



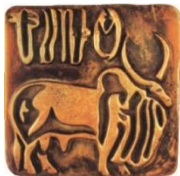
Reduction of drug residues in raw milk: management of bovine mastitis by ethno-veterinary medicine

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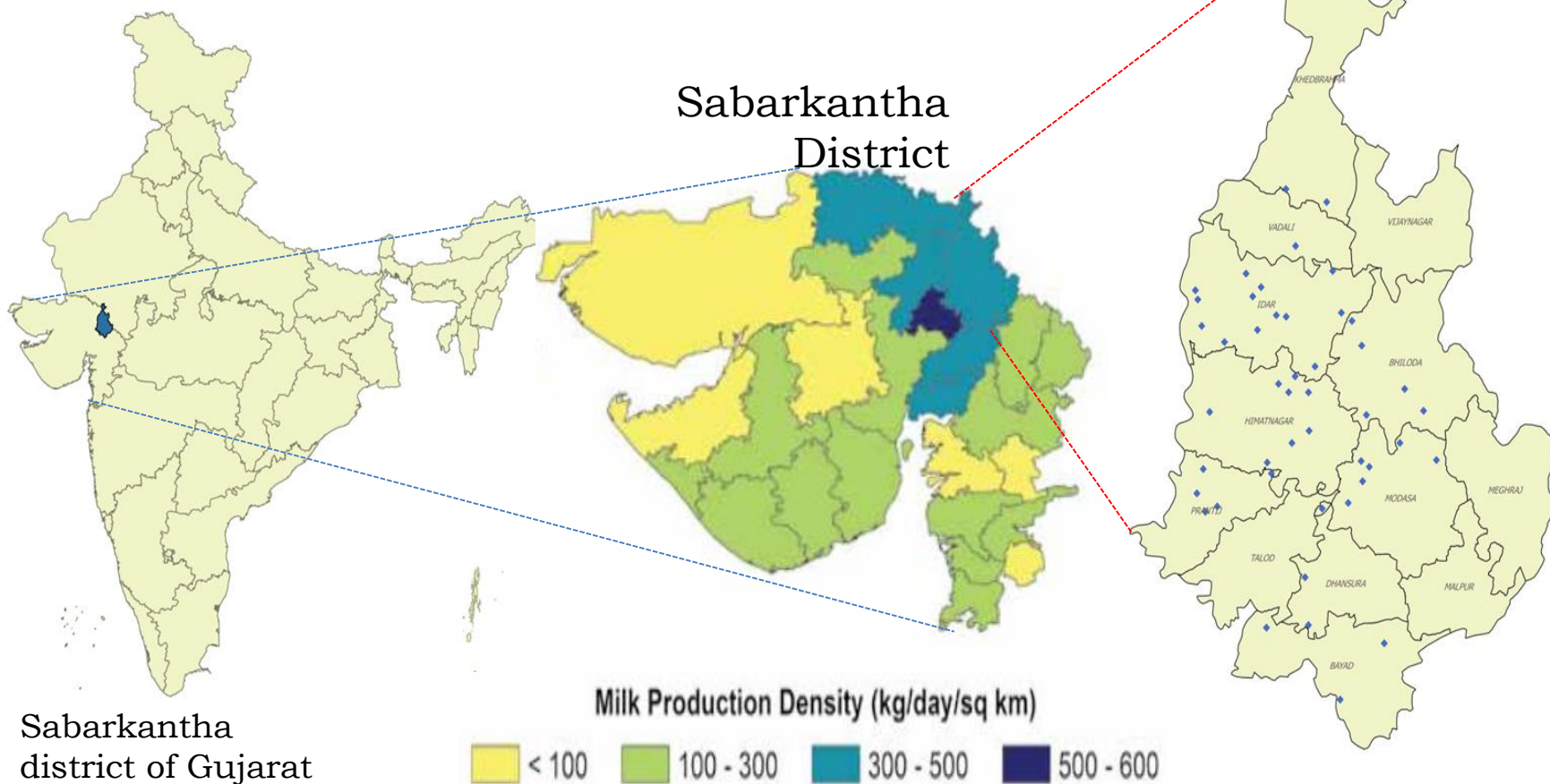
Overview

- Distribution of diseases, Bovine mastitis
- Detection and treatment of sub-clinical mastitis
- Use of Ethno-veterinary medicine in mastitis control
- Expansion of mastitis control project
- Microbiological investigations
- Conclusions



Information Network For Animal Productivity and Health (INAPH)

Area selected for initial study

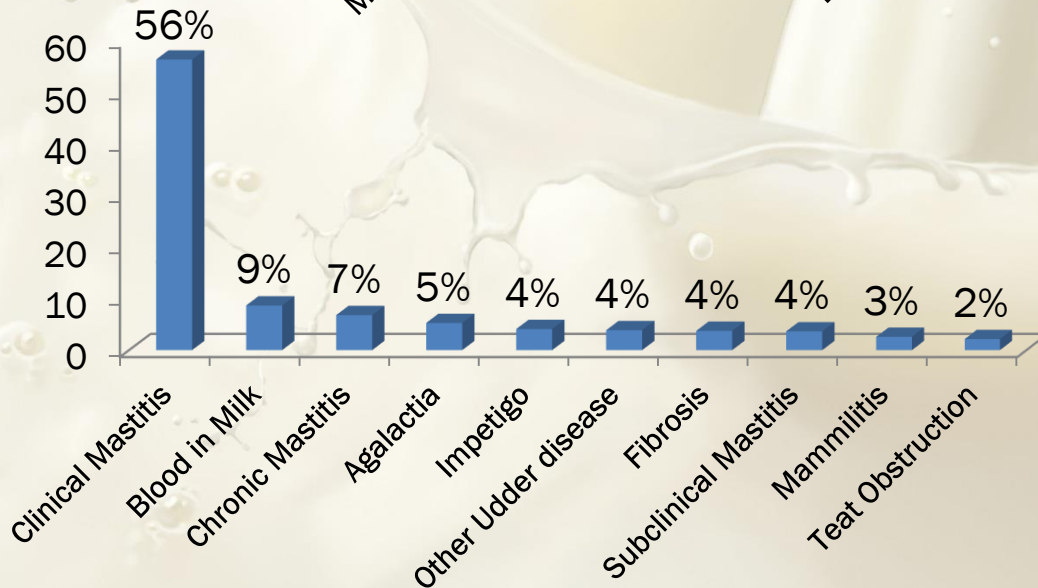
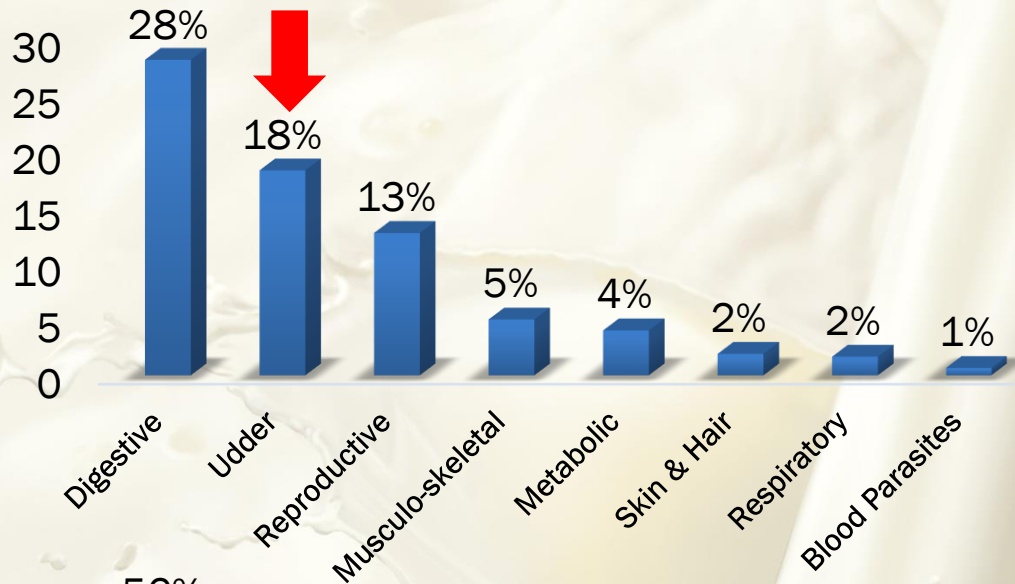


Sabarkantha
district of Gujarat

From more than 0.54
million records



Major disease conditions in bovine recorded Sabarkantha Milk Union



Mastitis emerged as leading economically important disease condition in bovine and required attention



Bovine Mastitis

Inflammation of one or more quarters of the udder



Subclinical Mastitis

- ~ 90-95% of all mastitis cases
- Udder/milk appears normal
- Elevated SCC
- Lowered milk output (~ 10%)
- Longer duration

Clinical Mastitis

- ~ 5 - 10% of all mastitis cases
- Inflamed udder
- Painful and swelling of udder
- Clumps and clots in milk
- Acute /Chronic type



Losses due to Mastitis in India



Annual losses due to mastitis in India- **Rs 7165.51 crores** (*Bansal & Gupta, 2009*)



Alternative approach for detection & control of sub-clinical mastitis



Awareness creation

- Village meetings
- Extension material- calendars, posters, stick-ups, individual farmer meetings etc.



Identification of CMT positives

- CMT of pooled cow & buff milk at DCS
- CMT of individual animal's milk at farmer's place if pooled milk CMT positive
- Bi-monthly testing at DCS

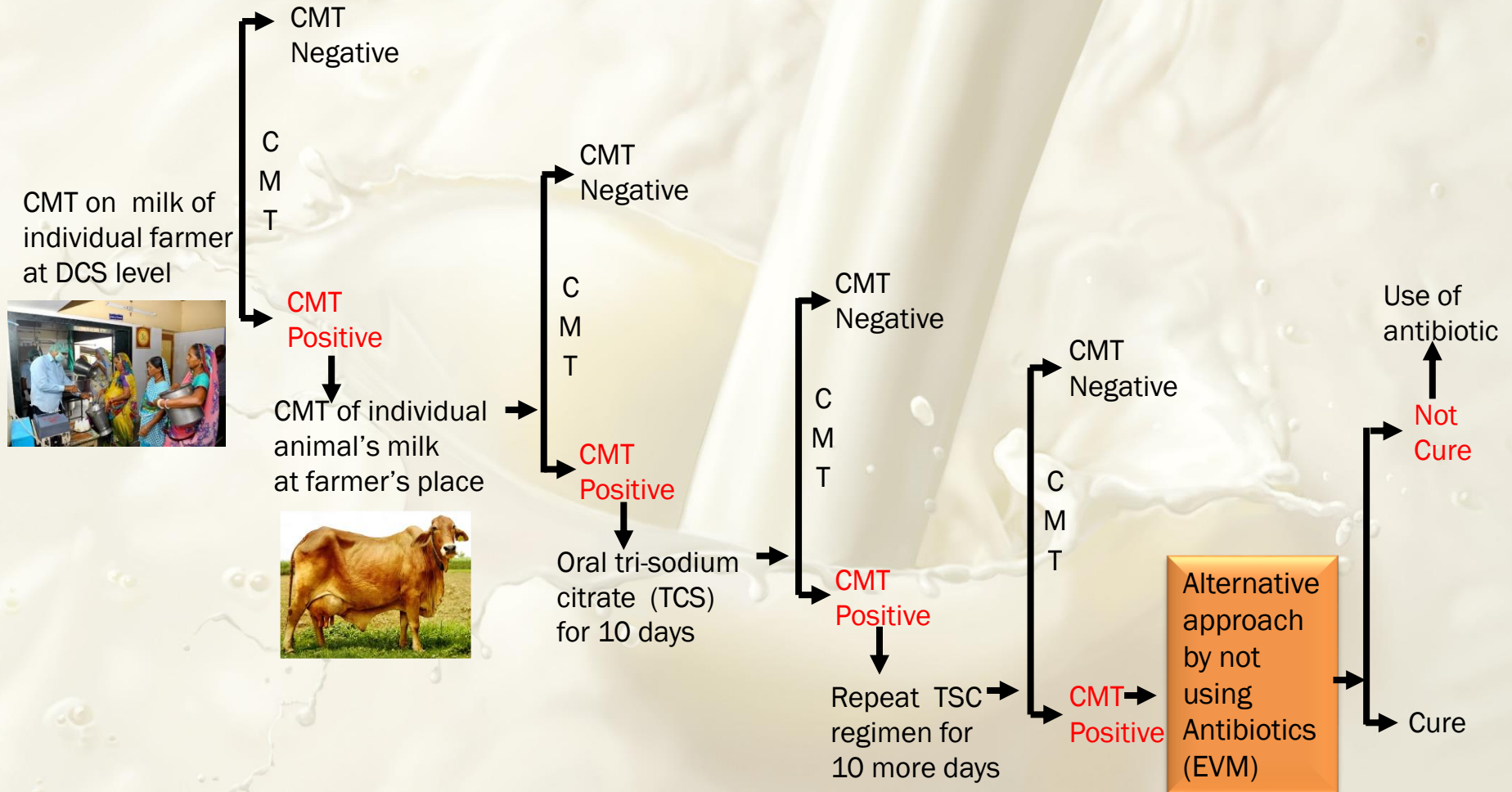


Treatment of CMT positives

- Tri-sodium citrate regimen
- Alternative approaches/Antibiotic course to TSC non-responsive animals
- Management of chronically infected animals

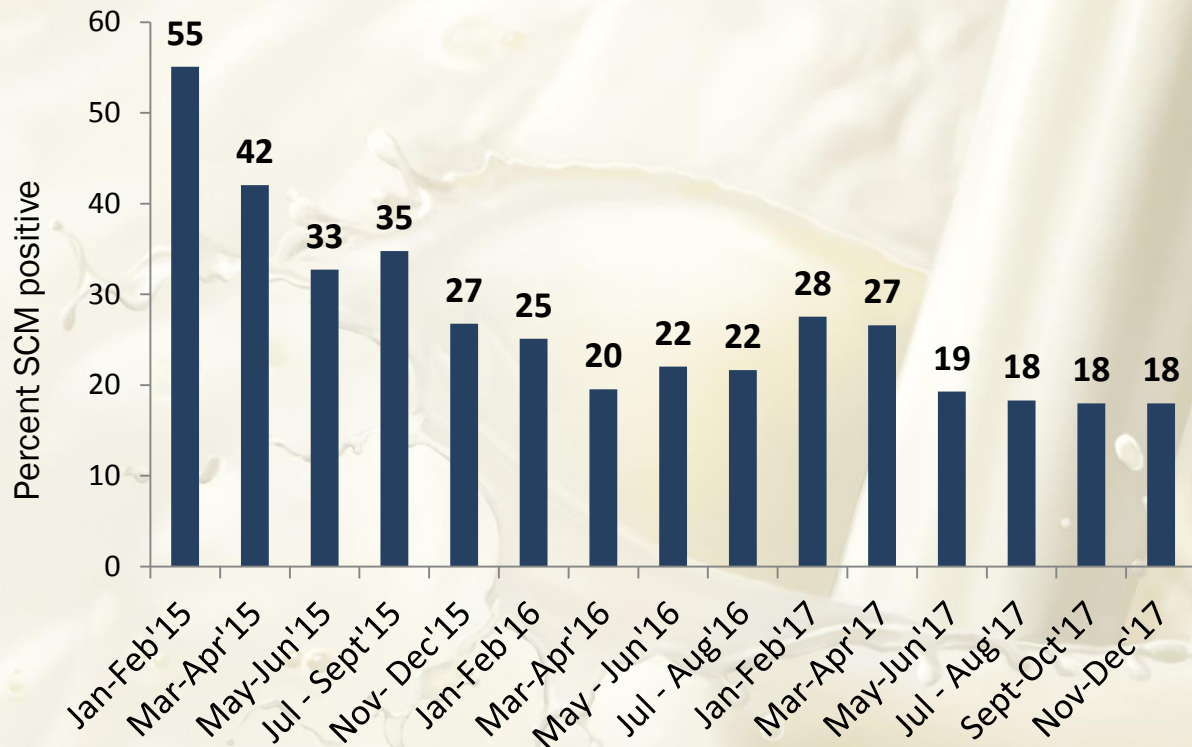


Work flow for management of mastitis by alternative approach -EVM

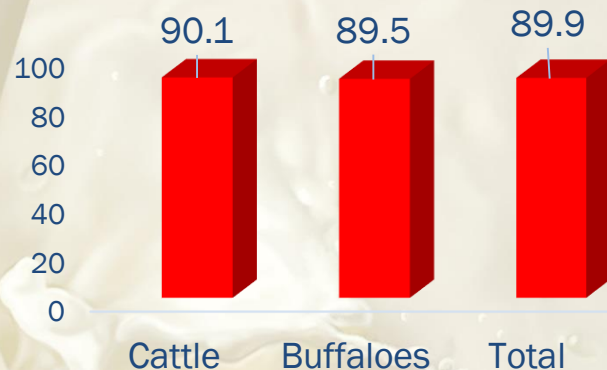




CMT Positivity of farmer's milk sample testing at DCS level



Total cure rate after using TSC



Cattle Milk Tested	CMT Positive	Percent CMT Positive	Buffalo Milk Tested	CMT Positive	Percent CMT Positive	Total Milk tested	CMT Positive	Percent CMT Positive
119637	35145	29	69486	12591	18	189068	47736	25



Effect of periodical CMT and TSC supplementation on mastitis and milk yield

- Sub-clinical mastitis cured by around 90 % as evident by CMT
- Effectively prevent the transformation of sub-clinical mastitis to clinical form
- Recorded an increase by 10-15% milk production in most of the animals that become CMT negative after TSC supplementation.
- Use of antibiotics for treatment of sub-clinical could be avoided



Alternative approach for management of clinical mastitis



Alternative approach for management of clinical mastitis

Mastitis (all types)



Aloe vera



Turmeric



Lemon



Curry leaves



Jaggery



Lime

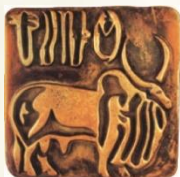
Botanical name	Proportion used
Aloe vera	250 gm
Curcuma longa (Turmeric)	50 gm
Calcium hydroxide	15 gm



Application of EVM paste on udder for treatment of clinical mastitis



- i) Prepare a handful of paste watery by adding 150-200 ml of water.
- ii) Wash and clean the udder and apply the mixture throughout.
- iii) Repeat application 5-10 times a day for 5 days

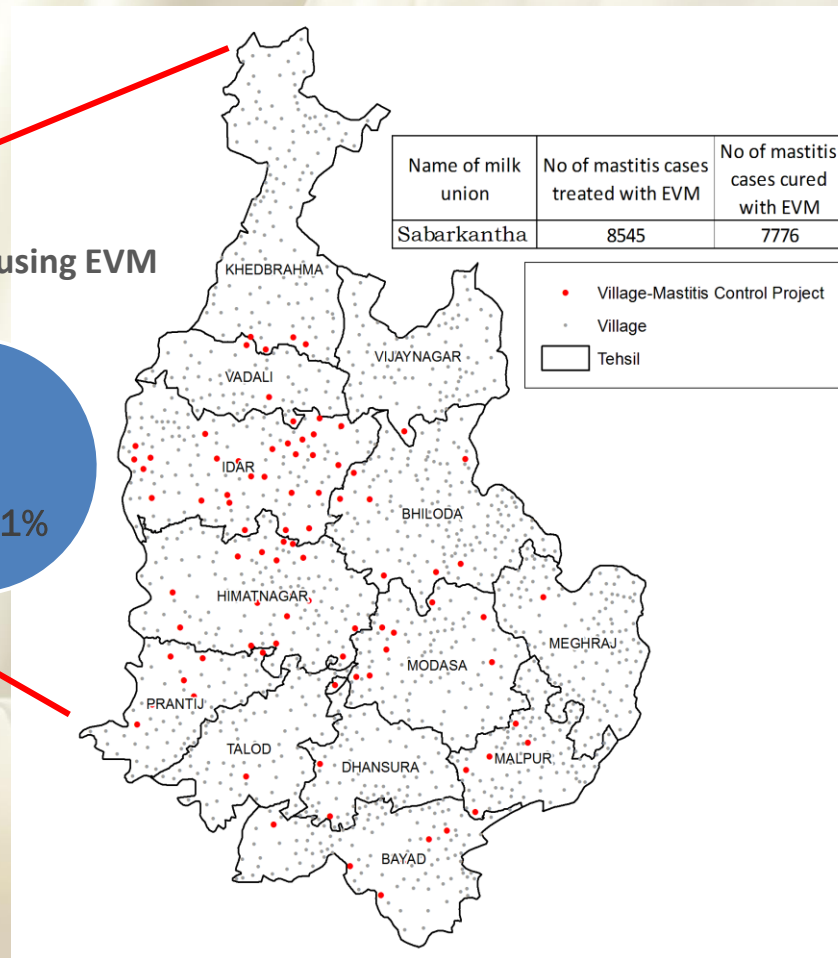
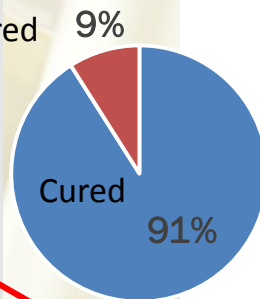


Treatment of clinical mastitis in Sabarkantha district in Gujarat using EVM



CM Cure rate using EVM

Not Cured 9%





Expansion of mastitis control project in total 27 districts 8 states



Gujarat

- Mehsana
- Bharuch
- Surat
- Banaskantha
- Sabarkantha

Maharashtra

- Kolhapur
- Aurangabad
- Pune
- Rajaram Bapu
- Warana
- Baramati

Karnataka

- Bangalore
- Chamrajnagar
- Shimoga
- Kolar
- Mysore
- Tumkur

Andhra Pradesh

- Krishna Producer Company
- Sri Vijaya Vishaka

Telangana

- Nalgonda Ranga Reddy Producer Company

Punjab

- Jalandhar
- Ludhiana
- Roper Milk Union

Kerala

- Malabar

Tamil Nadu

- Coimbatore
- Erode
- Salem Milk Union



Expansion of mastitis control project : using an alternative approach

Involving



**~1,04,400
Milk pourers**



~1510 DCS



**~1,78,000 in-milk
Bovines in 27 Milk Unions**



Components in expanded mastitis control project

1. Training and extension

- Training on EVM on a mass scale & at milk Union level
- Creation of medicinal plant plots at dairy plant / DCS

2. Mastitis detection and control

- California Mastitis Test (CMT) at DCS and farmers' homestead.
- Oral administration of Tri-sodium citrate
- Use of EVM for treatment of mastitis cases rationalizing use of antibiotics.
- Dry cow therapy

3. Monitoring

- Testing of antibiotic residues in bulk milk using field test kits at DCS level

4. Impact analysis

- Farmer awareness
- Bulk milk CMT positivity
- Antibiotic Residues in milk

5. Reporting : Web based reporting on all the above parameters



Training, extension, implementation and monitoring



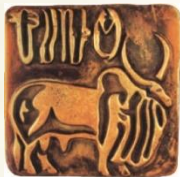
Field training of veterinarians on EVM



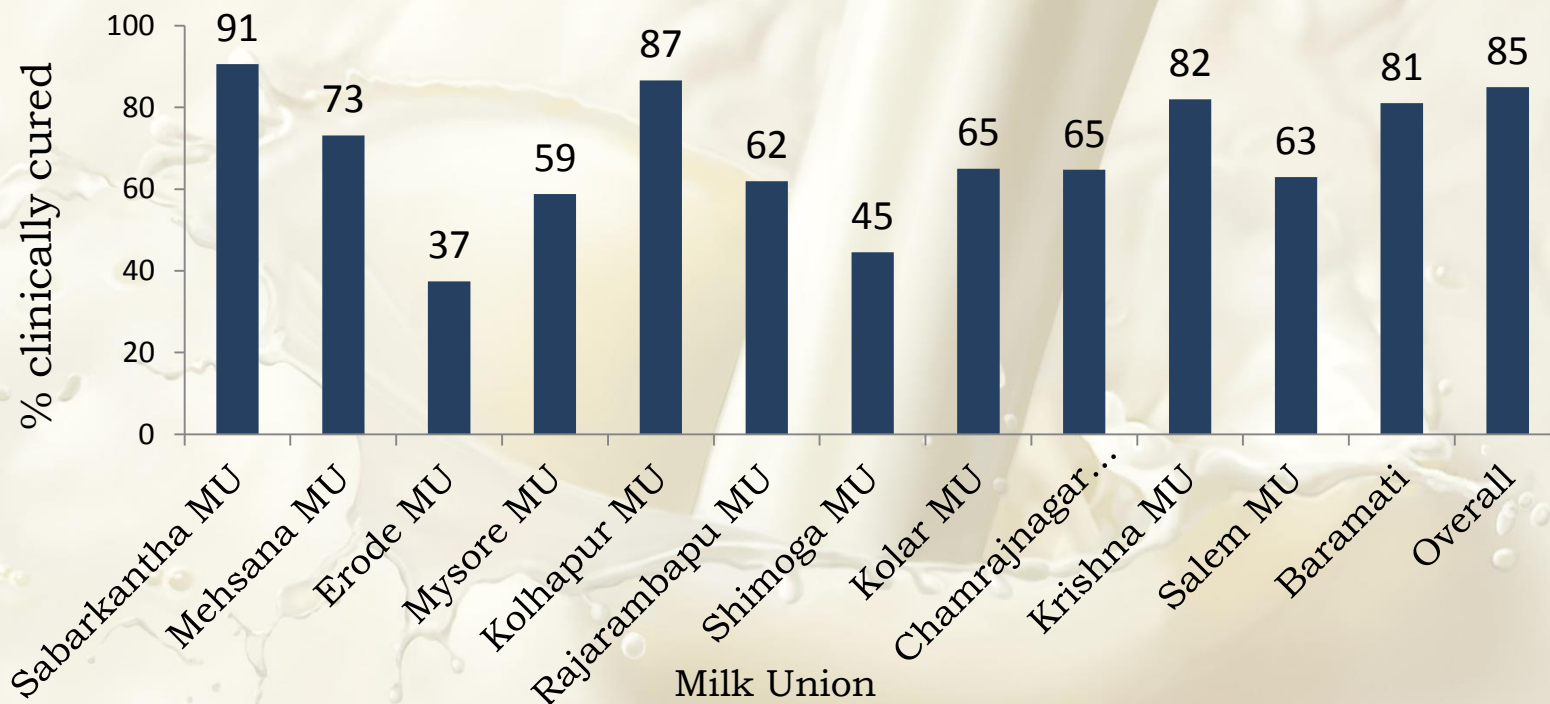
Demonstration on use of antibiotics residue test kit



Demo plot for medicinal plants

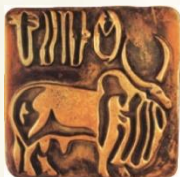


Cure rate of clinical mastitis using EVM in expanded project area

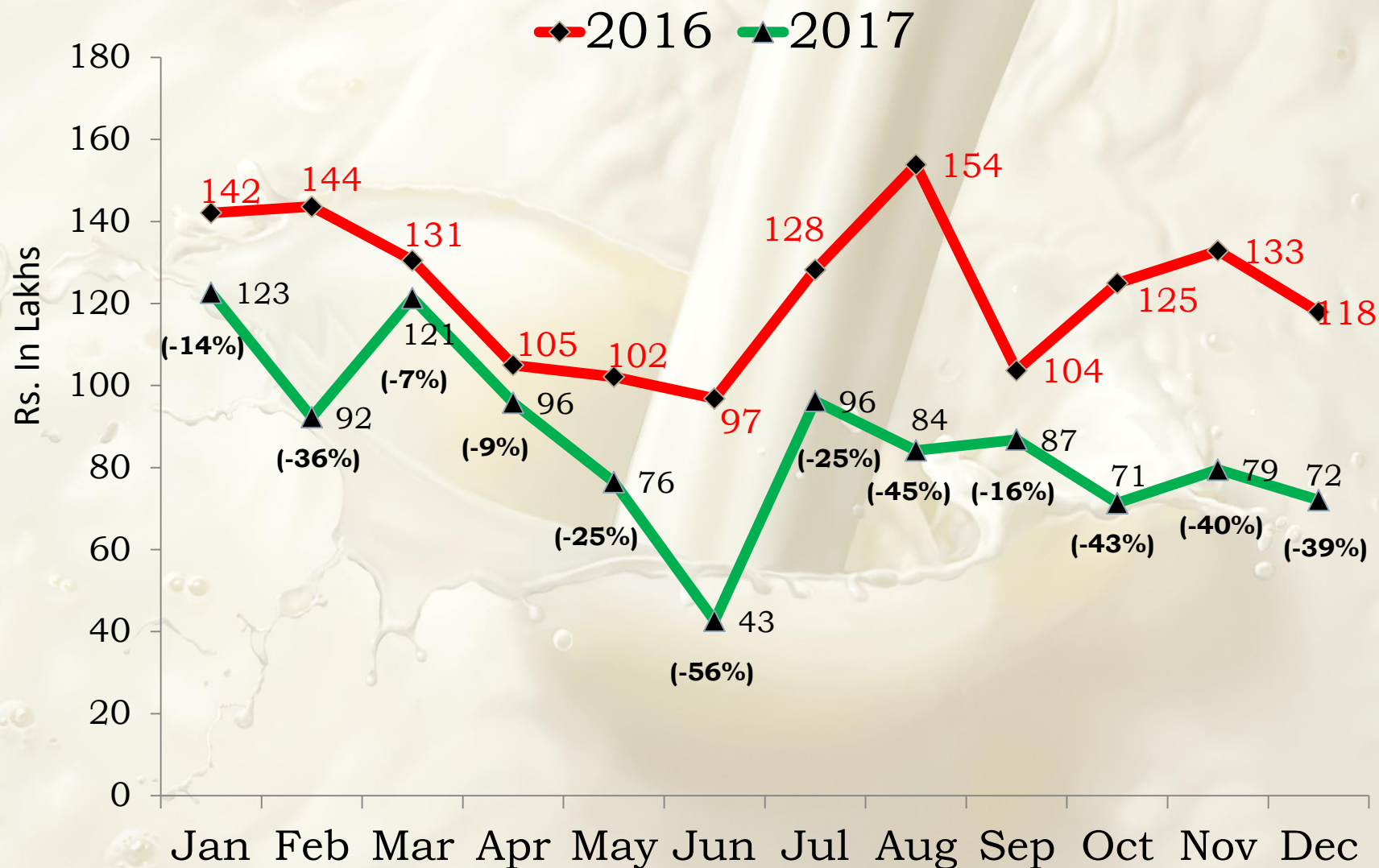


Total cases treated with EVM: 14,729

Total cases cured with EVM : 12,511(85%)



Medicine expenditure in 22 villages (~88 DCSs) in Kolhapur MU after implementation of EVM in 2017



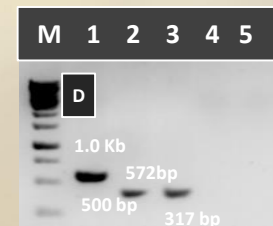
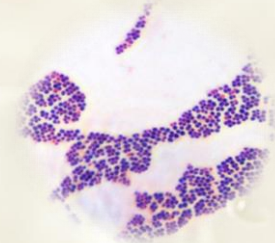


Microbiological investigations and antibiotic susceptibility



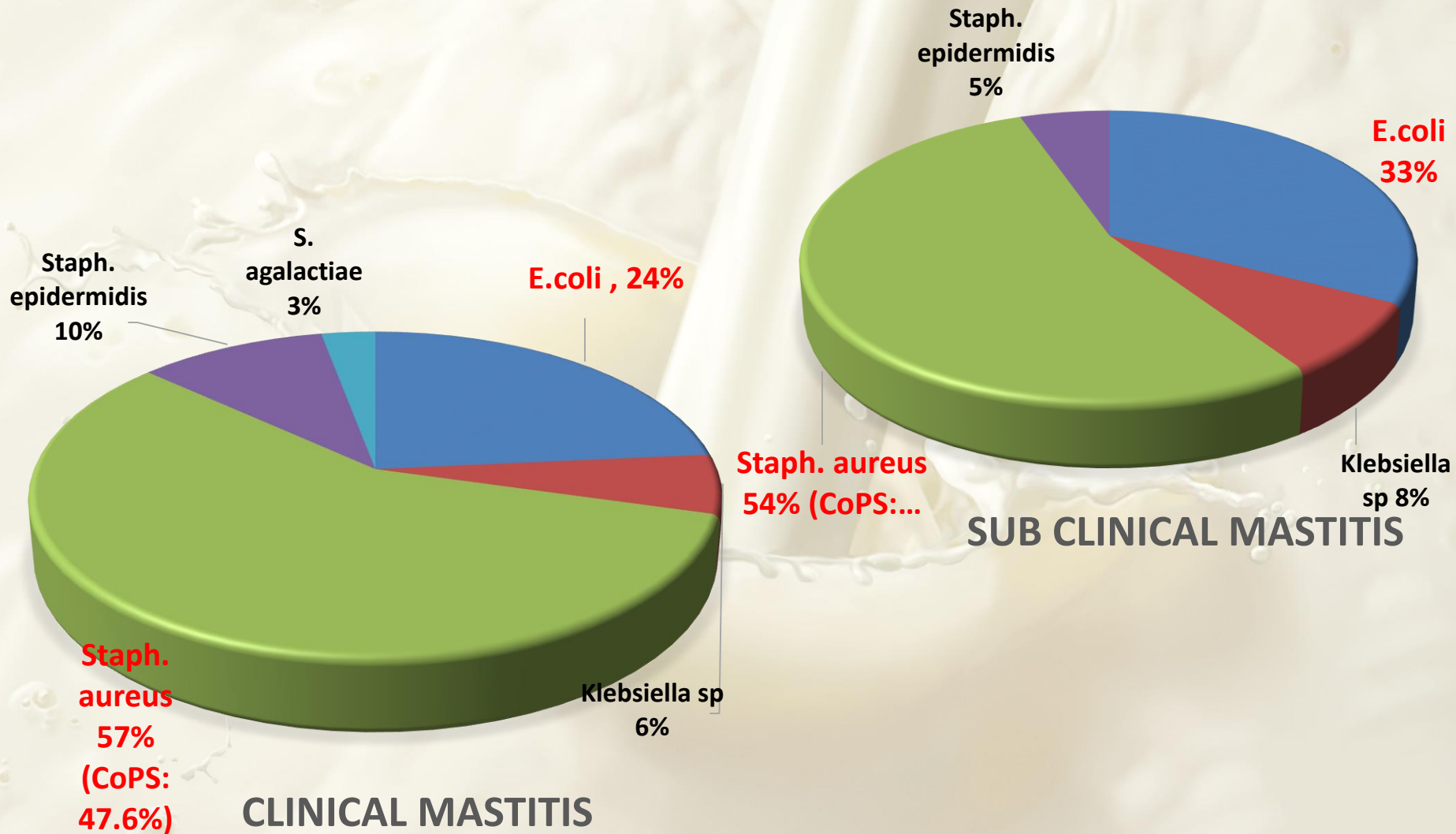
Microbiological investigations and antibiotic susceptibility

- Cultural Isolation : Bacteria from mastitis milk
- Identification of bacteria by Gram Staining
- Identification of bacteria on differential media
- Identification of mastitis pathogens
- By multiplex PCR assay





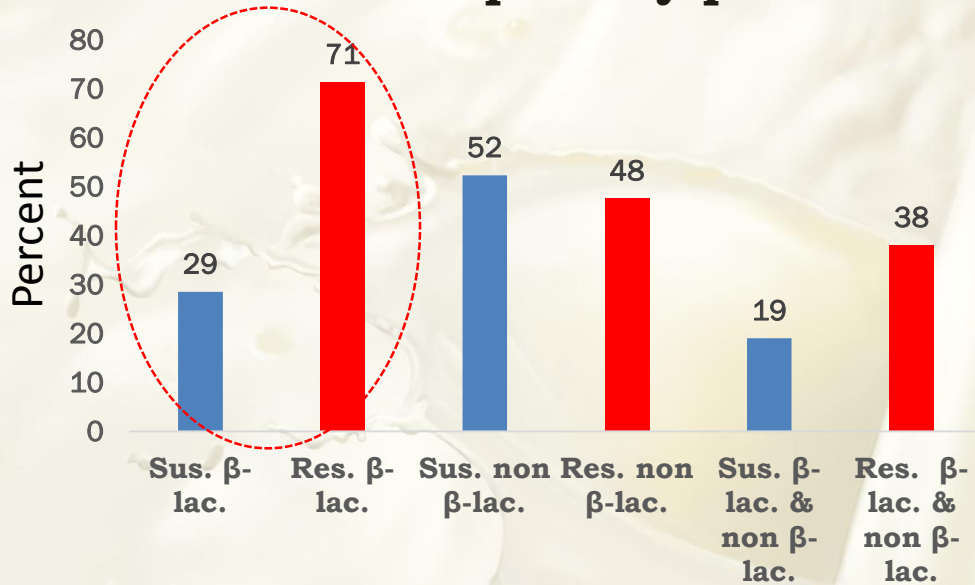
Distribution of mastitis causing organisms



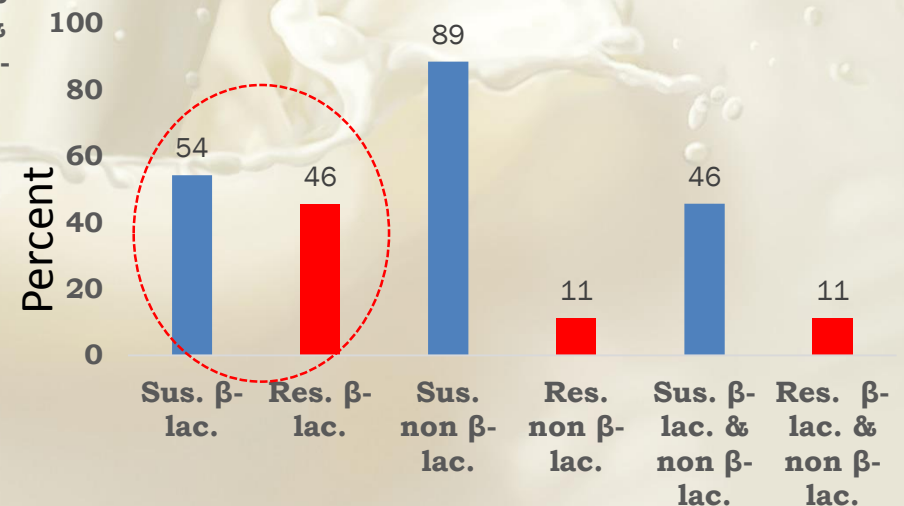


Antibiotic sensitivity of bacterial isolates from mastitis cases

***E. coli* susceptibility percent**



***Staph. aureus* susceptibility percent**





25.0

20.0

15.0

10.0

5.0

0.0

**Clinical
mastitis**

**Sub clinical
mastitis**

Over all

7.7

20

11.9

Distribution of
Methicillin Resistance
(MRSA) *Staph. aureus*

Distribution of
CoPS and CoNP
Staph aureus

70

60

50

40

30

20

10

0

**Clinical
mastitis**

**Sub clinical
mastitis**

Overall

38

62

40

60

39

61

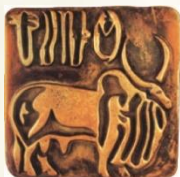
■ CoPS

■ CoNS



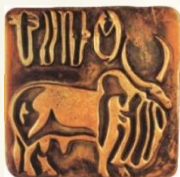
Genotypic characterisation of mastitis causing organism by WGS

Species	Genotypic AMR profile identified (by ResFinder)	Predicted phenotype (Resistance to)	Detected phenotype (Resistant to)
<i>Staph. aureus</i> (ID: MS003G)	Aminoglycoside		
	aph(3')-III	Kanamycin, Neomycin, Amikacin, Gentamicin B, Paromycin	Not Tested
	ant(6)-Ia	Streptomycin	Streptomycin
	aac(6')-aph(2'')	Gentamycin, Tobramycin, Amikacin	Gentamycin
	Beta-lactam		
	mecA	Methicillin	Methicillin, Cefoxitin
	blaZ	Penicillin	Penicillin
	MLS - Macrolide, Lincosamide and Streptogramin B		
	mph(C')	Macrolide	Not Tested
	msr(A)	Streptogramin B	Not Tested
	Tetracycline		
	tet(K)	Tetracycline	Tetracycline
	Trimethoprim		
	dfrG	Trimethoprim-resistant	Not Tested



Genotypic characterisation of mastitis causing organism by WGS

Species	Genotypic AMR profile identified (by ResFinder)	Predicted phenotype (Resistance to)	Detected phenotype (Resistant to)
E.coli (ID: MSG051-B)	Aminoglycoside		
	aph(3'')-Ib	Kanamycin, Neomycin, Paromycin	Not Tested
	aph(6)-Id	Streptomycin	Streptomycin Resistance
	Beta-lactam		
	blaTEM-1B	Ampicillin	Ampicillin Resistant
	Fluoroquinolone		
	QnrS1	Ciprofloxacin, Ofloxacin, Levofloxacin, Gatifloxacin, Moxifloxacin, Norfloxacin	Resistant to Ciprofloxacin & Ofloxacin
	Sulphonamide		
	sul2	Sulfamethoxazole	Not Tested
	Tetracycline		
	tet(A)	Tetracycline	Tetracycline Resistant



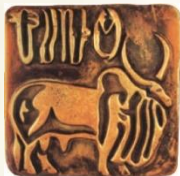
Anti-biogram of the mastitis causing organisms : cases clinically cured by EVM

Mastitis type	Causative organisms	AMR Pattern in the causal organism isolated	
		Description	No
Clinical mastitis (Clinically cured cases =26; Isolates : 29)	<i>E. coli</i> (n=11)	Susceptible to β -lactam and non- β -lactam group of Antibiotics	4
		Resistance to β -lactam group	5
		Resistance to non β -lactam group	4
	<i>Klebsiella Pneumonia</i> (n=2)	Resistance to β -lactam group	2
	<i>Staph. aureus</i> (n=13)	Susceptible to β -lactam and non- β -lactam group of Antibiotics	5
		Resistance to β -lactam group	8
		Resistance to non β -lactam group	3
	<i>Staph epidermidis</i> (n=2)	Resistance to β -lactam group	2
		Resistance to non β -lactam group	1
	<i>S. agalactasia</i> (n=1)	Susceptible to β -lactam and non- β -lactam group of Antibiotics	1
Sub-clinical mastitis (Cases Cured as evident by CMT=8; Isolates : 11)	<i>E. Coli</i> (n=4)	Resistance to β -lactam group	4
		Resistance to non β -lactam group	4
	<i>Staph aureus</i> (n=7)	Susceptible to β -lactam and non- β -lactam group of Antibiotics	3
		Resistance to β -lactam group	4



Conclusion

- Combination of CMT and oral regimen of TSC could drastically reduce the incidence of sub-clinical mastitis
- EVM emerged as effective in control of mastitis irrespective of causative agents viz. *E.coli* , *Klebsiella sp*, *Staph. aureus*, *Staph. epidermidis*, *S. agalactiae* and antibiotic susceptibility pattern of the organisms
- EVM as an alternative, cost effective mastitis management approach is found to be highly successful and acceptable by the farmers
- These alternative approaches of mastitis control significantly diminish the use of antibiotics in mastitis treatment
- Thus it minimizes the likelihood of antibiotic residues in raw milk as well as probabilities of development of anti microbial resistance



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