)

Values in the same column connected by a line are significantly different *(P<0.05); **(P<0.01); ***(P<0.001).

7.3.4 The influence of single or multiple quarter infections (with Staph. aureus) in individual cows at drying off on the effectiveness of antibiotic therapy during the dry period (1984-1989; 1990-1992)

7.3.4.1 Survey 1984-1989

Of the 259 *Staph. aureus* infections detected at drying off, 141 were diagnosed in a single quarter in each of 141 cows. Thirty-seven cows had two quarters infected, eight cows had three quarters infected and five cows had all quarters infected (Table 38). The elimination of *Staph. aureus* infections in the cows with single and multiple infections at drying off is presented in Table 38.

Single quarter infections were eliminated from 50 *per cent* of 141 treated cows. Thirty-two (43 *per cent*) infections were eliminated from cows with two quarters infected. Eighteen (75 *per cent*) infections were eliminated from cows with three quarters infected and eight (40 *per cent*) were eliminated from cows with all quarters infected (Table 38).

7.3.4.2 Survey 1990-1992

Of the 293 *Staph. aureus* infections detected at drying off, 88 were not sampled at the next calving (due to culling, animal transfers or 1993 calving data not being available). Results are presented, therefore, for this exercise on 205 infections with completed data after calving.

Single quarter infections were eliminated from 72 *per cent* of 126 cows. Fifty-five (79 *per cent*) infections were eliminated from cows with two quarters infected. Eight (89 *per cent*) infections were eliminated from cows with three quarters infected (Table 38). There was no animal in this survey with all quarters infected. In this survey there was no difference in efficacy associated with the number of quarters infected within cows (Table 38).

7.3.4.3 Combined survey data (1984-1989; 1990-1992)

In total, there were 464 quarters infected with *Staph. aureus* in 355 cows at drying off. Two hundred and sixty-seven infections were diagnosed in treated cows with a single quarter infected and 61 *per cent* of these were eliminated. There were 144 infections in the cows with two quarters infected and 60 *per cent* were eliminated. Thirty-three infections were diagnosed in the cows with three quarters infected and 79 *per cent* were eliminated. There were 20 infections in the cows with four quarters infected and 40 *per cent* were eliminated (Table 38). The treatment of *Staph. aureus* in cows with three quarters infected was more effective than in cows with either one quarter ($P<0.05$), two quarters ($P<0.02$), or four quarters ($P<0.02$) infected (Table 38).

There was a significant difference in the responses to treatment between cows with single quarters infected in 1984-1989 and 1990-1992 ($P<0.001$). There was a similar difference in efficacy for cows with two quarters infected at drying off ($P<0.001$) (Table 38).

7.3.5 The influence of lactation number on the response to antibiotic treatment of Staph. aureus in cows with single quarter infections at drying off (1984-1989; 1990-1992)

7.3.5.1 Survey (1984-1989)

Treated cows that had single quarters infected with *Staph. aureus* at drying off were selected for this analysis. A subset of 141 cows/quarters was selected from the complete data set. The elimination of *Staph. aureus* during the dry period was compared for animals in each of the six lactation age categories (first, second, third, fourth, fifth and greater than fifth). The results in Table 39 show that the effectiveness of antibiotic treatment was reduced with increases in the lactation number. Seventy-nine *per cent* of infections in first lactation and 39 *per cent* of infections in

animals greater than fifth lactation were eliminated (Table 39).

7.3.5.2 Survey (1990-1992)

The selection of cows and quarters and the method of data analysis in this survey was similar to that described for the 1984-1989 survey. One hundred and twenty-six cows/quarters were selected from the complete data set. Eighty-seven *per cent* of infections in first lactation animals and 68 *per cent* of infections in animals of greater than fifth lactation were eliminated (Table 39).

7.3.5.3 Combined survey data (1984-1989 : 1990-1992)

In total, 267 *Staph. aureus* infections were treated. Eighty-four *per cent* of 43 infections were eliminated in first lactation animals. In contrast, 51 *per cent* of *Staph. aureus* infections were eliminated in animals greater than fifth lactation (Table 39).

7.3.5.4 Statistical Analysis

The chi-square test of homogeneity was carried out on the three data sets (1984-1989; 1990-1992 and the combined data). There was a general trend in the 1984-1989 data that as the lactation number increased the effectiveness of the antibiotic treatment decreased ($P<0.02$). This trend was not significant in the 1990-1992 data. However, when the analysis was carried out on the combined data the trend was significant ($P<0.01$). The effectiveness of the antibiotic treatment in the combined data from the first and second lactation cows was also compared with the older cows. The treatment was more effective ($P<0.001$) in the first and second lactation animals.

Table 38 : The effectiveness of long-acting antibiotics in eliminating *Staph. aureus* in cows with one or more quarters infected at drying off (1984-1989; 1990-1992)

No. of quarters infected per cow	1984 - 1989				1990 - 1992				Combined data 1984 - 1992			
	Number infected		Number of quarter infections eliminated (%)		Number infected		Number of quarter infections eliminated (%)		Number infected		Number of quarter infections eliminated (%)	
	Cows	quarters			Cows	quarters			Cows	quarters		
1	141	141	71	(50)[a]	126	126	91	(72)[b]	267	267	162	(61)
2	37	74	32	(43)[c]	35	70	55	(79)[d]	72	144	87	(60)
3	8	24	18	(75)	3	9	8	(89)	11	33	26	(79)
4	5	20	8	(40)	0	0	-		5	20	8	(40)
All quarters	191	259	129	(50)	164	205	154	(75)	355	464	283	(61)

[a,b,c,d]Values with different superscripts on the same line are different ($P<0.001$)

Table 39 : The effectiveness of long-acting antibiotics in eliminating *Staph. aureus* infections in cows (in different lactation age categories) with only one quarter infected at drying off (1984-1989; 1990-1992)

Lactation Number	1984 -1989 No. of infections treated	1984 -1989 No. of infections eliminated (%)	1990 - 1992 No. of infections treated	1990 - 1992 No. of infections eliminated (%)	Combined data 1984-1992 No. of infections treated	Combined data 1984-1992 No. of infections eliminated (%)
1	20	16 (79)	23	20 (87)	43	36 (84)
2	19	12 (63)	22	17 (77)	41	29 (71)
3	23	12 (52)	21	14 (67)	44	26 (59)
4	29	14 (48)	21	13 (62)	50	27 (54)
5	19	5 (26)	17	12 (71)	36	17 (47)
>5	31	12 (39)	22	15 (68)	53	27 (51)
All lactations	141	71 (50)	126	91 (72)	267	162 (61)

7.4 Results and Discussion

At drying off, 98 *per cent* of the infections in the 1984-1989 survey and 91 *per cent* in the 1990-1992 survey were caused by staphylococci and streptococci in either pure or mixed culture. The combined data for both survey periods showed that 94 *per cent* of all infections diagnosed at drying off were caused by staphylococci and streptococci. Pure cultures of *Staph. aureus* were isolated from 56 *per cent* (range 53 *per cent* to 61 *per cent*) of infected quarters and streptococcal spp. from 27 *per cent* (range 26 *per cent* to 28 *per cent*) of infected quarters (Table 34). These data are in general agreement with other studies (Schultze and Mercer, 1976; Nickerson, 1990b). The infection pattern in the two surveys was similar. Infections caused by coliform spp. were diagnosed in two *per cent* (range 1 *per cent* to 2 *per cent*) of quarters and non-specific clinical mastitis was evident in four *per cent* (range 1 *per cent* to 7 *per cent*) of quarters at drying off (Table 34).

In the 1984-1989 survey, forty-eight *per cent* of *Staph. aureus* infections were eliminated with antibiotic therapy. This response was significantly ($P<0.001$) lower than the 72 *per cent* eliminated in the 1990-1992 survey (Table 37), but was similar to responses observed with a variety of products evaluated in infected cows of less than sixth lactation (Figure 6, **Chapter 4**).

The response in 1984-1989 was lower than the responses of 72 to 82.8 *per cent* reported by Storper and Ziv (1985). Curtis *et al.* (1977) and Boddie and Nickerson (1986) reported *Staph. aureus* elimination levels of 60 *per cent* and 45.4 *per cent*, respectively. These data were similar to the data observed in the 1984-1989 survey. Considerable variation between herds, however, has been reported in a number of studies, particularly in the treatment of *Staph. aureus* infections (Dodd and Griffin, 1975; Ziv *et al.*, 1981).

Seventy-five *per cent* (range 71 *per cent* to 78 *per cent*) of the streptococcal spp. were eliminated (Table 35). In general, these responses were lower than the responses obtained in the earlier experiments where the elimination of streptococcal spp. was usually in excess of 90 *per cent*. The number of streptococcal infections in these experiments, however, was generally too small to make accurate predictions on efficacy. Reports from other workers indicate streptococcal elimination levels in excess of 90 *per cent* (Hoare, Barton and Thompson, 1973; Phillips, 1979).

A response of 82 *per cent* was shown in the treatment of non-haemolytic staphylococcal spp. and this response was considerably higher than the response for *Staph. aureus* in each survey period (Table 35). The quarters with mixed *Staph. aureus* and streptococcal infections gave responses similar to those for quarters with *Staph. aureus* infections only (52 *per cent*).

A comparison was made on the effectiveness of antibiotic therapy in eliminating *Staph. aureus* infections in cows with single or multiple infections at drying off. When the data sets were combined for the two survey periods there was no difference in efficacy between the cows with one or two quarters infected at drying off. The treatment was more effective ($P<0.02$) in eliminating infections in cows with three quarters than in cows with four quarters infected at drying off. This effect was present only in the 1984-1989 survey data (Table 38) but had an influence on the data set for the combined survey periods. In the same survey data (1984-1989) the treatment in cows with three quarters infected was more effective than in cows with either one quarter infected ($P<0.05$) or in cows with two or four quarters infected ($P<0.02$). There was no difference in the response between cows with either one, two or three infected quarters in the 1990-1992 data (Table 38). The lower response in the cows with one quarter infected in the 1984-1989 survey cannot be explained.

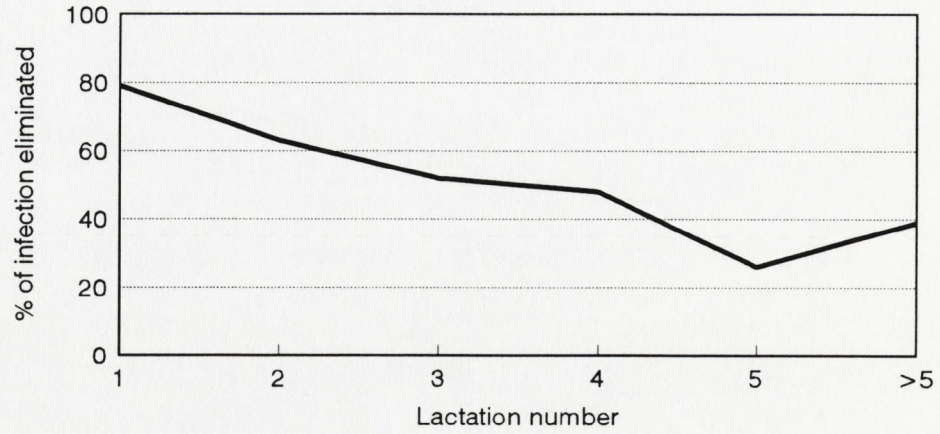

Figure 7: Elimination (*per cent*) of *Staph.aureus* mastitis in cows with single quarter infections in different lactation age categories (1984-1989)

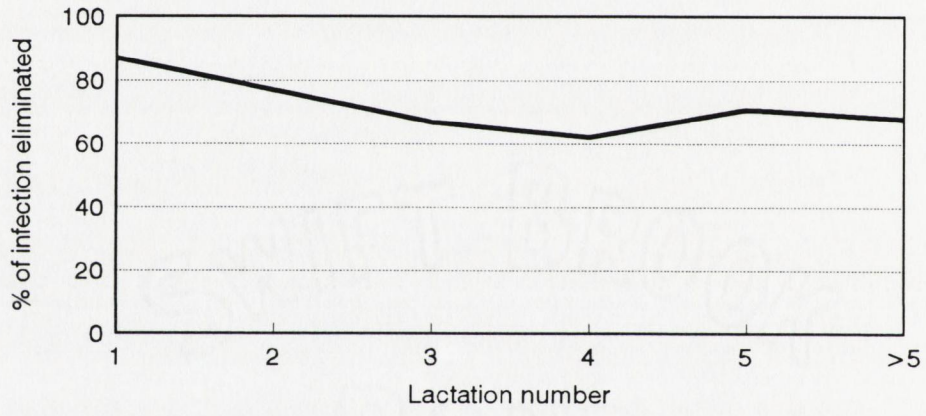

Figure 8: Elimination (*per cent*) of *Staph.aureus* mastitis in cows with single quarter infections in different lactation age categories (1990-1992)

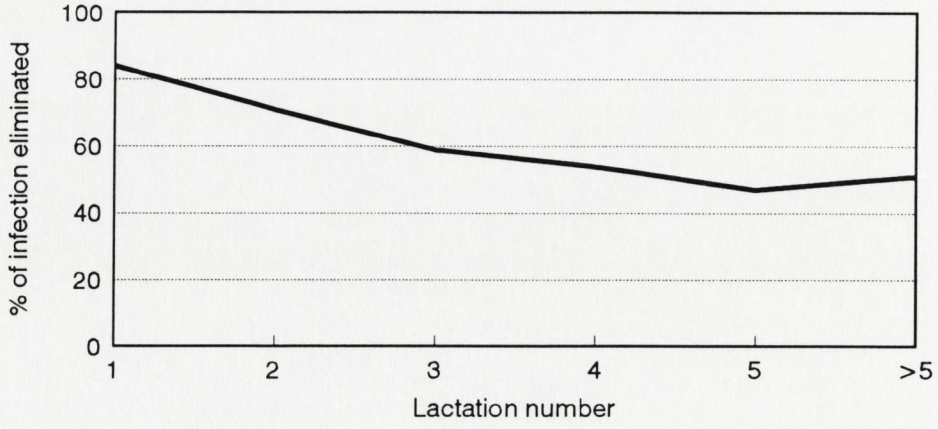

Figure 9: Elimination (*per cent*) of *Staph.aureus* mastitis in cows with single quarter infections in different lactation age categories (combined data 1984-1989; 1990-1992)

The treatment of *Staph. aureus* in the 1990-1992 period was more effective than in the 1984-1989 period (Table 38). The trend was shown in cows with one quarter infected or two quarters infected with the same level of significance ($P<0.01$). While this result is difficult to explain it may be related to variation in efficacy between herds (Dodd and Griffin, 1975; Ziv *et al.*, 1981).

For the final analysis, the cows were selected with only a single quarter infected with *Staph. aureus*. This data set was subdivided into first, second, third, fourth, fifth and greater than fifth lactation. In 1984-1989, there was an overall trend that as the lactation number increased the efficacy of the treatment decreased ($P<0.02$) (Figure 7). Even though the difference in efficacy between lactation numbers was not significant in the 1990-1992 data, there was a general trend in the data that was similar to the 1984-1989 data (Figure 8). When the data were combined for the two periods, again there was an overall trend of decreasing efficacy with increases in lactation number ($P<0.01$) as demonstrated in Figure 9. In this combined data set the antibiotic therapy was also more successful ($P<0.001$) in eliminating *Staph. aureus* infections in animals of first and second lactation than in older animals. This was the pattern both in infected cows (Table 36) and in infected quarters (Table 37). A similar trend was shown in both survey periods. Similar trends have been shown when treating either subclinical or clinical *Staph. aureus* infections in lactating cows (Griffin, 1971).

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

The EC standards scheduled to take effect from January, 1994 place major emphasis on the production of quality milk from disease-free animals (Council Directive 92/46/EEC, Anon (1992)). This Directive has changed the focus from the control of disease *per se* to the impact of disease on consumer attitudes as indicated by the present demands for quality milk and milk products. Many Irish dairy farmers are now confronted with major decisions on the most effective method for reducing the SCC levels in their bulk milk supplies. Reducing SCC is now firmly focused on achieving and maintaining standards of milk quality that will satisfy EC threshold levels and also the purchasers and consumers of dairy products within the European Community.

Staph. aureus is one of the major pathogens associated with bovine mastitis (Batra, 1988, Browning *et al.*, 1990; Bramley, 1992). *Staph. aureus* infections often persist as subclinical infections for weeks or months (Bramley, 1992) and thereby contribute to increases in SCC.

Mastitis control programmes have now become firmly established throughout the world and are usually based on the implementation of a five point plan (Bramley, 1992; Smith and Hogan, 1992). The routine use of dry period antibiotic therapy is one of the key elements of this plan. While there is disagreement among researchers on the issue of treating all quarters or selected infected quarters at drying off, all agree that long-acting antibiotics play a major role in reducing dry period infection. This topic was reviewed and discussed in recent years (Robinson, Jackson and Marr, 1988; Francis, 1989).

As already stated in **Chapter 1**, the primary objective of this study was to evaluate the effectiveness of a variety of antibiotic and non-

antibiotic formulations in reducing mastitis during the dry period. It was also proposed to classify the major pathogen types causing mastitis at drying off.

In **Chapter 4,** the efficacy of seven antibiotic formulations was evaluated between 1968 and 1989. The products were tested primarily on their ability to eliminate *Staph. aureus* infections at drying off. The elimination of *Staph. aureus* with a variety of products ranged from 25 *per cent* to 94 *per cent* of quarters. In most experiments there was no difference in efficacy between the products. The difference in efficacy between the benzathine cloxacillin and the neomycin/spiramycin products in Experiment 7 was not confirmed in Experiment 8 or Experiment 9. This emphasises the need to interpret data with caution particularly in situations where the number of infections treated and the number of herds enrolled is small.

The poor response of *Staph. aureus* to treatment, shown in some of the experiments, is not unusual and large variations in efficacy between herds have been shown by other workers (Dodd and Griffin, 1975; Ziv *et al.*, 1981). Sol, Harink, Schukken and Snoep (1992) reported that the bacteriological cure of *Staph. aureus* decreased with increases in SCC, age and the number of quarters infected per cow at drying off. When the younger animals were selected (less than sixth lactation) for treatment in Experiments 8 to 11, the efficacy of a range of antibiotic products was not improved.

In order to improve efficacy in the treatment of *Staph. aureus* some workers (Soback, Ziv, Winkler and Saran, 1990) experimented with systemic dry cow therapy. Results showed that norfloxacin nicotinate administered subcutaneously was more effective in eliminating *Staph. aureus* than a conventional dry period intramammary antibiotic (Soback *et al.*, 1990). Boddie and Nickerson (1986) also showed that full insertion of the cannula on the conventional intramammary syringe could result in the removal of the keratin plug from the teat duct,

allowing pathogens to enter the teat. As a result of their studies, they suggested the use of a modified syringe to accommodate partial insertion of the cannula into the teat. In contrast, a study by Sol and ter Balkt (1990) did not support this recommendation.

In the comparison of treated and untreated (control) quarters reported in Experiment 2, there was a significant benefit ($P<0.001$) in treating *Staph. aureus* with benzathine cloxacillin. This finding is in agreement with other studies (Smith *et al.*, 1967; Pankey *et al.*, 1982; Storper and Ziv, 1985).

The results of Experiment 6 showed that there was no advantage in infusing benzathine cloxacillin on two occasions during the dry period. Storper and Ziv (1985) and Cummins and McCaskey (1987) reported similar results. Re-infusing udder quarters during the dry period (particularly during the winter months) increases the risk of introducing contaminants into the teats at the time of infusion. There is also the added risk of antibiotic residues in the milk of treated cows after calving. This technique is not recommended.

In **Chapter 5,** a comparison was made between antibiotic treatment at drying off and an untreated control. A within-cow comparison was made in uninfected cows. The antibiotic was more effective ($P<0.001$) than the control in preventing new infections during the dry period. This result is in agreement with other studies (Bramley and Dodd, 1984; Batra, 1988). While some workers do not support the general recommendation of treating the uninfected quarters at drying off (Bratlie, 1973; Morse, 1975; Browning *et al.*, 1990) Robinson *et al.* (1988) stated that "*withholding dry cow therapy in uninfected and micrococcal infected quarters resulted in an unacceptably high rate of new major pathogen infection*". The results of Experiment 12 support this view. Armstrong (1977) concluded that dry cow treatment with antibiotics appeared to reduce the cow's resistance to mastitis and especially the coliform type. This concern was also expressed by Howell (1972). This trend, however, was not observed in Experiment 12 since

there were more new infections associated with environmental bacteria ($P<0.05$), including coliform spp., in the untreated quarters. There was no difference in the new infection levels between the treated and untreated quarters during the subsequent post-calving housed period.

A possible compromise to encompass all views may be the use of antibiotic therapy in the infected quarters and a non-antibiotic substance to protect the uninfected quarters.

In **Chapter 6,** the effectiveness of a bismuth subnitrate teat-sealer was evaluated in a series of eight experiments. The sealer was evaluated initially, either alone or in combination with an antibiotic, on its ability to prevent the development of new infection under conditions of bacterial challenge. The sealer, either alone or in combination with the antibiotic, was more effective ($P<0.05$) than the untreated control. When the sealer was compared with a standard 500mg benzathine cloxacillin under similar conditions of bacterial challenge there was no difference between the treatments. The results of these experiments indicated that the sealer could provide an alternative means of protecting uninfected quarters during the dry period.

X-ray examinations indicating the position of the sealers within the teats showed that some sealers remained in place (in the teat sinus and duct) for dry periods of ten to 25 weeks. Some seals were lost. There was also an indication that some seals moved upwards into the teat cistern. This was confirmed when particles of the seal was present in the milk of some cows for up to 21 days after calving. Further modification of the seal is required in order to overcome this problem.

Teat-sealers were also used in in-calf heifers as a method for reducing new infection levels at calving. There was no difference between sealed and control quarters. The new infection level in these animals was low by comparison with the incidence in another group of heifers examined during the same period (Meaney, 1981). The sampling of the

dry period secretion before infusing the teat seals did not predispose the animals to new infection. Oliver *et al.* (1956b) had already indicated that the breaking of the natural seal in cows did not increase infection. This may not be surprising since Trinidad, Nickerson, Alley and Adkinson (1990) found that micro-organisms survive in keratin and may produce harmful substances that are deleterious to the secretory tissue of the udder. Thus the removal of some of this material when sampling may have created a beneficial effect. It was possible to infuse 3.0g of sealing material into 63 out of the 64 quarters allocated to treatment; some at 110 days before calving.

The sealer was evaluated in three experiments (18, 19 and 20) under natural challenge conditions. Because some of the cows were infected at the time of infusion, an antibiotic formulation in either an oil or aqueous base was infused before the teat-sealer. The seal and antibiotic combinations were compared with a standard dry cow formulation containing penicillin and fradiomycin. In Experiments 18 and 20 the antibiotics and teat-sealers were presented in separate syringes and there was no difference between the treatments in the elimination of infection at drying off. The elimination of *Staph. aureus* ranged from 36 *per cent* to 74 *per cent* which was similar to the results in Chapter 4 and reports by other workers (Dodd and Griffin, 1975). When the antibiotic and teat-sealer were presented in the same syringe, however, the combination was more effective ($P<0.05$) than the antibiotic alone in eliminating *Staph. aureus* infections at drying off. This result was considered unusual since it was anticipated that the function of the teat-sealer was to prevent new infection. It is possible, however, that the teat-sealer, in this instance, altered the release profile of the antibiotic which may have retained the minimum inhibitory concentration for a longer period. This hypothesis would require further investigation.

On first analysis of the new infection data in Experiment 18 the antibiotic or the antibiotic and teat-sealer infused at drying off were significantly more effective than the antibiotic infused at drying off

and the teat-sealer infused 28 days later. When an adjustment was made to eliminate data from quarters with a history of infection during the previous lactation, there was no difference between the treatments. The results of this experiment emphasise the need to maintain a historical data base on the infection status of experimental animals in order to reduce the risk of misinterpreting infection data. The need to maintain infection history was also stressed by Pearson and Wright (1969). In Experiments 19 and 20 the antibiotic/sealant treatments were more effective ($P<0.005$; $P<0.05$) in reducing new infections than either of the antibiotics alone presented in oil or aequous bases.

In **Chapter 7,** data were assembled (to form the 1984-1989 survey) from some of the experiments reported in Chapter 4 and also from an additional commercial herd not reported previously. The 1990-1992 survey data was assembled from five herds attached to Moorepark. When the data were combined for the two survey periods, 94 *per cent* of all infections diagnosed at drying off was caused by staphylococci and streptococci. This finding has been consistent in studies at Moorepark over the past 25 years and is also consistent with reports from other workers (Smith *et al.*, 1967; Poutrel, 1977; Buddle *et al.*, 1987; Browning *et al.*, 1990).

Coliform spp. were isolated from one to two *per cent* of quarters at drying off. With this low percentage of Gram-negative bacteria in drying off samples it is difficult to condone the inclusion of broad spectrum and cocktail type antibiotics in dry cow formulations. Some commercial interests justify the inclusion of these products on the basis of *in vitro* antibiotic sensitivity testing. Several workers, however, have indicated that *in vitro* testing shows extremely poor correlation with the outcome of the treatment of mastitis (Sandholm *et al.*, 1990; Sears and Blackburn, 1992).

The survey data also showed that antibiotic therapy was more effective ($P<0.001$) in eliminating *Staph. aureus* mastitis in animals in first and

second lactation than in older cows. This result was similar to results reported by Griffin (1971) in the treatment of *Staph. aureus* in lactating cows and by Sol *et al.* (1992) in dry cows. When animals were selected from the survey material with single quarters infected with *Staph. aureus* at drying off, 84 *per cent* of first lactation animals and 51 *per cent* of animals greater than fifth lactation responded. There was a general trend of decreasing efficacy with increases in lactation number ($P<0.01$). This finding is in agreement with the observation made by Sol *et al.* (1992).

CONCLUSIONS

The studies on dry period intramammary antibiotics showed that in general there was no difference in efficacy between the formulations. However, there was considerable variation in the efficacy of the formulations between the experiments.

The treatment of uninfected cows with antibiotics at drying off made a significant ($P<0.001$) contribution to reducing the level of new infection at the next calving.

In general, the non-antibiotic teat-sealers were as effective as the antibiotics in preventing new infections during the dry period. In some instances the antibiotic combined with the seal formulation was more effective than an antibiotic alone in eliminating existing infections. The results of the experiments demonstrate the advantages of teat-sealing as an alternative to antibiotics for preventing new infections during the dry period.

The survey data showed that 94 *per cent* of intramammary infections which were diagnosed at drying off were caused by staphylococci and streptococci. Antibiotics which are formulated for the dry period

should, therefore, target the Gram-positive bacteria. Broad-spectrum antibiotics should be reserved for strategic applications particularly during winter-housing periods when acute infections are more likely to be caused by combinations of Gram-negative and Gram-positive bacteria.

Efficacy testing of products (especially under contract to commercial interests) is usually a compromise between the financial support available and the most effective experimental design. Because of this constraint it is not always possible to carry untreated controls or sufficient animal numbers or herds to show statistical significance.

There is also a requirement to standardize testing protocols, particularly in relation to the definition of infection. This subject was highlighted by Pearson and Wright (1969) when they stated that *"the word "infection" should not be confused with the word "isolation", as the organism may not always be associated with a pathological change"*. At present, an attempt is being made within the EC to develop protocols and standards for product evaluation.

One of the most novel topics in this thesis was the studies on the use of the non-antibiotic teat-sealers in the prevention of new infections. This testing programme was not supported after 1979 (Experiment 20). Further development on teat-sealers, however, re-commenced recently.

BIBLIOGRAPHY

A

Anon, (1992). Council Directive 92/46/EEC. *Official Journal of the European Communities* No L268/2 : 1-32.

Armstrong, K.R. (1977). Clinical experiences with dry cow therapy and coliform mastitis. *Bovine Practitioner* **12** : 85-87.

B

Batra, T.R. (1988). Effect of complete dry cow treatment on mastitis control in dairy cattle. *Canadian Journal of Animal Science* **68** : 553-556.

Beretta, C. and Carli, S. (1992). The pharmacological basis of mastitis therapy. *ATTI International Symposium on Bovine Mastitis* pp. 79-90.

Boddie, R.L. and Nickerson, S.C. (1986). Dry cow therapy : Effects of method of drug administration on occurrence on intramammary infection. *Journal of Dairy Science* **69** : 253-257.

Booth, J.M. (1988). Update on Mastitis. 1. Control measures in England and Wales. How have they influenced incidence and aetiology? *British Veterinary Journal* **144** : 316-322.

Booth, J.M. (1992). The importance and costs of mastitis in Europe. *ATTI International Symposium on Bovine Mastitis* pp. 11-18.

Bramley, A.J. (1992). Mastitis, In : *"Bovine Medicine, Diseases and Husbandry of Cattle"* . Edited by A.H. Andrews, R.W. Blowey, H. Boyd and E.G. Eddy. Oxford : Blackwell pp. 289-300.

Bramley, A.J. and Dodd, F.H. (1984). Reviews of the progress of dairy science : Mastitis control - progress and prospects. *Journal of Dairy Research* **51** : 481-512.

Bratlie, O. (1973). Dry cow therapy. *Veterinary Record* **93** : 430-431.

Browning, J. W., Mein, G.A., Barton, M., Nicholls, T.J. and Brightling, P. (1990). Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. *Australian Veterinary Journal* **67** : 440-442.

Buddle, B.M., Herceg, M., Ralston, M.J. and Pulford, H.D. (1987). Reinfection of bovine mammary glands following dry-cow antibiotic therapy. *Veterinary Microbiology* **15** : 191-199.

C

Cagienard, B. (1983). Mastitis prevalence and the selection of dry cow therapy programmes in dairy herds. *New Zealand Veterinary Journal* **31** : 31-32.

Cummins, K.A. and McCaskey, T.A. (1987). Multiple infusions of cloxacillin for treatment of mastitis during the dry period. *Journal of Dairy Science* **70** : 2658-2665.

Curtis, R., Hendy, P.G., Watson, D.J., Harris, A.M., Davies, A.M. and Marshall, M.J. (1977). A cerate containing cephalonium for the prophylaxis of dry udder infections in dairy cows. *Veterinary Record* **100** : 557-560.

D

Daniel, R.C.W. and Steffert, I.J. (1969). A comparison of four types of bovine mastitis therapy at drying off. *Australian Veterinary Journal* **45** : 530-532.

Dodd, F.H. and Griffin T.K. (1975). The role of antibiotic treatment at drying off in the control of mastitis. *Proceedings of Seminar on Mastitis Control 1975, Document 85,* International Dairy Federation, Brussels, pp. 282-302.

Dodd, F.H. and Neave, F.K. (1970). Mastitis control. *National Institute for Research in Dairying Biennial Review.* pp. 21-60.

Dodd, F.H. (1987). In: *"Bulletin, Environmental influences on Bovine Mastitis"* International Dairy Federation, Brussels, pp. 1-37.

du Preez, J.H. (1985). Teat canal infections. In : Progress in the Control of Bovine Mastitis - *Kieler Milchwirtscaftliche Forschungsberichte* **37** : 267-273.

E

Eberhart, R.J. (1986). Management of dry cows to reduce mastitis. *Journal of Dairy Science* **69** : 1721-1732.

Egan, J.W.A. (1989). An investigation of mastitis in Irish dairy cows and heifers with particular reference to *Actinomyces pyogenes* mastitis and summer mastitis. Ph.D. Thesis, University of Dublin.

Edwards, S.J. and Smith, G.S. (1967). The effect of treatment of the dry cow on the occurrence of clinical mastitis. *Veterinary Record* **80** : 486-487.

F

Farnsworth, R.J., Wyman, L. and Hawkinson, R. (1980). Use of a teat sealer for prevention of intramammary infections in lactating cows. *Journal of the American Veterinary Medical Association* **177** : 441-444.

Francis, P.G. (1989). Update on mastitis. III. Mastitis therapy. *British Veterinary Journal* **145** : 302-311.

Funke, H. (1975). Elimination of infections - Discussion opening *Proceedings of Seminar on Mastitis Control 1975. Document 85,* International Dairy Federation, Brussels, pp. 311-313.

G

Griffin, T. (1971). Antibiotic therapy in the control of mastitis - A summary of experimental results. In : *Control of Bovine Mastitis* (Eds. F.H. Dodd and E.R. Jackson) *British Cattle Veterinary Association,* pp. 81-91.

H

Hoare, R.J.T., Barton, M. D. and Thompson, R.J. (1973). Dry cow treatment for mastitis. *Australian Veterinary Journal* **49** : 497-498.

Howell, D. (1972). Survey on mastitis caused by environmental bacteria. *Veterinary Record* **90** : 654-657.

I

International Dairy Federation (1967). In : *"Annual Bulletin, Definition of Mastitis"*, - Part III, International Dairy Federation, Brussels, pp. 1-64.

International Dairy Federation (1971). In : *"Annual Bulletin, A Monograph on Bovine Mastitis* - Part 1. International Dairy Federation, Brussels, pp. 1-85.

International Dairy Federation (1981). In *"Bulletin, Laboratory Methods for Use in Mastitis Work"*, International Dairy Federation, Brussels, pp. 1-27.

International Dairy Federation (1984). In : *"Bulletin, Recommended Methods for Somatic Cell Counting in Milk"*, International Dairy Federation, Brussels, pp. 1-19.

International Dairy Federation (1987). In : *"Bulletin, Environmental Influences on Bovine Mastitis"*, International Dairy Federation, Brussels, pp. 1-37.

L

Langley, O.H., Meaney, W.J., Cullen, N.P. and Cunningham, J. F. (1971). The control of mastitis. *Veterinary Record* **89** : 315-316.

M

Meaney, W.J. (1974). Post-milking teat disinfection - a comparison of dipping and spraying. *Irish Journal of Agricultural Research* **13** : 157-161.

Meaney, W.J. (1976a). A comparison of two dry cow intramammary antibiotics. *Veterinary Record* **98** : 50 - 51.

Meaney, W.J. (1976b). Dry period teat seal. *Veterinary Record* **99** : 30.

Meaney, W.J. (1977). Effect of a dry period teat seal on bovine udder infection. *Irish Journal of Agricultural Research* **16** : 293-299.

Meaney, W.J. (1981). Mastitis levels in spring-calving dairy heifers. *Irish Veterinary Journal* **35** : 205-209.

Meaney, W.J. (1985). Mastitis control programme reduces level of infection. *Farm and Food Research* **16** : 118-119.

Meaney, W.J. and Nash, J.M. (1977). Comparison of two dry cow intramammary treatments. *Veterinary Record* **100** : 492-493.

Morse, G.E. (1975). Prophylactic and therapeutic benefits of dry cow therapy in mastitis control. *Proceedings of Seminar on Mastitis Control 1975, Document* **85,** International Dairy Federation, Brussels, pp. 358-361.

N

Natzke, R.P., Everett, R.W. and Bray, D.R. (1975). Effect of drying off practices on mastitis infection. *Journal of Dairy Science* **58** : 1828-1835.

Neave, F.K. (1971). The control of mastitis by hygiene. In : *Control of Bovine Mastitis.* Edited by F.H. Dodd, and E.R., Jackson *British Cattle Veterinary Association,* pp. 55-71.

Neave, F.K., Dodd, F.H. and Henriques, E. (1950). Udder infections in the "dry period". *Journal of Dairy Research* **17** : 37-49.

Nickerson, S.C. (1990a). Production of quality milk and control of mastitis in Mexico. *Dairy Research Report,* Louisiana Agricultural Experiment Station, pp. 3-13.

Nickerson, S.C. (1990b). Mastitis and its control in heifers and dry cows. *Dairy Research Report,* Louisiana Agricultural Experiment Station, pp.59-73.

O

Oliver, J., Dodd, F.H. and Neave, F.K. (1956a). Udder infections in the "dry period". V. The effect of teat disinfection at drying-off on the incidence of infections in the early dry period. *Journal of Dairy Research* **23** : 212-216.

Oliver, J., Dodd, F.H., Neave, F.K. and Lee, J.M. (1956b). Udder infections in the "dry period". II. The effect of withdrawing secretion from the dry udder on the incidence of infection. *Journal of Dairy Research* **23** : 194-196.

Oliver, J., Dodd, F.H. and Neave, F.K. (1956c). Udder infections in the 'dry period'. IV. The relationship between the new infection rate in the early dry period and the daily milk yield at drying-off when lactation was ended by either intermittent or abrupt cessation of milking. *Journal of Dairy Research* **23** : 204-211.

Oliver, J., Dodd, F.H. and Neave, F.K. (1956d). The importance of the dry period in the control of mastitis. *Proceedings of British Society of Animal Production,* pp.33-41.

Oliver, J., Neave, F.K. and Sharpe, E. M. (1962). The prevention of infection of the dry udder. *Journal of Dairy Science* **29** : 95-100.

Oliver, S.P., Lewis, M.J., Gillespie, B.E. and Dowlen, H.H. (1992). Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. *Journal of Dairy Science* **75** : 406-414.

P

Pankey, J.W., Barker, R.M., Twomey, A. and Duirs, G. (1982). A note on effectiveness of dry cow therapy in New Zealand dairy herds. *New Zealand Veterinary Journal* **30** : 50-52.

Pankey, W.N. (1984). Economics of mastitis control In: *"The Veterinary Clinics of North America; Large Animal Practice"* **6** : 233-245.

Pearson, J.K.L. (1949). Bovine mastitis in Northern Ireland with particular reference to *Corynebacterium pyogenes* mastitis in dry cattle and *Corynebacterium pyogenes* infections. Ph.D Thesis, Queens University, Belfast.

Pearson, J.K.L. (1950). The use of penicillin in the prevention of *C. pyogenes* infection of the non-lactating udder. *Veterinary Record* **62** : 166-168.

Pearson, J.K.L. (1951). Further experiments in the use of pencillin in the prevention of *C. pyogenes* infection of the non-lactating bovine udder. *Veterinary Record* **63** : 215-220.

Pearson, J.K.L. and Wright, C.L. (1969). Dry cow therapy as a means of controlling bovine mastitis. *Veterinary Record* **84** : 294-298.

Phillips, J.M. (1979). The combination of nafcillin with penicillin and dihydrostreptomycin : Subclinical trials in infected bovine quarters. *Veterinary Record* **104** : 371

Poutrel, B. (1977). Antibiotic treatments at drying-off of induced and natural infections of the bovine mammary gland. *Annales de la Rechercha Vétérinaire* **8** : 333-340.

Prescott, S.C. and Breed, R.S. (1910). The determination of the number of body cells in milk by a direct method. *Journal of Infectious Diseases* **7** : 632 - 640.

Preston, N.W. and Morell, A. (1962). Reproductible results with the Gram stain. *Journal of Pathology and Bacteriology* **84** : 241-243.

Pugh, K.E., Harris, A.M., Marshall, M.J. and Evans, J.M. (1973). Evaluation of a long-acting intramammary preparation containing high doses of penicillin and streptomycin for use in dry cows. *Veterinary Record* **93** : 212-216.

R

Rindsig, R.B., Rodewald, R.G., Smith, A. R. and Spahr, S.L. (1978). Complete versus selective dry cow therapy for mastitis control. *Journal of Dairy Science* **61** : 1483-1497.

Roberts, S.J., Meek, A.M., Natzke, R.P., Guthrie, R.S., Field, L.E., Merrill, W.G., Schmidt, G.H. and Everett, R.W. (1969). Concepts and recents developments in mastitis control. *Journal of the American Veterinary Medical Association* **155** : 157-166.

Robinson, T.C., Jackson, E.R. and Marr, A. (1988). Mastitis incidence in quarters with different infection status at drying off and calving in two treatment groups. *British Veterinary Journal* **144** : 166-173.

Rosenzuaig, A. and Mayer, E. (1970). A note on dry cow therapy in an Israeli dairy herd. *Veterinary Record* **87** : 409-410.

Sandholm, M., Kaartinen, L. and Pyorala, S. (1990). Bovine mastitis - why does antibiotic therapy not always work ? An overview. *Journal of Veterinary Pharmacology and Therapy* **13** : 248-260.

Schalm, O.W. and Norlander, D.O. (1957). Experiments and observations leading to development of the California Mastitis Test. *Journal of the American Veterinary Medical Association* **130** : 199-204.

Schultze, W.D. and Mercer, H.D. (1976). Nonlactating-cow therapy with a formulation of penicillin and novobiocin : Therapeutic and prophylactic effects. *American Journal of Veterinary Research* **37** : 1275-1279.

Sears, P. and Blackburn, P. (1992). Promising avenues in mastitis control. *Proceedings XVII World Buiatrics Congress* **2** : 95-100.

Serieys, F. and Roguinsky, M. (1975). Comparative tests with dry cow therapy for some or all cows in a herd. *Proceedings of Seminar on Mastitis Control 1975 Document 85,* International Dairy Federation, Brussels, pp. 349-351.

Siegel, S. (1956). *Nonparametric Statistics for the Behavioural Sciences.* New York : McGraw-Hill.

Smith, A., Rautenbach, H.F.P., Dodd, F.H. and Brander, G.C. (1975). The effect on udder infection of varying the levels and persistency of antibiotic in the dry period. *Proceedings of Seminar on Mastitis Control 1975 Document 85,* International Dairy Federation, Brussels, pp. 345-348.

Smith, A., Westgarth, D.R., Jones, M.R., Neave, F.K., Dodd, F.H. and Brander, G.C. (1967). Methods of reducing the incidence of udder infection in dry cows. *Veterinary Record* **81** : 504-510.

Smith, K.L. and Hogan, J.S. (1992). Control of environmental mastitis. *ATTI International Symposium on Bovine Mastitis* pp. 37-52.

Soback, S., Ziv, G., Winkler, M. and Saran, A. (1990). Systemic dry cow therapy - A preliminary report. *Journal of Dairy Science* **73**: 661-666.

Sol, J., Harink, J., Schukken, Y.H. and Snoep, J.J. (1992). Factors in cure of subclinical *S. aureus* after dry cow therapy. *Proceedings of XVII World Buiatrics Congress 2* : 105-110.

Sol, J. and ter Balkt, J.H. (1990). Effects of intramammary cannula design on dry cow therapy. *Veterinary Record* **126** : 438.

Storper, M. and Ziv, G. (1985). Multiple and combination dry period antibiotic therapy of *Staphylococcus aureus*. IDF Seminar Progress in the Control of Bovine Mastitis. *Kieler Milchwirtschaftliche Forschungsberichte* **37** : 533-536.

T

Trinidad, P., Nickerson, S.C., Alley, T.K. and Adkinson, R.W. (1990). Mastitis in dairy heifers. *Dairy Research Report* Louisiana Agricultural Experimental Station, pp.23-25.

U

Uvarov, O., Evans, J.M., Marshall, M. and Sizer, D. (1967). Dry cow therapy. *Veterinary Record* **81** : 241-242.

W

Wilson, C.D. (1973). Dry cow therapy. *Veterinary Record* **93** : 616.

Z

Ziv, G., Storper, M. and Saran, A. (1981). Comparative efficacy of three antibiotic products for the treatment and prevention of subclinical mastitis during the dry period. *Veterinary Quarterly* **3** : 74-79.