From

The Head,

Deptt of Vety Clinical Medicine.

CCS HAU, Hisar

To

The Director of Research

CCS HAU, Hisar

Memo No ; VCM/ 964-66

Dated 30.8.2005

Sub: Final Report of Research Scheme

Unclosed please find herewith the final report of the research scheme C(g)\VCM-I-OA "Therapeutic efficacy of Mastacure^{TM}, a homoeopathic Medicine, in mastitis and theft eat conditions in cattle and buffaloes" for your kind information and necessary action

Head of Department

CC to : 1. M/s Gurdev Veterinaries C/o Jindal Homeo Clinic, Old Courts Colony, Sirsa

2. Dr. (Mrs.) Anshu Sharma, Scientist Incharge; College Central Laboratory, COV'S CCS HAU, Hisar for her information please.

Table 1: Therapeutic efficacy of Mastacure inclinical mastitis^{TM} in clinical mastitis ……. and buffalo

S.No. .	**Type of mastitis (animals)**	Quarters involved	Therapeutic efficacy (quarter-wise)			Mean somatic cell count ± SE per ml x 10^5		
			Cured		Uncured	Before treatment	After treatment	
			Clinical + bacteriological	Clinical only			Cured	Uncured
1.	**Acute (n=25)**	25	21 (84.00%)	2 (8.00 %)	2 (8.00 *%)*	34.12 ±0.25	2.25 = 0.28	20.21 ±0.22
2.	Sub acute (n=80)	105	68 (64.76 %)	31(29.52%)	68 (5.72 %)	25.32 ±0721	31.1 ±0.11	12-33 =.0.12
3.	Chronic (n=82)	85	43 (50.59 %)	23 (27.06%)	19 (22.35%)	39.20=0.11	4.82 ± .22	23.20 ± 0.31
**Total * **	**187**	**215**	132 (61.40 %)	56 (26,05%)	**27(12.55%)**			

A homeopathic medicine manufactured by Gurudev Veternaries, Sirsa, Haryana

Table 2: Therapeutic efficacy of Mastacure™ in teat affectionser other than mastitis

No.	Teat affections (animals)	Teats involved	Therapeutic efficacy (quarter-wise)	
1.	Blood in Milk (32)	20	26 (81.25)	6 (18.75)
2.	Teat stenosis (30)	30	25(83.33)	5(16.67)
3.	Milk letdown problems (25)	30	24(80.00)	6(20.00)
4.	Teat obstruction (25)	25	21 (84.00)	4 (16.00)
5.	Teat fibrosis(20)	20	10 (50.00)	10 (50.00)
6.	Teat atrophy)	20	10 (50.00)	10 (50.00)

Table 7: Somatic cell count (Mean LSE.) per ml of milk samples before and alter treatment in sub- clinical mastitis in buffaloes'

Groups	Before treatment	After treatment
1*	$23.3 \pm 4.07 \times 10^{5a}$	$4.4 \pm 1.75 \times 10^{5b}$
2**	$25.33 \pm 5.37 \times 10^{5a}$	$1.0 \pm 0.552 \times 10^{5b}$
3***	$29.166 \pm 2.60 \times 10^{5a}$	$1.75 \pm 0.391 \times 10^{5b}$ by day 10 $0.583 \pm 0.216 \times 10^{5b}$ by day 20 $0.458 \pm 0.208 \times 10^{5b}$ by day 30

Means with different superscript differ significantly at 5% level of significance

* infra mammary infusion of procaine penicillin G.I.P. 100000 units, streptomycin 100mg, Sulphamerazine 0.5 gm and hydrocortisone acetate 20 mg (Pendistrin – SH, M/s Sarabhai Zydus) two times per day after each milking of three days

** Intra mammary infusion of ampicillin Sod. 75 mg and cloxacillin 20 mg (Vetclox plus M/S sarabhai Zydus) two times per day after each milking for three days.

*** Given mastacure TM a homeopathic medicine (manufactured by Gurudev Veterinaries, Sirsa, Haryana) orally 30 drops three times a day for 20 days.

Proceedings of the Technical Programme Committee Meeting of the Departments of Central Vety. Laboratory, Teaching Vety. Clinical Service Complex, Animal Biotechnology and Vety. Surgery & Radiology held on 5.8.2005.

The meeting was chaired by Dr. Jit Singh, Addl. Director of Research and was attended by the Dean and HODs, COVS; HODs of APP, BMB, AN, LPM, Director, TVCSC, Director, NRCE, faculty of all concerned Departments and Associate Director (V&AS), Directorate of Research

The salient research findings, observations and decisions taken w.r.t. different research schemes in operation these departments are as under:

Central Vety. Laboratory

Research Schemes:

1. C(a)VCL-1 -NP(Agri.) " Etiology and diagnosis of mastitis and infectious abortions in animals"
2. C(g), COVS-4-OA "Testing of sheep flock for brucellosis"
3. C(a)COVS-1-Plan (Agri.) "Establishment of Central Diagnostic Laboratory"

Salient Research Findings 2004-2005:

- Monoclonal antibody based competitive ELISA was standardized and used for detecting brucellosis in cattle.
- Testing of all the 6742 sheep belonging to Central Sheep Breeding Farm for brucellosis was achieved using ELISA. It was more effective in detecting brucellosis infected sheep than RBPT.
- A total 3448 clinical and milk samples (2391 clinical 955 sub clinical mastitis & clinical material) were culturally examined Resulting isolates were identified and subjected to antimicrobial sensitivity testing.
- Microbial load of 280 semen samples received from different semen banks in Haryana

were determined and antibiogram of resulting organism were communicate.

- DAS-ELISA was standardized for detection of staphylococcal mastitis in cows and 279 milk of samples CCSHAU farm were screened using this assay.

- Out of eight d iagnostic tests subjected to 100 milk samples, *lactate dehydrogenase* (LDH) enzyme assay was found to be the best test for detecting sub clinical mastitis in cows, followed by spot TIA *(trypsin inhibition assay)* and MAMP *(modified aulendorfer mastitis probe)* test

Evaluation:

The research work under taken during the year 2004-05 was found very good and evaluated as most satisfactory.

Recommendations generated for field application:

- *Spot Trypsin Inhibition .Assay* (TIA) and *bromothymol blue* (BTB) plale test can be conducted in the field conditions by the side of animals to detect sub clinical mastitis Demonstration and practice of these tests was imparted to forty field veterinarians.

- *Mastacure* gave encouraging results m treating cases of blood in milk, teal *stenosis,* teat obstruction, milk let down problems and sub acute *streptococcal* mastitis The drug is economical when more than one quarter of animal is affected

College Central Laboratory

Dr. (Mrs.) Anshu Sharma

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Dr. (Mrs.) Anshu Sharma

MVSc., Ph.D. Post Doctorate (U.K.)

Scientist In-Charge

To

Dr. Kamal Jindal

Gurudev Veterinaries

Old Courts Colony, Sirsa- 125 055,

Haryana

No. CCL/2005/142-143 Dated: 30-8-2005

Subject: Submission of Report of project entitled "To evaluate Immune response of Mastacure^{TM}, a homoeopathic medicine bearing company code Hivet-gv-1DK in mastitic animals".

Sir,

Kindly find enclosed herewith final report of the of the research work conducted under above mentioned scheme on Mastacure ™ (homoeopathic medicine), bearing company code Hivet-gv- 1DK. in the Veterinary College Central Lab.

Yours Sincerely

Scientist In-Charge College Central Laboratory

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To

Dr. Kamal Jindal
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Old Courts Colony, Sirsa- 125 055,
Haryana

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Yours Sincerely

Anshu 30-8-2005

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RESEARCH ARTIC

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BOVINE SUB CLINICAL MASTITIS: PREVALENCE AND TREATMENT WITH HOMEOPATHIC MEDICINE

ANSHU SHARMA[1], PREM DHINGRA[1], BL PANDER[1] AND RAKESH KUMAR[2]

CCS Hisar Agricultur University, Hisar, Haryana, 125004, India

ABSTRACT

A total of 423 quarters of 109 apparently healthy lactating crossbred cows (Sahiwal x Jersey) were screened for sub-clinical mastitis. Of these, 40 cows (36.69%) and 71 (16.78 %) quarters

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ANSHU SHARMA[1], PREM DHINGRA[1], BL PANDER[1] AND RAKESH KUMAR[2]

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ABSTRACT

A total of 423 quarters of 109 apparently healthy lactating crossbred cows (Sahiwal x Jersey) were screened for sub-clinical mastitis. Of these, 40 cows (36.69%) and 71 (16.78 %) quarters were found culturally positive. As per the International Dairy Federation criterion, 58 (13.71%) were found to suffer from sub clinical mastitis. Relative frequency of various micro-organisms among 60 isolates, from the 58 apparently healthy quarters revealed isolation of *Staphylococcus epidermidis* (28) *Staphylococcus aureus* (22), *Streptococcus agalactiae* (6), *Streptococcus dysgalactiae*(3) and *Streptococcus uberis* (1) .Two quarters harbored mixed infection . Cows found positive for sub-clinical mastitis were divided into three groups on the basis of antimicrobial sensitivity towards penicillin and cloxacillin. As many as 80 per cent of the quarters treated with Vetclox plus in group I were bacteriologically negative five days after withdrawal of therapy, whereas 70.58 per cent of quarters revealed bacteriological cure in group II cows treated with Pendistrin SH. Corresponding figure for treatment with Mastacure ™ in group III on day 5 post-treatment was recorded as 47.61 per cent However, on days 10 and 20 post-treatment, bacteriological cure rates in Mastacure ™ treated quarters of group III were found to be increased to 57.14 per cent and 66.67 per cent respectively, whereas no change in bacteriological cure rates were observed in groups I and II treated with antibiotics when compared with day 5 post-treatment values. No side effects of drug administration were observed in any of the groups of treated cows.

Key words: Bovine, mastitis, staphylococcus, homeopathy

INRODUCTION

Mastitis continues to be the most economically important disease of dairy animals. It can be classified into clinical and sub clinical. The occurrence of clinical mastitis is proportional to the prevalence of sub-clinical mastitis, because an existing sub-clinical phase of intra-mammary infection predisposes the former. Recently in India, Dua (2001) has reported annual losses due to sub clinical mastitis to the tune of Rs 4369.32 crores, Therefore, it is of paramount importance that a particular dairy herd/organized farm should be screened for the disease at regular intervals so as to adopt an effective treatment regimen and sound prophylactic measures, that would prevent serious economic losses in terms of decreased milk production, cost of treatment and culling of animals. Along with the financial implications of mastitis, the public health importance of the disease cannot be ignored. The extensive and indiscriminate use of antibiotics in the treatment and control of mastitis can lead to undesirable presence of drug residues in milk, emergence of antibiotic resistant bacteria and necessary withholding period. To overcome this problem, one of the alternatives to antibiotic therapy is homeopathy

Therefore, the study was planned to screen all the lactating cows of an organized cattle farm located in Hisar (Haryana), and to assess the therapeutic efficacy of a homeopathic medicine, Mastacure™ (manufactured by Gurudev Veterinaries, Sirsa, Haryana).

MATERIAL AND METHODS

A total of 423 quarters of 109 apparently healthy lactating crossbred cows (Sahiwal x Jersey) were screened for sub-clinical mastitis following International Dairy Federation criterion.

Collection of milk samples: The udder of the animals were cleaned thoroughly with water and allowed to dry The hands were washed with soap and water and rinsed

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with 70 per cent alcohol. After discarding first few streams of milk, the teat apices were thoroughly scrubbed with a cotton swab soaked in 70 per cent alcohol. About 20 ml of milk from respective quarters was collected separately into sterilized test tubes and were marked as right fore (RF), right hind (RH) left fore (LF) and left hind (LH). The collection was first done from the near side then from the off side to avoid contaminating the disinfected teat apices.

Bacteriological Examination: The milk samples collected aseptically were shaken thoroughly. With the help of a 4.00 mm diameter platinum loop, 0.01 ml of the milk sample was streaked on 5 per cent sheep blood agar (BA) and MacConkey's lactose agar plates. The plates were incubated aerobically at 37°C for 24 to 48 hours. The resulting growth from the respective plates of media was purified and identified on the basis of Gram's reaction, morphology and colony characteristics. Further characterization to the species level was carried out on the basis of biochemical tests.

Treatment trail: Cows found positive for sub-clinical mastitis were divided into three groups on the basis of antimicrobial sensitivity towards penicillin and cloxacillin. In Group I, 20 quarters were infused with cloxacillin (200mg) and ampicillin sodium 75 mg (Vetclox plus) and in Group II, 17 quarters were infused with intra-mammary preparation containing procaine penicillin G (100000 IU), streptomycin 100 mg, sulphamerazine 500 mg and hydrocortisone acetate 20 mg (Pendistrin SH) for 3 days at 12 hr. intervals after milking. In Group III animals, Mastacure ™ was given @ 30 drops four times daily with wheat flour dough orally for 20 days.

RESULTS AND DISCUSSION

Out of 423 quarters of 109 lactating cross bred cows, 40 cows (36.69%) and 71 (16.78 %) quarters were found culturally positive. As per the International Dairy Federation (IDF) criterion, 58 (13.71%) were found to suffer from sub clinical mastitis. The Results of screening are presented in Table 1. The rate of prevalence of SCM in this investigation is lower in comparison to the findings reported by Roy *et al.* (1989), Thirunavukarasu and Prabharan (1998), Nagal *et al.* (1999), Saravanan *et al.* (2000) Maiti *et al.* (2003), Saluja *et al.* (2004) and Jain (2005). The differences in the prevalence rates of sub-clinical mastitis as reported by different workers are perhaps, due to different managemental and hygienic practices, adopted in different dairy herds. Besides farm practices and sanitation, incidence of mastitis has been found associated significantly with animal factors such as breed, milk yield, parity, stage of lactation and udder morphology (Thirunavukarasu and Prabaharan, 1998). The climatic conditions also affect the prevalence of mastitis (Schultze, 1985). Thus the higher rate of prevalence as observed by other workers might be attributed to intensive management and lack of proper hygienic and control measures.

Relative frequency of various micro-organisms among 60 isolates, from the apparently healthy milk quarters including 2 quarters harboring mixed infection is given in Table 2. In the present study Staphylococci were found to be the major pathogens accounting for *Staph. epidermidis* (28) and *Staph. aureus* (22). The high prevalence of staphylococci has also been reported by several workers (Kalorey *et al.*, 1983, Javed and Siddique, 1999; Tijare *et al.*, 1999; Tuteja, 1999 and Kaya *et al.*, 2000). Several workers (Tuteja 1999, Bulla 2002 and Jain 2005) have also observed the prevalence of *Staph. epidermidis*, higher than *Staph. aureus* in sub-clinical mastitis. However, many workers have found *Staph. aureus* to be more prevalent than *Staph. epidermidis* (Char *et al.* 1983; Saini *et al.* 1994).

In the present study, frequency of isolation of streptococci was *Streptococcus agalactiae* (6), *Streptococcus dysgalactiae* (3) and *Streptococcus uberis* (1). Almost the same pattern of occurrence of *Str. agalactiae* and *Str. dysgalactiae* has been reported by Saxena (2000) and Bulla (2002). Contrary to this, the higher prevalence of *Str. dysgalactiae* than *Str. agalactiae* was reported by Kalra and Dhanda (1964) and Tuteja

Table 1: Cultural examination and somatic cell count (SCC) of cow milk samples for detection of sub-clinical mastitis

	Animals culturally positive	Quarters culturally positive	Animals showing SCC>500000 per ml.	Quarters Showing	
				SCC >500000 /ml	SCC >500000 /ml and Culturally positive (sub-clinical mastitis)
Numbers positive	40	71	25	78	58
Per cent positive	36.69	16.78	22.93	18.43	13.71

Table 2: Micro-organisms isolated from 58 quarter-milk samples of apparently healthy cows.

Organism isolated	No. of isolates (%)
Staph. epidermidis	28(46..67)
Staph. aureus	22(36.67)
Str. agalactiae	6(10)
Str. dysgalactiae	3(5)
Str. uberis	1(1.66)

(1999). Javed and Siddique (1999) found higher prevalence rate of *Str. uberis* than that recorded in this study. Similar to our findings, Sharma and Kapur (2000) and Bulla (2002) also reported higher incidence of mixed infections with staphylococci and streptococci.

Homeopathy is widely used and recommended for both mastitis prevention and treatment. In the present study, 80 per cent of the quarters treated with Vetclox plus in group I were bacteriologically negative five days after withdrawal of therapy, whereas 70.58 per cent of quarters revealed bacteriological cure in group II cows treated with Pendistrin SH. Corresponding figure for treatment with Mastacure ™ in group III on day 5 post-treatment was recorded as 47.61 per cent However, on days 10 and 20 post-treatment, bacteriological cure rates in Mastacure ™ treated quarters of group III were found to be increased to 57.14 per cent and 66.67 per cent respectively, whereas no change in bacteriological cure rates were observed in groups I and II treated with antibiotics when compared with day 5 post-treatment values (Table 3) . No side effects of drug administration were observed in any of the groups of treated cows. Clinical trials with homeopathic medicine in cows by Sonnenwald (1986), Klocke *et al.* (2002), revealed 34 per cent and 21.70 per cent bacteriological cure, respectively which are lower as compared to our study except for the cure rate 77.77% reported by Manjesh (2005) in buffaloes. In contrast to our findings Dorenkamp (1992) achieved a very high bacteriological cure rate (97.90%) four days post treatment with 4.80 per cent showing relapse after eight weeks. Homeopathic treatment of 50 cows suffering from acute mastitis, showed positive results in favor of homeopathy, especially in the treatment of cases of *E.coli* mastitis (Merck *et al*, 1989). A placebo controlled clinical trial, on treatment of sub-clinical mastitis, showed a more than 50 % reduction in the frequency of mastitis, in cows treated with homeopathy, compared to placebo (Searcy *et al*, 1995).The difference in cure rate among cows and between cows and buffaloes in different studies might be due to differences in the nature of clinical and sub clinical mastitis as well as nature of homeopathic medicine used in these trials. Sonnenwald (1986) found homeopathic preparations more successful than antibiotics in treating mastitis cases. He found it more effective in treating Gram negative bacteria than mastitis cases caused by gram positive bacteria. In our study all the organisms isolated were Gram positive and the homeopathic medicine gave encouraging results. In foreign countries, some research on homeopathic treatment of animals has been and is being done and Homeopathy is emerging as the main alternative or complement to antibiotic therapy. An increasing number of farmers, in particular organic farmers, are using Homeopathic remedies instead of conventional drugs to treat their animals. A survey showed that 39% of Norwegian homeopaths treat animals, mainly pets, but also farming animals (Norske, 1999). A Swedish survey showed that 14% of milk producing farmers are regularly using homeopathy to treat their animals (Ståhl, 1997). Another survey showed that the main reasons why farmers use homeopathic remedies are the risk of bacterial resistance, a wish to diminish the use of antibiotics, their own interest and economic advantages. (Henriksson, 1997). There are three reasons for increased cost effectiveness from homeopathic treatment of animals. First of all one of the aims of the

Table 3: Therapeutic efficacy of Mastacure ™ and intra-mammary antibiotic preparations in Sub-clinical mastitis in cows

Group –1* Quarters		Group-2** Quarters		Group-3*** Quarters			
Treated	Cured	Treated	Cured	Treated	Cured by day 5	Cured by day 10	Cured by day 20
20	16 (80.00%)	17	12 (70.58%)	21	10 (47.61%)	12 (57.14%)	14 (66.67%)

*Intra-mammary infusion of ampicillin Sod. 75 mg and cloxacillin 200 mg (Vetclox plus M/S Sarabhai Zydus) two times per day after each milking for three days.

**Intra-mammary infusion of Procaine penicillin G. I.P. 100,000 units, Streptomycin 100mg, Sulphamerazine 0.5 gm and hydrocortisone acetate 20 mg (Pendistrin– SH, M/S Sarabhai Zydus) two times per day after each milking for three days.

***Given Mastacure ™ a homoeopathic medicine, (manufactured by Gurudev Veterinaries, Sirsa, Haryana) orally 30 drops three times a day for 20 days.

treatment is to prevent the recurrence of diseases, which results in reduced frequency of disease and therefore reduced expenses for medicinal products. Secondly, homeopathic remedies are of low cost. Thirdly, homeopathic remedies do not leave medicinal traces in products like meat and milk, which therefore reduces the withdrawal period after treatment. In spite of all these advantages of Homeopathy, scientific information on the effectiveness of homeopathic remedies for the treatment of bovine mastitis is too limited to justify a definite conclusion. Hence, further studies are required on the effect of these homeopathic medicines on the immune response of mammary gland to explain the large variation recorded on therapeutic efficacy of homeopathic medicines in bovine mastitis.

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