

PILOT STUDY TO INVESTIGATE THE USE OF COMPLEMENTARY MEDICINES TO REDUCE HERD SOMATIC CELL COUNTS AND TREAT INDIVIDUAL HIGH CELL COUNT COWS.

Anne Baldock and Vanessa Hart*

*Consultant Veterinarian, 71 O'Shea Road, RD6, Te Awamutu 3876,
Ethical Extracts Ltd, 276 Racecourse Road, RD1, Cambridge 3493**

INTRODUCTION.

There are a large number of complementary medicines available to treat mastitis and many opinions about which is the most effective. The purpose of this pilot study was to consider some of the options and investigate whether any of the treatments warrants further study. Treatments using homeopathic remedies and/or herbal extracts were given to 5 groups of cows on 5 farms. On one property the decision on what treatment to use was made by using kinesiology.

The aim of the trial was to achieve a reduction in somatic cell count to below 150,000 cells per ml (cpm) and a bacteriological cure.

Homeopathy.

The problem with many trials using homeopathy is that they may be scientific but are often not homeopathic because each case is not treated individually and the group is blanket treated with one or a combination of remedies. The homeopathic approach is further complicated by the lack of symptoms for homeopathic analysis in cases of subclinical mastitis.

Current research includes:

Hektoen et al (2004) compared homeopathic treatments to placebo and to antibiotic treatments of clinical mastitis. The homeopathic treatments were selected on an individual basis by skilled homeopaths. They found that there were differences between the groups in the initial response to the treatment of the mastitis but by day 28 there was little difference between the groups. However the numbers were too small for statistical analysis.

Andrew et al (2004) looked at the use of homeopathic remedies (plant based but not stated as to which plants) in combination with a mastitis nosode compared to a placebo in treating subclinical mastitis in Holstein Friesians over a 60 day period. They did not find any significant difference between the homeopathic treated group and the placebo group over the period. During the treatment period there were differences in the Somatic Cell Count (SCC) of the homeopathic group and changes in IgG levels (an immunoglobulin in milk associated with infection and used to monitor immune responses to infection) compared to the placebo. This suggests that the homeopathy may have had a short term affect during the period of treatment but was too short to provide a long term benefit.

There are a number of trails carried out in India assessing the value of homeopathy Varshney et al (2005) concluded that a combination homeopathic remedy was effective and economical in the management of mastitis in lactating dairy cows.

Klocke et al (2002) compared homeopathic treatments with antibiotic treatment over a 6 month period. They found poorer success in the homeopathic group in over all cure rate but found there was no difference between the homeopathic group and the antibiotic group when assessing "remaining in lactation and acceptable cell count".

Klocke, et al (2007) tried to treat high cell count cows with four treatment groups: homeopathic combination therapy, a one off dose with tuberculinum, a placebo group and an untreated control group. There was no significance difference between the groups.

There are a number of trials looking at homeopathy and clinical mastitis but very little in the literature on reducing high somatic cell counts.

Herbal Medicine.

Traditional uses of plant remedies are familiar to most people in some form and have a history of use in most if not all cultures. The way in which information about herbal use has been passed between generations has been generally empirical and unquestioning. This has tended to give herbal medicine a tainted image amongst the scientific community. However generations of experience and accrued insight should not be taken lightly as it is this very folk knowledge that has given the world powerful medicines. The use of Sweet Annie (*Artemisia annua*) in the treatment of malaria and Wild Yam (*Dioscorea spp.*) as a source of hormone precursors are examples.

Despite modern biochemical ability about 25% of present pharmaceuticals are still plant derived, (Fact sheet 134 May 2003 WHO) and the WHO maintains that over 60% of the world's population still use herbs as their primary source of medicine. As one would expect herbal medicine is widely used for the treatment of animals and research continues. See below.

The use of herbal treatments for mastitis in New Zealand is limited by convention, regulation and lack of qualified advisers. However there appears to be strong interest from a small but significant part of the dairy industry for reliable advice and products.

The world wide trend continues to be one of increasing importance of herbal medicine. Total worldwide sales are hard to measure but in 2001 were estimated at \$16-20 billion (Journal of Nutrition. 2001; 131: 1120S-1123S)

More reliable figures are available from the US where sales grew from \$2 to \$4.4 billion over the period 1994 to 2005. (Ferrier et al. Nutrition Business Journal 2006)

The following is a selection of recent trials.

Treatment Trial of Subclinical Mastitis with the Herb *Persicaria senegalense*.

Tropical Animal Health and Production, Volume 33, Number 6 / December, 2001
D. Abaineh and A. Sintayehu, National Animal Health Research Centre, Sebeta, Ethiopia

The remedial effect of *Persicaria senegalense* in bovine subclinical mastitis was studied by in vitro and in vivo antimicrobial tests., using crude extracts and the leaf in different forms. The in vivo trial feeding 0.77 kg of leaf powder, equivalent to 3 kg of wet leaf, was fed per day for 5 days resulted in an apparent cure rate of 92.8% (52.8% actual as there was a 40% spontaneous cure rate in the negative control group, in contrast to 80% (40% actual) in the positive control group treated with an intramammary antibiotic preparation. The difference in cure rate between the negative control group and the experimental group given 0.77 kg leaf powder was significant ($p = 0.008$).

Antimicrobial Activity of Plants Used in the Prevention and Control of Bovine Mastitis in Southern Brazil.

Lat. Am. J. Pharm. 27 (6): 894-9 (2008)
Avancini et al.

Ethnoveterinary information about plants used in the prevention and control of bovine mastitis in Southern Brazil was obtained by informal interview. In order to validate the traditional practice, the decoctions obtained with the plants were analysed for the *in vitro* antimicrobial activity against *Staphylococcus aureus* and *Salmonella choleraesuis* by the agar dilution method. *Alternanthera brasiliana*, *Achillea millefolium*, *Baccharis trimera* and *Solidago chilensis* extracts were active against *S. aureus* while *Symphythum officinale*, *Sambucus nigra*, *Mentha sp.*, *Ocimum basilicum*, *Parapiptadenia rigida* and *Cuphea carthagenensis* extracts were active against both microorganisms. For all the cited species, scientific data were reviewed aiming to establish a correlation between popular use and biological properties. The data found in literature for several of these plants could justify the use in the bovine mastitis treatment for antimicrobial, anti-inflammatory and wound healing activities.

Antibacterial Activity of some Herb Extracts against *Staphylococcus aureus* causing Bovine Mastitis.

The 4th Chulalongkorn University Veterinary Annual Conference 15 February 2005.
Chaidate Inchaistri et al.

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration of various herbal extracts against resistant penicillin-*Staphylococcus aureus* (8 strains) and sensitive penicillin-*S. aureus* (7 strains) causing both subclinical and clinical mastitis were determined. *Andrographis paniculata* leaf extract could not inhibit or kill all *S. aureus* in this study. Whole plant of Asiatic pennywort and turmeric rhizomes extract could inhibit *S. aureus* at high concentration level but it could not kill most of resistant and sensitive penicillin-*S. aureus* causing both subclinical mastitis and clinical mastitis. Guava leaf (*Psidium guajava* Linn.) and mangosteen fruit-skin (*Garcinia mangostana* Linn.) extracts could inhibit both resistant and sensitive penicillin-*S. aureus* at low concentration level and kill at high concentration level. Moreover, the most concentration of herb extracts (MIC and MBC) against sensitive penicillin-*S. aureus* was lower than the concentration of herb extract against resistant penicillin- *S. aureus*.

High SCC is a multi factorial problem, but from a herbal perspective because depressed immune function increase the risk for disease, the immunological status of the cow is a priority.

There are some particularly stressful periods, e.g. around calving, when the immune functions of the dairy animal are more likely to be suppressed (Sordillo et al. 1997, Mallard et al. 1998). This period, i.e. the peri-partum and early lactation period, is associated with a high susceptibility to udder infections and mastitis (Sordillo et al. 1997). High blood levels of glucocorticoids, as well as of other hormones, are present around parturition, and the risk for metabolic stress is high. Examples of stress factors during this period are parturition, onset of lactation, and changes in feeding and management regimes.

Other diseases, such as viral infections, can also cause immune suppression, which also increases the risk for other health problems like mastitis (Niskanen et al. 1995). Local stress due to injuries or skin diseases of the teats and udder is also an important risk factor for udder infections.

Stress can lead to milk retention by inhibiting oxytocin-mediated reflexes (McCaughan et al. 1981). New environment, new milkers, painful milking technique and pain due to udder oedema are factors, which can also be stressful for the animal. Inhibition of milk-let-down can cause subclinical mastitis to become clinical and will affect milk production.

There are many herbs which can benefit immune function and support the cow during stress. Herbal practitioners would usually treat chronic conditions such as high SCC for an extended period. However for ease of administration in this trial the treatment period was limited to 15 days.

The herbs chosen were *Phytolacca decandra/americana*, *Artemisia annua* and *Eleutherococcus senticosus*.

Phytolacca decandra/americana

In European herbal medicine *Phytolacca* has been extensively used for the topical treatment of mastitis (Bone 2000). Its specific ethnobotanical indications are for painful enlarged glands, soreness of mammary glands and mastitis. (Cress 2009). Other indications are for suppressed immunity. It is an immuno-stimulant (Bone 2000, Ganora 2008), inflammatory conditions such as rheumatism and arthritis (Bone 2000, Ganora 2008), and for its strong antioxidant activity (Ganora 2008).

Franklin, Young and Nonnecke (1994) evaluated cell proliferation in cultures of bovine peripheral blood stimulated by *Phytolacca* mitogen. Cell numbers at day 6 in stimulated cultures increased to 96% of the original number and remained elevated from day 6 to 14. The numbers in the unstimulated cultures from days 4 to 14 decreased to <20% of the original number. Overall, IgM secretion induced by pokeweed by B cells in cultures is associated with proliferation of CD4+ (helper-inducer) T cells, B cells, and increased expression of activation antigens.

Several studies have shown *Phytolacca* to have antiviral proteins. Aron and Irvin, 1980, found *Phytolacca* antiviral protein inhibited the multiplication of herpes simplex virus type 1.

Artemisia annua

There has been suggestion that high SCC may be influenced by protozoal and/or viral pathogens.

Artemisia annua has been used in traditional Chinese medicine as an anti malarial for hundreds of years and has been seriously investigated as an alternative treatment of chloroquine-resistant parasites since the 1970's (Lee et al. 2002) and is used in the treatment of protozoal gut infections in humans. (Bone 2000). The first definitive trials of artemisinin were carried out in 1979 by the Chinese Coordinating Research Group. They treated 2,099 patients with malaria with the terpene (ratio *Plasmodium falciparum*:*Plasmodium vivax* is 3:1). They reported an astonishing result: clinical cure in all patients! In addition they treated 143 cases of chloroquine-resistant falciparum malaria and 141 cases of cerebral malaria with good results. (Qinghaosu Antimalaria Coordinating Research Group 1979).

Wynn and Fougere (2007) suggest that the herb has potential veterinary use for some blood and intestinal parasites.

The antiviral activity versus flaviviruses of artemisinin has been investigated using as an in vitro model bovine epithelial cells from embryonic trachea (EBTr) infected with the cytopathic strain Oregon C24V, of bovine viral diarrhoea virus (BVDV). Treatment of infected cells with IFN- α , ribavirin and artemisinin markedly reduced BVDV-induced cell death. A combination of these drugs resulted in an additive protective effect. These drugs induced a significant reduction in the production/release of BVDV virions by infected EBTr cells; there was also an additive effect when combinations of them were assayed. These results suggest a potential usefulness of artemisinin in combination with current pharmacological therapy for

the treatment of human and veterinary infections by flaviviruses. (Romero et al. 2006).

Eleutherococcus senticosus

Also commonly known as Siberian Ginseng, *Eleutherococcus* is one of a group of herbs known as adaptogens which are primarily stress modulators but often have other benefits such as reduced fatigue and better immune function. The use of *Eleutherococcus* was pioneered and researched in depth by the Russians.

In a single-blind, placebo-controlled crossover study, supplementation with *Eleutherococcus* was evaluated in regards to maximal working capacity in adolescent males. The results indicated a 23.3% increase in total work with *Eleutherococcus* supplementation. This increase in total work seemed to be partially due to the improvement of subjects' oxygen metabolism reflected by an increase in maximal oxygen uptake and in maximal oxygen pulse. (Asano et al. 1986).

Animal studies measuring forced swimming time show that supplementation with *Eleutherococcus* inhibits stress-induced cortisol increase, inhibition of stress-induced immune suppression, and improved endurance demonstrated by increased swimming time. (Kimura et al. 2004).

More recent research postulates that adaptogens work primarily by affecting the Hypothalamic/ Pituitary/Adrenal (HPA) axis and the Sympathoadrenal System (SAS) (Panossian 2003). Thus, adaptogens modulate our response to stress (physical, environmental, or emotional) and help regulate the interconnected endocrine, immune, and nervous systems. This re-regulation of a disordered or highly stressed system is achieved by metabolic regulators such as cytokines, catecholamines, glucocorticoids, cortisol, serotonin, nitric oxide (NO), cholecystokinin, corticotrophin-releasing factor (CRF), and sex hormones (Winston 2004)

The herbal extracts used were sourced from and manufactured by Ethical Extracts Ltd. They were manufactured from dried plant material by cold percolation techniques using ethanol as the extraction medium. The strength of extracts were *Phytolacca* 200mg/ml dry plant equivalent (DPE), *Artemisia* 500 mg/ml DPE and *Eleutherococcus* 500 mg/ml DPE.

Calendula oil and Clove oil

Traditional use of *Calendula officinalis* has included assistance with wound healing.

Calendula extract appears to cover the wound with a 'skin' which stimulates the correct tissue response. This observation may be explained by the work of Schmidgall et al. (2000) on the bioadhesive effects of polysaccharides and polysaccharide-containing herbs. They devised a test system to discriminate the adhesive effects of different raw polysaccharides, obtained from a variety of medicinal plants. While polysaccharides from *Althea officinalis*, *Plantago lanceolata*, *Malva moschata*, or *Tilia cordata* showed only moderate bioadhesion to epithelial tissue, strong adhesive processes were observed with polysaccharides from *Fucus vesiculosus* and *Calendula officinalis*. The adhesive effects were concentration-dependent. Histological studies of membranes, incubated with a fluorescence-labelled rhamnogalacturonan, indicated the presence of distinct polysaccharide layers on the apical membrane surface. With these results, adsorption effects of certain polysaccharides on mucus membranes were shown for the first time.

Studies now show the vulnerary activity of Calendula is attributed to an increase in angiogenesis (Patrick et al. 1996), collagen, nucleoprotein, and glycoprotein metabolism (Brown et al. 1998, Kloucek-Popova et al. 1982).

A controlled murine study (Rao et al. 1991) explored the wound healing effect of an ethanolic extract of *C. officinalis*, paravertebral incisions treated with topical Calendula demonstrated significantly greater wound breaking strength than controls after 10 days of treatment. In full-thickness excised wounds, the period to complete epithelialization with topical calendula also was significantly less than control wounds. These results suggest topical calendula may facilitate collagen maturation and epithelialization.

In a double blind RCT (Kartikeyan et al. 1990) on 18 patients with trophic ulceration, 10% Calendula ointment was compared to topical neomycin and placebo paraffin ointment. Topical Calendula prevented secondary infection and demonstrated a 40% reduction in wound diameter and volume within 4 weeks. However, the comparative effects of neomycin and placebo ointment on wound healing were not detailed.

Clove oil (*Eugenia caryophyllata*) has been traditionally used for its topical anodyne effect and has been included in many commercial topical treatments to reduce pain, notably a gel for baby teething.

The topical anaesthetic effect of the Clove oil (Ganora 2008) combined with the healing attributes of calendula oil promote rapid healing as well as pain reduction.

Applied Kinesiology.

Muscle testing or applied kinesiology is a non invasive way of accessing the body's imbalances and needs. It is based on the concept of internal energy and the flow of energy along meridians; a concept fundamental to Chinese medicine.

Kinesiologists use an indicator muscle to tap into the body's energy and a "yes" "no" answer is obtained to challenges to the body with verbal statements or substances. The response is seen as a strengthening or a weakening of the muscle.

Muscle testing is used in human medicine and includes Touch for Health and Nambutripad's Allergy Elimination Technique (NAET). NAET has also been adapted for the use in animals (Harris V. 2001).

Setting up a session.

Someone is chosen who has a close emotional and physical link with the cows and is willing to stand in for the transfer of energy in the form of information in an unbiased way. It is important to check that the person's personal energy integrity is balanced to optimise accurate muscle checking responses. This is achieved by a series of pre-checks of meridian switching points, checking the integrity of the Central Meridian (CV) and body hydration levels. Water is an electrical conductor within the body and so if hydration is compromised muscle checking responses will not be clear. Personal stress can reverse the CV and the switching points which will once again give false responses to testing.

Once this clearing is achieved then permission of the body, mind, spirit must be obtained and locked in with a positive muscle test. If permission is not given then clearing may be needed with flower essences, or other techniques known to a trained Kinesiologist. It may even be necessary to choose another person.

The next step is making contact with the cows. Once again permission must be sort and obtained. If it is not given then further clearing of the surrogate cow may be necessary or once again it may require the choosing of another person to work with them. The responses obtained via the muscle checking must be respected to keep the energy channels open and operating. It is possible to check that contact is established by asking the surrogate person to state "my name is (cows number)". This should produce a positive muscle check repeat with the persons real name - this should evoke a weak or switched off muscle check.

Choosing the goal.

This is established with the aid of muscle checking to establish the correct goal. The goal is then rephrased as a statement of established fact. The correct goal will show as a stress response or switched off muscle check to indicate it is not a true statement. This muscle check is then locked into circuit and held for the duration of the session. A change in muscle tension gives a yes or no response. When all avenues have been explored (see the protocol list below) the goal is again stated as positive fact. If everything has been covered this will test as a strong response. This indicates that if the support tested for is given, then the goal is likely to be achievable.

Ending the session.

It is important to thank the cows for their participation in the partnership. Sometimes thanks is required before the goal will test strong. Physical contact is then disconnected and the surrogate person is asked to mentally disconnect from the cows and be themselves. To check this is complete the person is asked to state "my name is (cows no)" = weak muscle check. "My name is (real name)" = strong muscle check. It is also wise to repeat pre-checks as post-checks i.e. the switch points and CV are correct.

The protocol has evolved over the time of the study to that outlined below;

Modality	Specifics	Yes	No
Homeopathy	Organ remedy		
	Immune remedy		
	Nosode		
	Constitutional remedy		
	Disease specific remedy		
	Other		
Herbs			
Flower essences	Bach flowers		
	Earth song		
	Australian bush flower essences		
Isopathy			
Acupuncture			
Aromatherapy			
Orthobionomy			
Aura soma			
Colour therapy			
Cell salts			
Homoebotanicals			
Nutritional			
Minerals			
Water quality			

Vitamins			
Biodynamic preparations			
Milking machines			
Stray electricity			

If for example homeopathy is identified as a need then it will be further refined as to the type of homeopathy required as listed above. Then if an organ remedy is identified a list of organ remedies will be tested. (For the herbs and Flower essences sample were use to test rather than the name.)

Once the remedy is identified dose rates etc are clarified. See protocol below.

Once the remedy is identified, establish:

Dilution: Decimal X Centesimal C

Potency: 6, 12, 30, 200, 1M

If an odd potency is established chose the one closest.

Dispensing
 In the water? Trough?
 Dosetron?

Dose:
 Drops per day. Once a day? Twice a day?

How many days.
 If more than one remedy chosen; can they be given in combination?

Summary

Remedy

Potency and dilution

Drops per dose to be given individually/in trough/via dosetron

Once twice a day

Duration of treatment

RESULTS AND DISCUSSION

Herd A

This herd is certified organic. It was treated using homeopathy and a herbal rub.

A drying off protocol using homeopathy and manuka honey was also investigated.

Treatment protocol.

After first herd test: 2 Feb 2007

High cell count cows were treated with a spray on vulval mucous membrane of HFS Mastitis nosode twice a day for 4 to 5 days, as per normal farm practice.

Samples were taken from high somatic cell count cows for culture after treatment with homeopathy.

After second herd test: 25 Oct 2007

High SCC cows were treated with homoeopathic remedies to the vulval mucous membranes:

SSC remedy (combination remedy of Sulphur, Silica and Carbo veg)

HFS mastitis nosode,

Strep uberis / *Staph aureus* nosode and

Herbal rub to the udder.

At drying off:

Cow numbers 166 and 59 were treated with manuka honey, aloe vera and tea tree oil intra mammary.

High cell count cows and those with a history of mastitis were treated with 5ml of 20+ UMF honey intramammary.

Homeopathic Lac caninum for 2 days in the water trough.

Apple Cider Vinegar and Garlic for approximately one month.

Drenched with ACV at drying off.

Table:1 Showing somatic cell counts and culture of cows sampled 5th September 2007.						
Date	5 th Sept 07	27 th Sept 07	25 th Oct 07	8 th Jan 08	10 th Apr 08	13 th Oct 08
Cow No.	SCC	Culture	SCC	SCC	Culture	SCC
23	749	No growth	106	107	<i>Coryneforms</i>	129
29	623	No growth	44		Dry	49
59	1647	No growth	82	1071	Dry	117
72	812	No growth	55	1736	<i>Bacillus</i> <i>Coryneforms</i>	45
79	670	No growth	105	62	CNS <i>Bacillus</i> <i>Coryneforms</i>	

140	4446	No growth	69	137	Dry	
166	2021	No growth	37	sold	Sold	
Ave.	1567		71	623		135
SCC Somatic Cell Counts CNS Coagulase Negative Staphs						

Table: 2 Showing somatic cell counts and culture of cows sampled 12 th November 2007.						
Date	2 nd Aug 07	25 th Oct 07	12 th Nov 07	8 th Jan 08	10 th Apr 08	13 th Oct 08
Cow No.	SCC	SCC	Culture	SCC	Culture	SCC
6	NC	957	<i>Pasturella haemolytica</i>		<i>Staph aureus</i>	
22	NC	1950	<i>Staph aureus</i>	758	<i>Staph aureus</i> <i>Strep uberis</i>	559
28	NC	525	<i>Staph aureus</i> <i>Strep uberis</i>	227	Dry	159
112	385	2976	<i>Staph aureus</i>	306	<i>Staph aureus</i>	401
119	NC	385	No growth	486	<i>Coryneforms</i>	127
122	NC	709	<i>Staph aureus</i> <i>Coryneforms</i>	247	Dry	47
132	NC	1089	<i>Staph aureus</i> <i>Strep agalactiae</i> <i>Strep uberis</i>	Culled High cell count	Culled	Culled
Ave.		1227		405		259
SCC Somatic Cell Counts						

Comment.

No statistical analysis has been carried out on these results but there are some interesting observations.

After the initial herd test 7 cows were treated with HFS mastitis nosode and milk samples were cultured on 5th September 2007. None of the samples cultured any bacteria. This has been seen this on other occasions where animals with high cell counts are treated with homeopathy and when cultured no growth is seen (personal communication). McDougall (2002) has found that 29% to 50% of glands with clinical mastitis will not culture bacteria.

This may suggest that the homeopathy is having an effect and is reflected in the second herd test where the average cell count of these cows had dropped from 1567 to 71. On the subsequent herd test some cows returned to high cell counts and cultured *Coryneforms*, CNS and *Bacillus sp.* These bacteria are usually associated with a breakdown in teat spray technique or a complete absence of teat spraying. (Pankey 1997). Unfortunately there was no culture prior to treatment for the initial animals so it is not possible to know if these were new infections or failure of an initial bacteriological cure.

The high cell count cows on the second herd test had a high number of cows culturing *Staph. aureus*. There was some reduction in SCC counts between the two herd tests but those animals that were still in the herd in April did not show a bacteriological cure.

Spontaneous bacteriological cure rates vary, and are difficult to assess. In trials using antibiotic treatment there is a reluctance to leave untreated controls for welfare reasons.

Clinical cure rates were 75% for non contagious pathogens (contagious pathogens were considered to be *Staph aureus*, *Strep agalactia* and *Mycoplasma sp.*) were the same for antibiotic treated cows compared to animals stripped more frequently using oxytocin (McDougall 2005). House (2003) quotes self cure rates for environmental bacteria of 20% and 73% for CNS.

It is therefore impossible with this small sample to look at cure rates but the lack of bacteriological culture after homeopathic treatments could warrant more study.

The cows culturing *Staph aureus* appeared to have a small drop in SCC counts but not a bacteriological cure.

Cow number	Last test March 08	SCC > 300,000	Herd test 15 th Oct 2008	Cow's SCC falling below 250,000 after treatment
1	364	*	247	****
10	349	*	212	****
12	174		102	
13	516	*	129	****
14	625	*	149	****
15	237		159	
21	1317	*	595	
22	1396	*	559	
24	371	*	58	****
26	399	*	117	****
44	288		118	****
47	277		79	****
58	643	*	299	
59	157		49	
60	354	*	99	
64	30		109	
72	104		117	
73	85		46	
74	25		83	
79	51		45	
86	77		49	
87	364	*	1528	
91	327	*	135	****
92	414	*	153	****
94	381	*		
97	215		60	
98	560	*	112	****
99	104		37	
102	100		42	
104	290		671	
106	79		59	
110	278		87	
112	410	*	401	
117	233		73	
119	1365	*	127	****
124	534	*	174	****
125	241		100	
135	368	*	84	
136	348	*	272	
139	239			
145	506	*		
153	268		80	
175	464	*		

22 cows treated with manuka honey had cell counts of over 300,000 on the last herd test in March 2008. On the first herd test in October 2008 59% had cell counts below 250,000.

Assessment of the success or failure of this treatment is hard to assess as there is a natural cure rate over the dry period. The three cows sampled with *Staph aureus* two still had high cell counts. Manuka honey does have activity against *Staph aureus* in in vitro studies (Cooper et al and Allen et al).

Herd B

Herd B was not an organic herd but the use of antibiotics was minimal and homeopathy was used as part of the mastitis control program.

There was a severe problem in this herd due to incorrect milking machine cup liners being inserted at the beginning of the season. The liners were too short for the cups, providing an inadequate relaxation phase during milking. Despite there being severe teat end damage and a serious mastitis problem identified as predominantly *Staph aureus*, the teat liners were not changed until the end of November just prior to the second milk samples being taken.

A combination of herbs and homeopathy were used on this property.

The herbal treatments were divided into two groups; 10 animals were treated with *Phytolacca americana* only and the second group of 30 were treated with a combination of herbs: *Phytolacca americana* , *Artemisia annua* , and *Eleutherococcus senticosus*

Additional treatments:

In addition to the individual herbal treatments the herd was treated with HFS homeopathic mastitis nosode as per normal farm policy.

A second homeopathic mastitis nosode was made up from mastitic milk from the cows sampled and the cows were treated on a daily basis for one month. There was no change in the herd bulk somatic cell count during this treatment.

Calendula oil and clove oil

This was used as a teat dip for cows with sores on their teats. Due to the affects of the vacuum on the teat ends there were a large number of black pock lesions. No evaluation was carried out on this treatment. In the herd manager's opinion the calendula / clove oil mixture was very affective in treating the teats with lesions. The cows stopped kicking the cups off and the lesions cleared up.

Table 4 Results for Treatment 1 : Phytolacca only					
Dose: 15ml per day for 15 days					
Date	26 th Sep 07	5 th Oct 07	20 th Nov 07	13 th Dec 07	10 th Jan 08
Cow No.	SCC	Culture	SCC	Culture	SCC
54	1137	<i>Staph coag -ve</i>	408	<i>Staph coag -ve mixed coliforms</i>	35
296	1166	<i>Strep dys</i>	199	<i>Staph aureus Staph coag -ve</i>	62
387	2967	<i>Staph aureus</i>	3888	<i>Staph aureus</i>	1413
541	1760	<i>Strep uberis Staph coag -ve</i>	3149	<i>Staph coag -ve strep uberis</i>	364
576	9999	<i>Staph aureus Strep dys</i>	2157	<i>Staph aureus</i>	538
603	1488	<i>Staph aureus Staph coag -ve</i>	626	<i>Staph aureus</i>	390
670	1072	<i>Staph aureus Strep</i>	693	<i>Mixed coliforms</i>	602

680	1296	<i>Staph aureus</i>	3176	<i>Staph aureus</i>	1611
687	2508	<i>Arcanobact pyogenes</i>	159	<i>Staph coag -ve</i>	50
760	1724	<i>Staph aureus</i> <i>Strep dys</i>	9999	<i>Staph aureus Strep dys</i>	died
Averages	2512		2445		562
SCC Somatic Cell Counts					

Table 5 Results for Treatment 2 : Phytolacca mix Dose: 30ml per day for 15 days Phytolacca americana 20%, Artemisia annua 40%, Eleutherococcus senticosus 40%					
Date	26 th Sep 07	5 th Oct 07	20 th Nov 07	13 th Dec 07	10 th Jan 08
Cow No.	SCC	Culture	SCC	Culture	SCC
15	3986	<i>Staph coag -ve</i>	25		27
44	2295	<i>Staph coag -ve</i>	261		244
48	1058	<i>Staph aureus</i>	1136	<i>Staph aureus</i> <i>Staph coag -ve</i>	138
58	2351	<i>Staph aureus</i> <i>Strep uberis</i>	2513		2104
70	1578	<i>Staph aureus</i> <i>Strep uberis</i>	91		66
72	1186	<i>Staph aureus</i> <i>Strep dys</i>	102		570
149	1350	<i>Staph aureus</i> <i>Strep uberis</i>	1975		5259
162	4495	<i>Staph aureus</i> <i>Strep uberis</i>	3163	<i>Strep dys</i>	509
211	1083	<i>Staph aureus</i> <i>Strep dys</i>	1270		729
217	1113	<i>Staph aureus</i> <i>Strep dys</i>	1401	<i>Staph aureus</i> <i>Staph coag -ve</i>	352
226	4524	<i>Strep dys Arcono pyogenes</i>	277	<i>Staph coag -ve</i>	111
303	1848	<i>Staph coag -ve</i>	195	<i>Staph coag -ve bacillus</i>	106
341	1266	<i>Staph aureus</i>	3232	<i>Staph aureus</i>	1588
380	1728	<i>Staph aureus</i> <i>Strep dys</i>	1762		205
405	3344	<i>Staph aureus</i>	55		56
412	36	<i>Bacillus</i>	137	<i>Staph coag -ve coryneforms</i>	Died/culled
417	1630	<i>Staph aureus</i>	85		70
424	1321	<i>Staph aureus</i>	2986	<i>Staph coag -ve</i>	153
432	2944	<i>Strep dys</i> <i>Staph coag -ve</i>	447	<i>Bacillus</i>	Died/culled
471	1132	<i>Staph aureus</i> <i>Strep dys</i>	1216	<i>Staph aureus</i>	287
526	3174	<i>Staph coag -ve coryneforms</i>	400	<i>Staph coag -ve</i>	74
528	1249	<i>Staph coag -ve mixed coliforms</i>	62		69
543	1625	<i>Staph aureus coryneforms</i>	1554		992
573	4194	<i>Staph coag -ve coryneforms</i>	136	<i>Staph coag -ve</i>	3524
717	1676	<i>Staph aureus</i>	1642		302

720	1166	<i>Staph aureus</i> <i>mixed colif</i>	4077	<i>Staph aureus</i>	4428
734	1143	<i>Staph aureus</i> <i>Strep dys</i>	105	<i>Bacillus</i> <i>mixed coliforms</i>	50
741	3293	<i>Staph aureus</i>	508		443
748	1984	<i>Staph aureus</i> <i>Strep dys</i>	9482	<i>Staph aureus</i> <i>Strep dys</i>	829
753	1036	<i>Staph aureus</i> <i>Strep dys</i>	1209	<i>Staph aureus</i>	180
Ave.	2027		1383		838
SCC Somatic Cell Counts					

Somatic cell count trends of cows not treated with herbs.			
These cows received the homeopathic treatments as they were given on a herd basis.			
Date	26 th Sep 07	20 th Nov 07	10 th Jan 08
Cow No.	SCC	SCC	SCC
134	705	62	285
177	772	61	99
199	2226	897	85
275	715	120	63
308	880		1039
348	907	33	
467	788	485	404
517	793	120	297
567	9999	207	126
610	956	3434	629
699	783	23	71
700	781	4258	9642
718	3109	91	230
751	1419	279	66
Average:	3202	774	1002

Discussion:

No statistical analysis has been carried out on these results.

Treatment 1

At the first herd test after treatment 30% of the cows had reduced their cell counts to below 500,000. The average cell count for the group had not dropped to any great extent, from 2,511,000 to 2,445,000 but the problem with the cup liners still had not been addressed. In the January herd test there was a large reduction in the somatic cell count from an average of 2445 in November to 562,000. 55% of the cows on the third herd test were below 500,000. However there was no bacteriological cure.

Treatment 2

After the first herd test 43% of the cows had reduced their cell count to below 500,000. By the January herd test there was 61% of this group below 500,000. The average somatic cell counts however stayed above 500,000. There was also no bacteriological cure.

Non Treated group

At the first herd test 70% of the cows had reduced their cell count to below 500,000. By the herd test in January 77% of the cows were below 500,000. No bacteriological culture carried out on these cows.

Conclusion

Interestingly the herb treated group the SCC remained below the November values, whereas the untreated group had started to increase again.

Herd C

This herd is certified organic.

Muscle testing or applied kinesiology was used to establish the treatment chosen. The treatment protocols were aiming to reduce SCC. Mastitis treatments were looked at briefly on one occasion.

First session: 20th June 2007

The remedies / problems identified/ chosen were:

The goal set was to: "Maintain the bulk somatic cell count below 200,000 cells per ml." It was identified that this needed to be addressed at a herd level rather than by working on an individual basis with high cell count cows.

Flower essences:

- ! Earth Song essences
- ! Winter Jasmine "Prepare joyfully for the unexpected"
- ! Japanese Anemone "Courage"

Minerals:

- ! Vanadium; Yes
- ! Cobalt; sort of
- ! Cobalt; as B12 Yes
- ! Iodine; Yes
- ! Sulphur; Yes

These minerals could be supplied by supplementing Arcadian sea weed in a lick.

Cell salts

- ! Calc flur.
- ! 11 drops in trough twice a day from when first cow calved until the end of calving.

Colloidal silver

- ! Yes (this was not followed through due to certification issues)

Nutrition

- ! Yes
- ! It was identified that there was a requirement for more plantain and chicory in the pasture and an increase in hay/fibre in the diet.

A requirement for specific herbs were identified but not followed through due to the practicalities of supplying herbs on a whole herd basis.

22nd July 2007 Muscle testing Results.

Herbs required on an individual basis.

Flower essences:

- ! Earth Song essences.
- " Heart Charka.
- " At calving.
- " 9 days from the 1st August.
- " 16 drops in 500mls.

" Dose: 17ml per day in dosatrone.

! Continue Winter sweet and jasmine for a week after 1st August.

Cell salts:

! Continue cal flur until end of season.

Nutrition:

! More plantain and chicory.

! Haylage.

! Psyllium seed (Plantain).

! Endophytes are an issue.

Minerals

! Cobalt as Vit B₁₂.

! Iodine.

! Iron too much.

! Manganese too little.

Seaweed kelp as Archadian sea weed.

Sea water 1st priority to sea weed.

! 1l in trough for 89 days.

! Open ocean water.

18th August 2007. Muscle testing results.

The SCC for the herd is already below 200,000 cells /ml, so we established a goal to lower the cell count to below 100,000 per ml. This was accepted by the herd.

On a herd basis:

! The milking machines were having a 15% influence on the SCC. We were not able to identify the problem

! Water quality was having a 66% affect. This was identified as the additives. The homeopathic liver remedies are to be stopped. The drum in the dosetron needed to be cleaned.

! Flower essences were identified as having a 26% affect. Required Grape up until Christmas

" Grape (the sweetener of unconditional love starting with self): 2 drops per 500ml. Two drops on Monday.

" Papaya. (for burn out) 12 drops per 500ml. 8 drops per day but not Mondays.

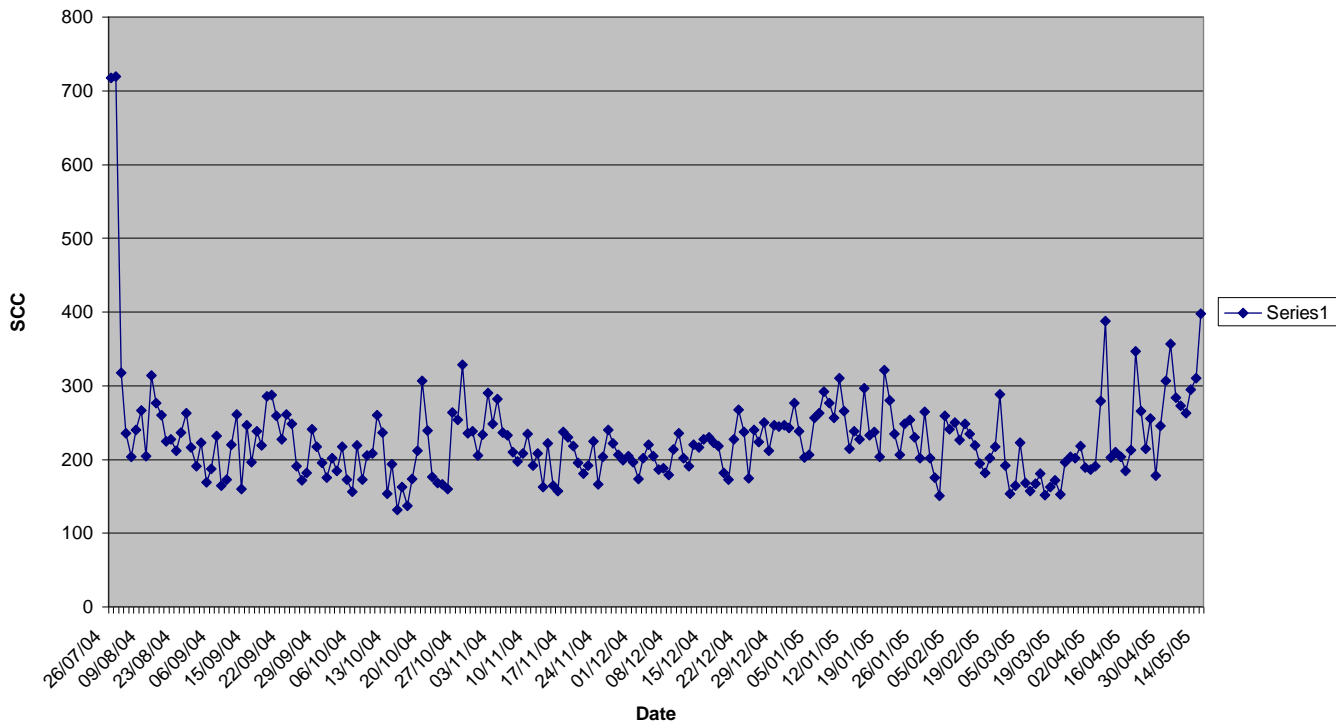
Table:7 Results from high cell count cows.						
Cow no.	SCC 9 th Sept 2007	Cultured 16 th Sept 2007	SCC 12 th Nov 2007	SCC 7 th Sept 2008	Cultured 6 th Nov 2008	SCC 29 th Jan 2009
14	1503	Strep uberis	36	Not calved	CNS bacillus	163
56	769	Coryneforms		119	Staph aureus	275
63	342	Strep dysgalactia	123	499	Staph aureus CNS	421
64		Coryneforms	23	78	No growth	27
78	2075	Step uberis	195	129	coryneforms	489
82	483	Coryneforms	77	34	Staph aureus	241
85	259	Bacillus Strep uberis	114	129	Strep uberis	42
102	881	Strep uberis	724		Strep uberis	1801
104	2097	CNS	2746	123	No growth	629
112	1122	CNS	114	36	CNS	685
125		Coryneforms	36	54	CNS	205
155	548	Strep uberis	1317	Not calved		1354
159	1178	Staph aureus Strep uberis	562	Not calved		1521
194		Strep uberis, coryneforms	95	Not calved	Strep uberis	45
199	1216	strep uberis	4902	culled	No growth	142
207	217	Coryneforms	133	147	Staph aureus CNS	316
205	33	Strep uberis	80	40	Strep uberis	95
252	430	Staph aureus	153	Not calved	CNS	704
268	268	Strp uberis CNS	94		Staph aureus CNS	193
Av.	839		640	126		492

Cows were not sampled at the end of 07/08 season as they were dried off early due to drought.

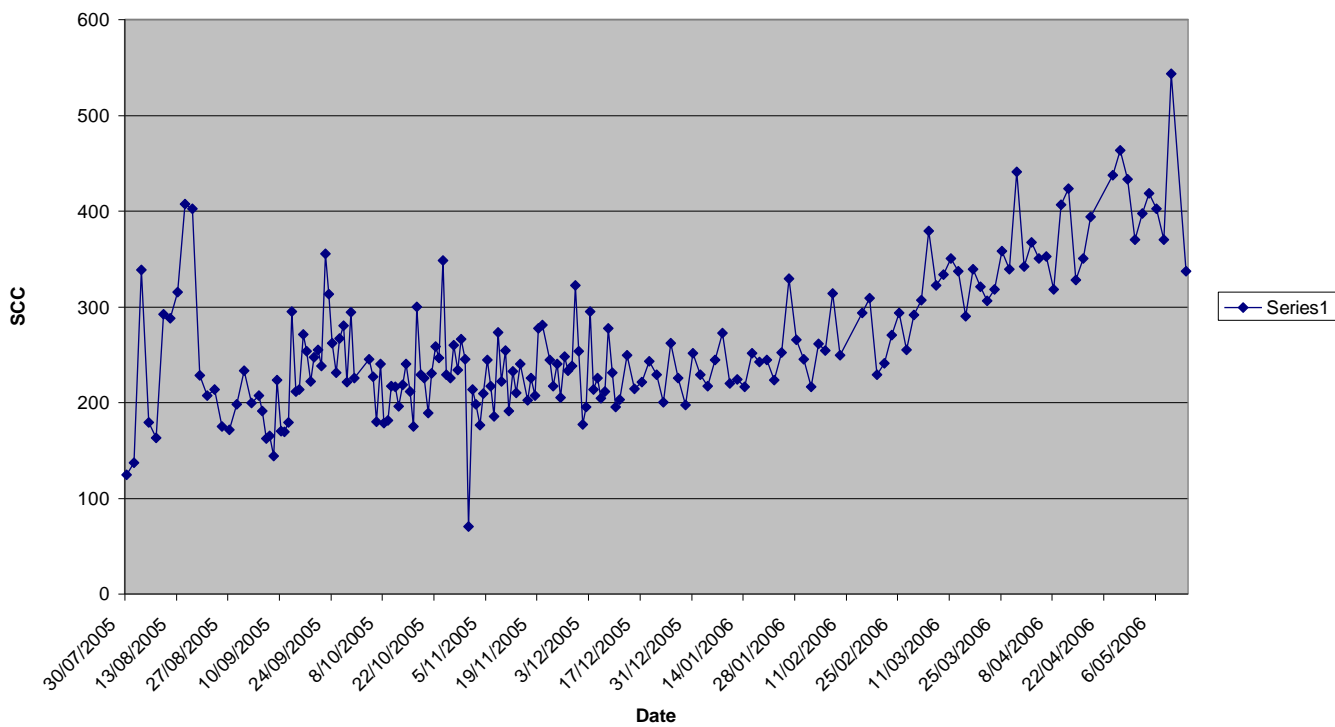
Table:8 Somatic cell count trends over the; 04/05, 05/06, 06/07, 07/08 and 08/09 seasons Bold illustrates seasons during trial					
Season	40301	05/06	06/07	40396	08/09
Average	227	256	231	246	224
Highest	719	543	434	495	515
Lowest	131	70	113	130	115

Table: 9 Somatic cell count trends all cows. Bold indicate seasons during trial										
Cell count ranges	Nov 05	Jan 06	March 06	Sept 06	Nov 06	Jan 07	March 07	Sept 07	Nov 07	Jan 08
	%	%	%	%	%	%	%	%	%	%
0 - 149	75	74	51	78	81	73	38	71	75	43
150 - 249	11	13	17	7	11	13	17	12	11	19
250 - 499	7	8	23	8	5	8	29	9	8	24
500 plus	6	5	10	7	4	6	16	8	6	14
Est bulk count	185	176	256	177	140	176	302	229	190	300

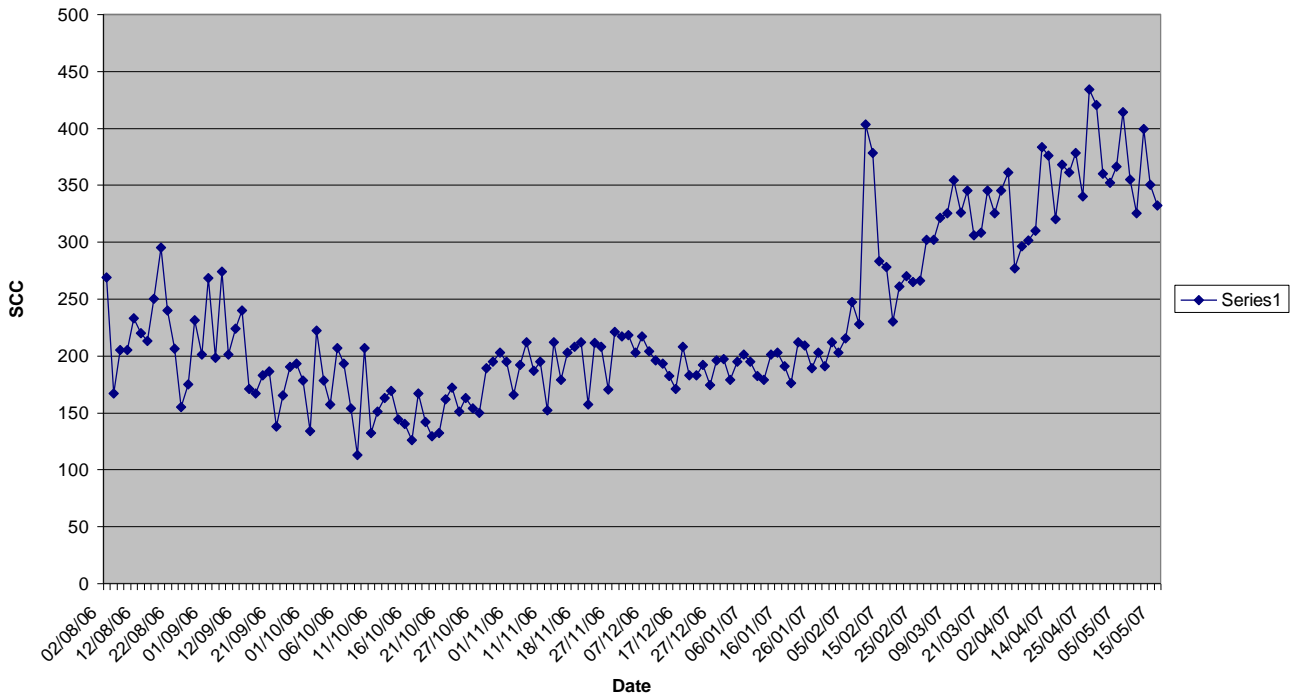
SCC 04/05



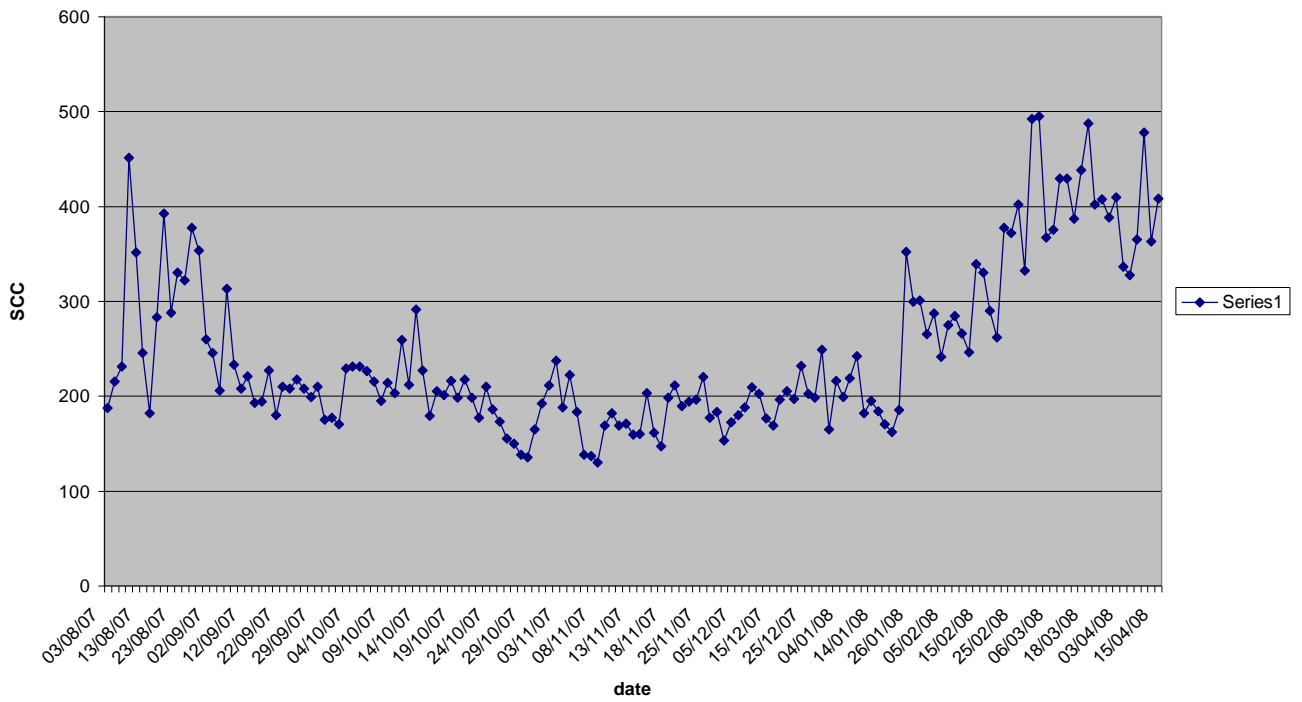
ScC 05/06



SCC06/07



SCC 07/08



SCC 08/09

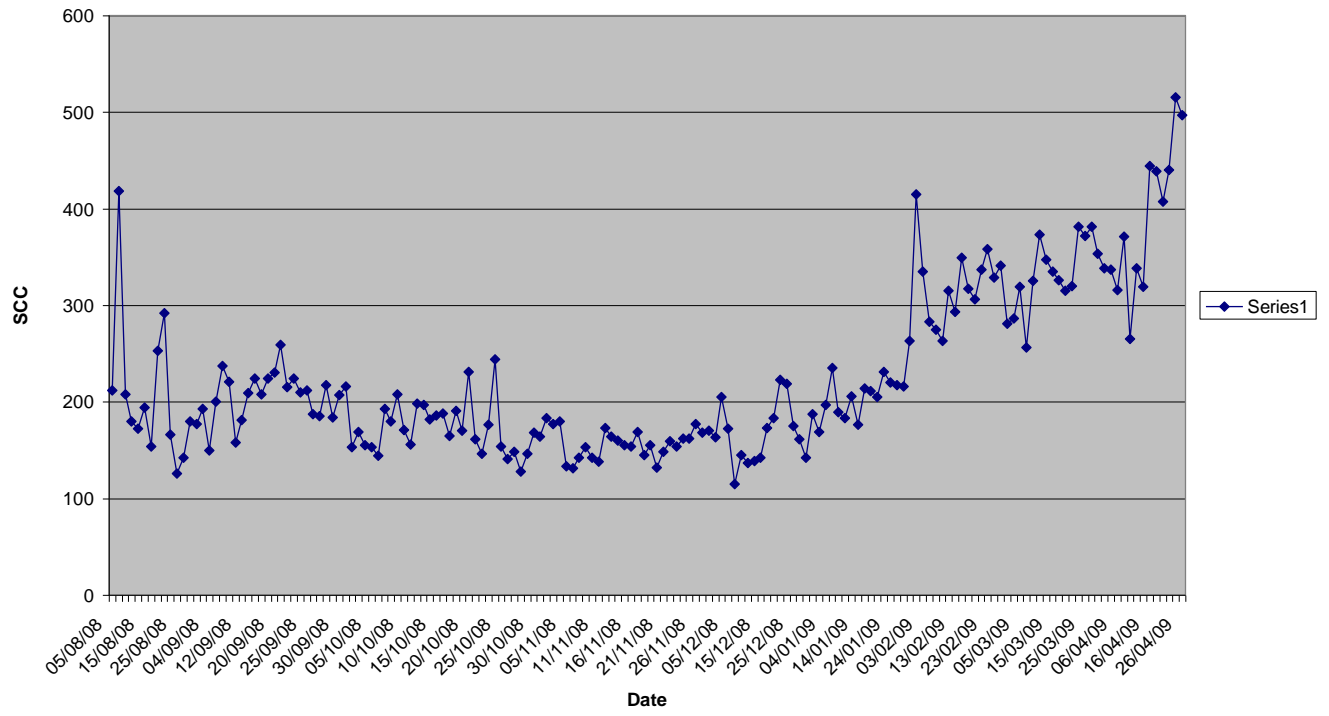


Table: 10

Somatic cell count trends 2 year olds

Bold indicate seasons during trial

Cell count ranges	Nov 05	Jan 06	March 06	Sept 06	Nov 06	Jan 07	March 07	Sept 07	Nov 07	Jan 08
	%	%	%	%	%	%	%	%	%	%
0 - 149	100	100	100	80	90	82	54	78	80	70
150 - 249				4	4	10	19	14	9	15
250 - 499				4	4	4	19	4	8	11
500 plus				11	2	4	8	4	3	5
Est bulk count	38	26	58	242	136	129	195	117	179	189

Discussion.

From the data there appears to be very little change in the herd somatic cell counts over the 5 year period, two years before and three years of muscle testing. The average SCC for the seasons have remained consistent and number of times the SCC have peaked over 400,000 has been confined to the start and finish of lactation. The 2004/05 season had the least spikes but the following season, 2005/06 prior to the start of the trial shows some major fluctuations in SCC through out the seasons. The 2006/07, 2007/08, 2008/09 seasons the SCC remained below the 300,000 level except at the beginning and end of the lactation.

The farmer however has said he has not had to cull any cow on somatic cell count during this trial as the cell counts have remained within the desired level in spite of animals in the herd with SCC levels consistently over 1 million. He also commented that the animals were generally more settled at calving, general health problems appeared reduced, the incidence of clinical mastitis appeared lower and response to treatment was quicker.

During the session on 8th August 2008, muscle testing was carried out to develop a herbal formula for clinical mastitis. The formula indicated (formula A) consisted of two herbs, an Ayurvedic herb with traditional ethnoveterinary usage for the treatment of mastitis, and a European herb traditionally used as a diuretic and anti-inflammatory.

A second formula (formula B) was also given to the farmer. It consisted of herbs traditionally used in European herbal medicine for the treatment of mastitis in humans and stock.

Alternated cases were treated with the two formulas. Formula B was soon abandoned due to poor response and formula A has continued to be used successfully in subsequent seasons.

It has to be noted that the muscle tester was a very important part of this trial and a non bias person is required. Our muscle tester was not from a farming background and had no preconceived ideas regarding the outcome. Results were consistent and acceptable.

Herd D

This herd was treated using homeopathic remedies on a herd and an individual basis and aloe vera on an individual basis. This herd had a major *Staph aureus* problem.

Treatment protocol:

The approach used a combination of homeopathic remedies to try and eliminate the *Staph aureus* from the high cell count cows. The cows were also drenched with Aloe Vera.

Aloe Vera : 10ml a day for 10 days

Homeopathy on a herd basis:

Staph aureus nosode.

Calc flur cells salts.

Individual cows:

Mercurius

Thiosianamum

Results

Cow No.	Cultured 3 rd Nov 2007	Cultured 10 th Apr 2008	Cow No.	Cultured 3 rd Nov 2007	Cultured 10 th Apr 2008
7	CNS	dry	117	Staph aureus Step dysgalactiae Coryneforms	dry
8	Staph aureus CNS Coryneforms	dry	118	CNS Coryneforms	dry
26	CNS	Staph aureus	122	Staph aureus Coryneforms	dry
29	Staph aureus Coryneforms	dry	140	Staph aureus Coryneforms	dry
30	Staph aureus	Staph aureus	151	Coryneforms	dry
54	Coryneforms	dry	198	Staph aureus Coryneforms	dry
62	Staph aureus CNS	Staph aureus	200	Staph aureus CNS	Staph aureus
71	Staph aureus CNS Coryneforms	dry	207	CNS Coryneforms	dry
80	Staph aureus Coryneforms	Staph aureus	208	Staph aureus Coryneforms	dry
96	Staph aureus	Staph aureus	223	Staph aureus	dry
110	CNS Coryneforms	dry	271	CNS	dry
111	Staph aureus	Staph aureus	386	Staph aureus CNS	Staph aureus

Discussion

The CNS appeared to be eliminate by this regime (5 cows) , but there was no change in bacteriology of the *Staph aureus* samples.

Herd E

The treatment protocol for this herd was to use *Strep uberis* and *Staph aureus* nosode on a herd basis.

Cow no.	5 th Nov 2007	22 nd Dec 2007	28 th Apr 2008
18		Staph aureus	Staph aureus
24	Strep uberis	Mixed coliforms	coryneforms
45		Staph aureus	coryneforms
75		Staph aureus	Not cultured
88		No growth	Staph aureus
90		Staph aureus	Not cultured
100	No growth	No growth	No growth
105		Staph aureus	Staph aureus
111	Strep uberis Staph aureus	Staph aureus	Not cultured
126	Strep uberis	Staph aureus	Staph aureus coryneforms
130	No growth	Bacillus sp	Coryneforms

Discussion for herds D and E.

For herd E the *Strep uberis* cleared up between 5th November and 22nd December, number 126 then cultured *Staph aureus* and 5 cows became high cell count between herd test and cultured *Staph aureus*. The numbers are too small to be significant but it is interesting that this herd as well as herds A and C developed *Staph aureus* later in the season. Some of these cases are new, some are in cows already identified as high cell count cows.

Herd D. There was no response to treatment.

General conclusions:

Consistently through out this study we have seen very poor response to *Staph aureus* subclinical mastitis. This is similar to the problems seen with conventional treatments. The incidence of *Staph aureus* mastitis however was very high across all 5 farms. McDougall (2002) found an incidence of <20% for *Staph aureus* in his study on clinical mastitis. *Strep uberis* was the most common isolate in this study of >45%. The table below summarizes the incidence of *Staph aureus* across all 5 farms.

Herd	Number of cows Sampled	Cows culturing <i>Staph aureus</i>	% of total	<i>Strep uberis</i>	% of total
Herd A	14	5	36%	3	21%
Herd B	41	26	68%	1	2%
Herd C	19	7	40%	4	21%
Herd D	24	15	64%	0	0%
Herd E	11	7	69%	3	27%
Totals	109	60	55%	11	10%

It would be interesting to see if *Staph aureus* has a similar prevalence across all organic farms. The incidence of other mastitis pathogens appears lower may be a reflection that these other pathogens are responding to organic treatment approaches. This may well warrant more investigation.

The response to clinical mastitis herbal treatments was encouraging and further investigation is warranted.

Similarly the Calendula/Clove treatment for the black pock was also very encouraging.

The use of kinesiology was helpful and the farmer felt that the treatments chosen had a beneficial effect on his herd. Unfortunately this approach needs a trained and unbiased practitioner. This makes it an expensive diagnostic tool an difficult for farmers to practise themselves.

The homeopathic treatments were disappointing but similar results ave been seen in other overseas trials. There does appear to be some response as seen in herd A where high cell count cows were bacteriologically negative after homeopathic treatment. Due to the nature of homeopathy it is ideally used on an individual basis which will always make it difficult when used on a herd basis.

The overall success of the trial was encouraging in all areas and further investigation would be worthwhile. As reflected in other trials finding enough participants to make the results statistically valid is challenging. This is further complicated by the multi factorial nature of mastitis and high cell counts.

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Val MacArthur from Herbal Touch carried out the muscle testing and wrote the introduction to muscle testing;

Fiona Lane from Biopet provided some of the homeopathic remedies and helped with discussion on appropriate remedies and,

Bruce Barwell gave us his time to discuss homeopathic remedies and possible approaches to treatment of chronic mastitis problems.

WINTEC Animal Ethics Committee (AEC) has provided ethical approval for the completion of this project and gave its support.

References

1. Allen K. L. and Molan P. C. The sensitivity of Mastitis-causing Bacteria to the Antibacterial Activity of Honey. *New Zealand Journal of Agricultural Research*, 1997, Vol. 40: 537-540
2. Andrew S M and Rastani R R. Evaluation of Homeopathic Therapy for Subclinical Mastitis in Holstein Cows. *Alternative Health Practices of Livestock*. Morris T F, Kelly M T. 2004. ISBN 13 978-0-08138-1764-4.
3. Aron G M and Irvin J D. Inhibition of herpes simplex virus multiplication by the pokeweed antiviral protein. *Antimicrob Agents Chemother* 1980. 17(6): 1032-1033.
4. Asano K, Takahashi T, Miyashita M, et al. Effect of *Eleutherococcus senticosus* Extract on Human Physical Working Capacity. *Planta Med* 1986 Jun;52(3): 175-7.
5. Bone K and Mills S. *Principles and Practice of Phytotherapy*. Churchill Livingstone. 2000: 178, 515-516.
6. Brown D, and Dattner A. Phytotherapeutic Approaches to Common Dermatological Conditions. *Arch Dermatol*. 1998; 134(11): 1401-1404.
7. Cooper R A, Molan P C, and Harding K G. Antibacterial Activity of Honey against strains of *Staphylococcus aureus* from Infected Wounds. *J R Soc Med*. 1999 June; 92(6): 283-285.
8. Franklin S T, Young J W, and Nonnecke B J. Proliferation and Phenotype of Bovine Mononuclear Leukocytes in Cultures Stimulated by Pokeweed Mitogen 1994. *Journal of Dairy Science* Vol. 77 No. 12 3592-3600.
9. Ganora L. Herbal Constituents. *Foundations of Phytochemistry* 2008: 110, 144, 156-7.

10. Harris V. Treating Skin Allergies without drugs using NAET (Nambutripad's Allergy Elimination Technique). Proceedings of the Holistic Veterinary Society NZVA Conference 2001: 43-52
11. Hertoen L, Larsen S, Ødegaard A and Løken T. Comparison of Homeopathy, Placebo and Antibiotic treatment of Clinical Mastitis in Dairy Cows. J.Vet.Med. A51 439-446 2004.
12. House J K and Smith B P. Mastitis Therapeutics. Proc 20th Annual Seminar Society of Dairy Cattle Veterinarians NZVA 2003.
13. Kartikeyan S, Chaturvedi R and Narkar S. Effect of Calendula on Trophic Ulcers. Lepr Rev. 1990; 61(4):399.
14. Kimura Y and Sumiyoshi M. Effects of various *Eleutherococcus senticosus* cortex on swimming time, natural killer activity and corticosterone level in forced swimming stressed mice. J Ethnopharmacol 2004 Dec; 95(2-3): 447-53.
15. Klocke P, Garbe S, Spranger J and Merck C. Effects of Homeopathic and Antibiotic Mastitis Treatment Considering Medium Term Parameters in an Organic Dairy Herd. World Buiatrics Congress, Hannover, Germany, 2002; 18.-23.8.2002. [Unpublished]
16. Klocke P, Ivemeyer S, Heil F, Walkenhorst M I and Notz C. Treatment of Bovine Sub-clinical Mastitis with Homeopathic Remedies. 3rd QLIF Congress: Improving Sustainability in Organic and Low Input Food Production Systems, University of Hohenheim, Germany, March 20-23, 2007.
17. Kloucek-Popova E, Popov A, Pavlova N and Krusteva S. Influence of the Physiological Regeneration and Epithelialisation using fractions isolated from *Calendula officinalis*. Acta Physiol Pharmacol Bulg 1982. 8(4):63-67.
18. Lee M.R and Coll J R. Plants Against Malaria Part 2: *Artemisia Annu* (Qinghaosu Or the Sweet Wormwood) Physicians Edinb 2002; 32: 300–305
19. McCaughan C.J. and Malecki J.C. 1981. Milk retention in chronically stressed dairy cows. Australia Veterinary Journal 57: 203–204.
20. McDougall S. Bacteriology: Cure Rate and Herd Risk Factors for Clinical Mastitis in Dairy Herds. Proc 19th Annual Seminar, Society of Dairy Cattle Veterinarians NZVA. 2002 : 1-16.
21. McDougal S and Compton C. Bacteriology: Cure Rate and Herd Level Risk Factors for Clinical Mastitis in Dairy Herds. Proc Annual Seminar Society of Dairy Cattle Veterinarians. NZVA 2005 : 17- 32.
22. Mallard B.A., Dekkers J.C., Ireland M.J., Leslie K.E., Sharif S., Van Kampen C.L. Wagter L. and Wilkie B.N. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. Journal of Dairy Science 81:585–595.
23. Niskanen R. Emanuelson U., Sundberg J., Larsson B. and Alenius S. 1995. Effects of infection with bovine virus diarrhoea virus on health and reproductive performance in 213 dairy farms in one county in Sweden. Preventive Veterinary Medicine 23: 229–237.
24. Pankey J W. Teat Spraying for Mastitis Control. Proc 14th Annual Seminar Society of Dairy Cattle Veterinarians NZVA, 1997.

25. Panossian A. Adaptogens: Tonic Herbs for Fatigue and Stress. *Alternative & Complementary Therapies* 2003; 9(6): 327-331.
26. Patrick K, Kumar S, Edwardson P and Hutchinson J. Induction of Vascularisation by an Aqueous Extract of the flowers of *Calendula officinalis* L, the European marigold. *Phytomedicine*.1996; 3(1): 11-18.
27. Qinghaosu Antimalaria Coordinating Research Group. Anti-malaria studies on qinghaosu. *Chinese Med J* 1979; 12:811–16.
28. Rao S, Udupa A, Udupa S, Rao P, Rao G and Kulkarni D. *Calendula* and *Hypericum*: Promoting Wound Healing in Rats. *Fitoterapia*.1991;62(6): 508-510.
29. Romero M, Serrano M, Valleo M, Efferth T, Alvarez M and Marin J. Antiviral effect of Artemisinin from *Artemisia annua* against a model member of the Flaviviridae family, the Bovine Viral Diarrhoea Virus (BVDV). *Planta medica* 2006, vol. 72, no13, pp. 1169-1174 [6 page(s) (article)] (29 ref.)
30. Schmidgall J, Schnetz E and Hensel A. Evidence for Bioadhesive Effects of Polysaccharides and Polysaccharide-containing herbs in an ex vivo Bioadhesion Assay on Buccal Membranes. *Planta Med.* 2000 Feb;66(1): 48-53.
31. Sordillo L.M., Shafer-Weaver K. and DeRosa D. 1997. Immunobiology of the mammary gland. *Journal of Dairy Science* 80: 1851–1865.
32. Varshney J.P. and Naresh R. Comparative Efficacy of Homeopathic and Allopathic systems of medicine in the management of Clinical Mastitis of Indian Dairy Cows *Homeopathy* Vol 94, Issue 2, April 2005, pg 81-85.
33. Winston D. *Harmony Remedies; An Overview of Adaptogens* 2004 :1
34. Wynn S and Fougere B; *Veterinary Herbal Medicine*, Mosby 2007; 646