

TRANS-DISCIPLINARY VALIDATION OF ETHNO-VETERINARY FORMULATION FOR MASTITIS

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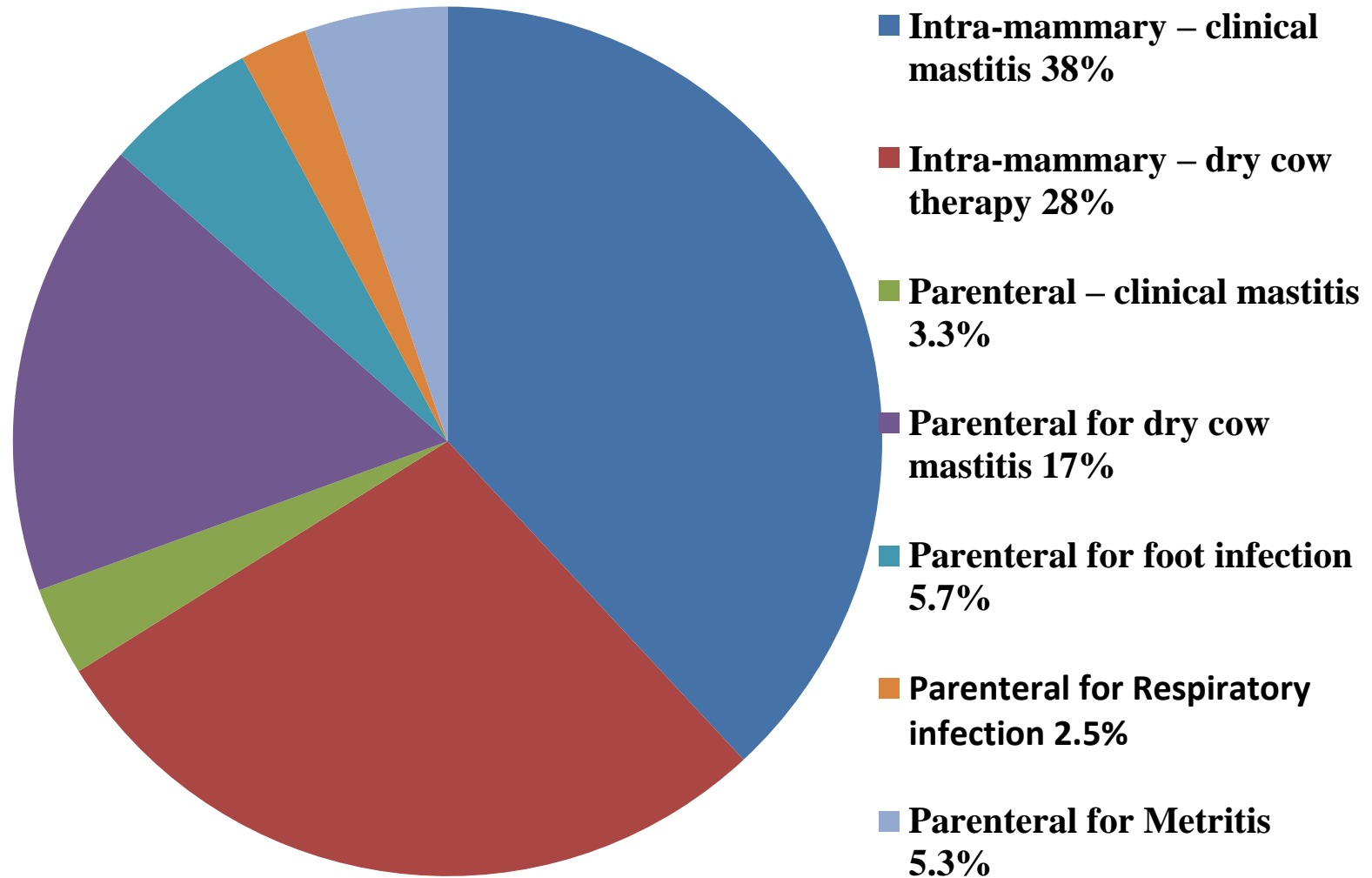


Mastitis

- Mastitis is an inflammation of the mammary gland generally associated with intra-mammary infection (IMI).
- Average drop in income due to mastitis is from Rs306- 413 per cow per day (Sharjeel Ashraf Wani, et al. 2022)
- 70% of all avoidable losses during milk production
- Multi-etiological disease - 95% - *Streptococcus*, *Staphylococcus* and *E. coli*.
- Other microbes such as fungal species (*Aspergillus fumigatus*; *A. nidulans*; *Candida spp*; *Trichosporon spp*, etc.), certain microscopic algae (*Prototheca spp.*), viruses and Physical injury can cause mastitis
- 20-35% of clinical cases of bovine mastitis have unknown etiology

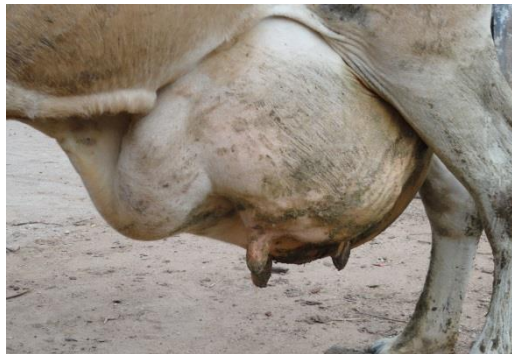
(Contreras, G.A., Rodríguez, J.M. Mastitis: Comparative Etiology and Epidemiology. *J Mammary Gland Biol Neoplasia* 16, 339–356 (2011).

Proportion of defined daily doses of antimicrobial per cow per year administered on conventional dairy farms in Wisconsin (n = 20) for treatment of selected diseases by route and indication. (Pol and Ruegg 2007a).

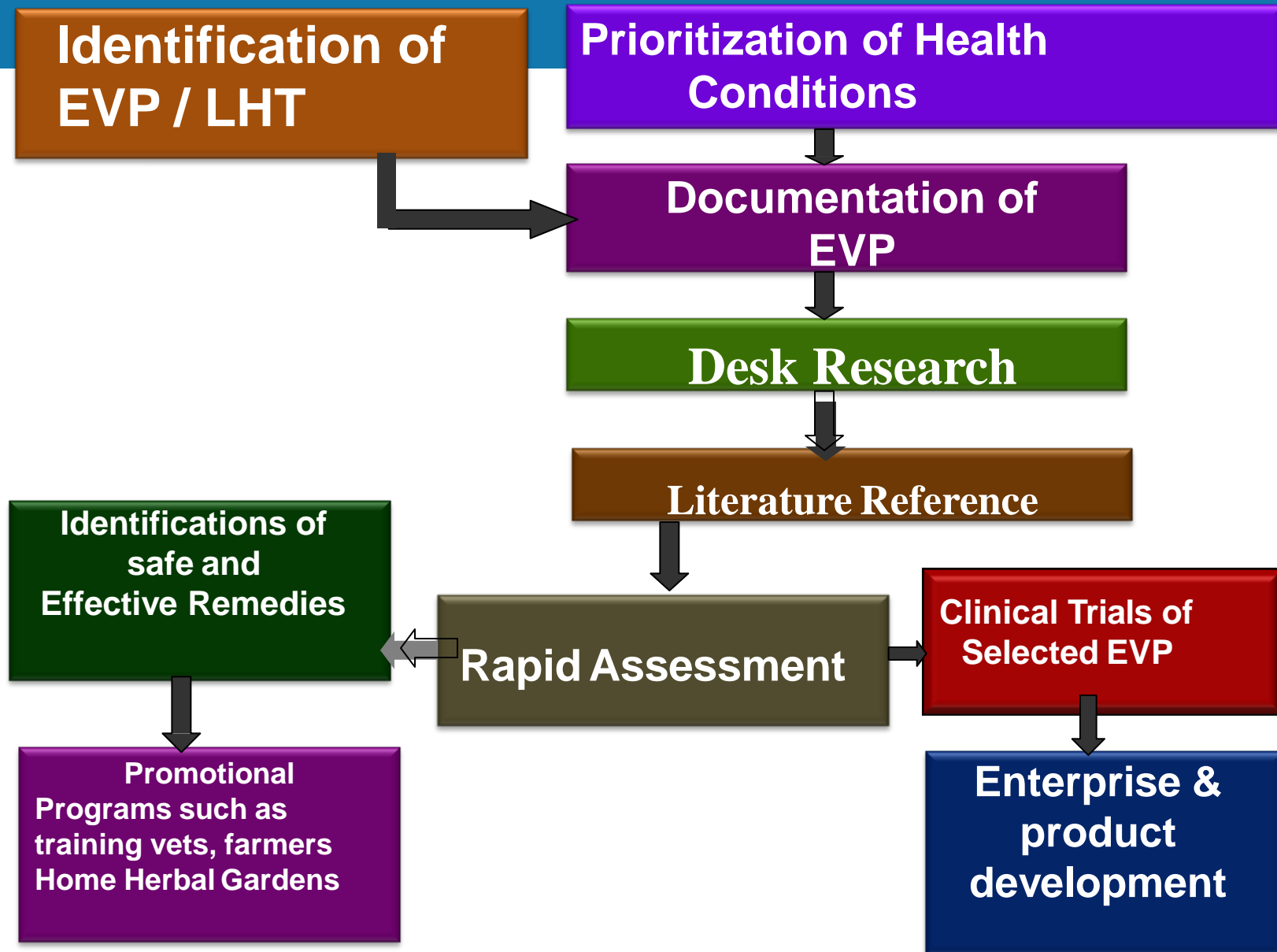


Alternative Approach

- **Cost effective, efficacious and safe Ethno-veterinary Practices (herbal formulations) are available in India**
- **They are used in preventing and curing Mastitis**
- **Reduce the use of antibiotic thereby reduces the antibiotic residues in the milk**



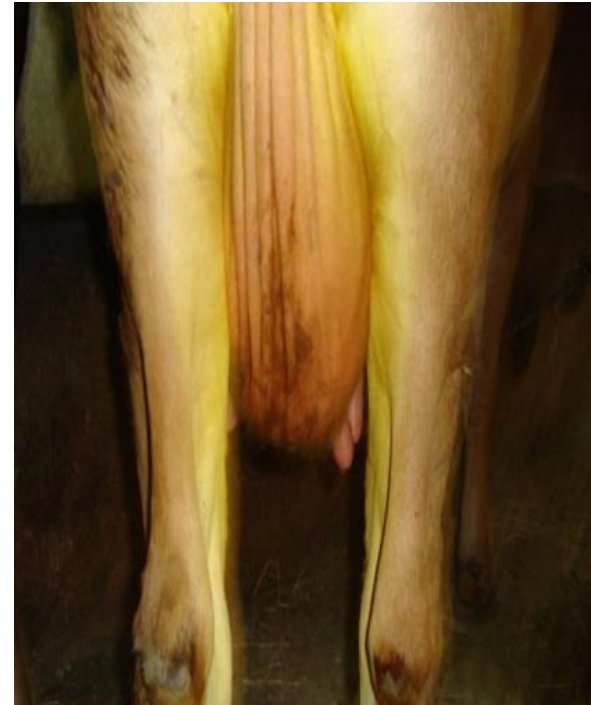
Steps in implementing strategy



Example of EVP remedy for Mastitis







Note: In the case of chronic mastitis add *Cissus quadrangularis* in the formulation and the treatment should be continued till the hardness of the udder is completely disappear

Assessments - Ayurveda

- ❖ This formulation consists of *Curcuma longa*, *Aloe vera* and Calcium hydroxide
- Have properties of Vrana shodaka (Wound cleanser), Vrana ropaka (Wound healing) and very good shotha hara⁴ (anti-inflammatory), Puti rodhaka (anti-infective) and Krimihara (anti-microbial)
- ✓ This remedy is very much beneficial in Mastitis.

***In-Vitro* Antimicrobial Activity of Ethno-veterinary Herbal Preparation for Mastitis**

The antimicrobial activity of aqueous, ethanol and ethyl acetate extracts obtained from *Aloe vera* and *curcuma longa* assayed in-vitro using agar well diffusion method exhibited antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aurogenosa*.

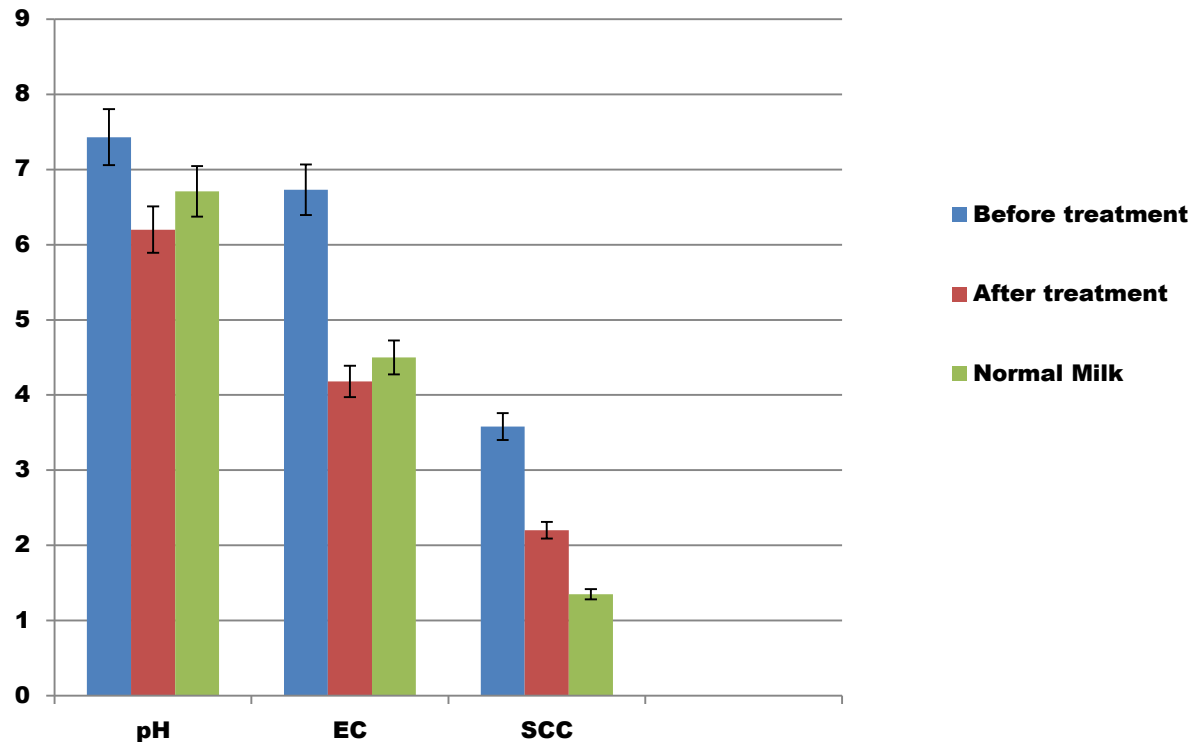
Dairy and Vet Sci J

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reduction of pH, Electrical Conductivity (EC) and Somatic Cell Count (SCC) before and after treatment with herbal formula in comparison with normal values.

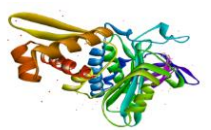
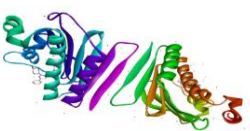
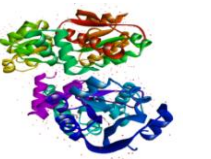





Nair M N B, et al. 2017. Ethno-veterinary Formulation for Treatment of Bovine Mastitis, *RRJVS/ Journal of Veterinary Sciences* S1. 25-29.

Reverse pharmacology

The bioactive compounds were tested for its effect against the target proteins of *Staphylococcus aureus* using molecular docking studies.

Punniamurthy et al.
2017.IJANS Vol. 6, Issue 5, Aug
– Sep 2017; 23-30

Target	PDB ID	Structure of target	Total binding sites
BPL	3V7S		7
DNA gyrase	3G7B		5
opuCB	3O66		14
sirA	3MWF		7
SrtA	1T2W		14
PBP	3VSL		44

- Many bioactive components of *Aloe vera* and turmeric interact with target protein
- The pharmacodynamics study using online server PASS reveal that the compound in the preparation posses **anti-inflammatory and anti-microbial properties**
- Component of lime had lesser interaction with lesser affinity
(Punniamurthy et al. 2017)

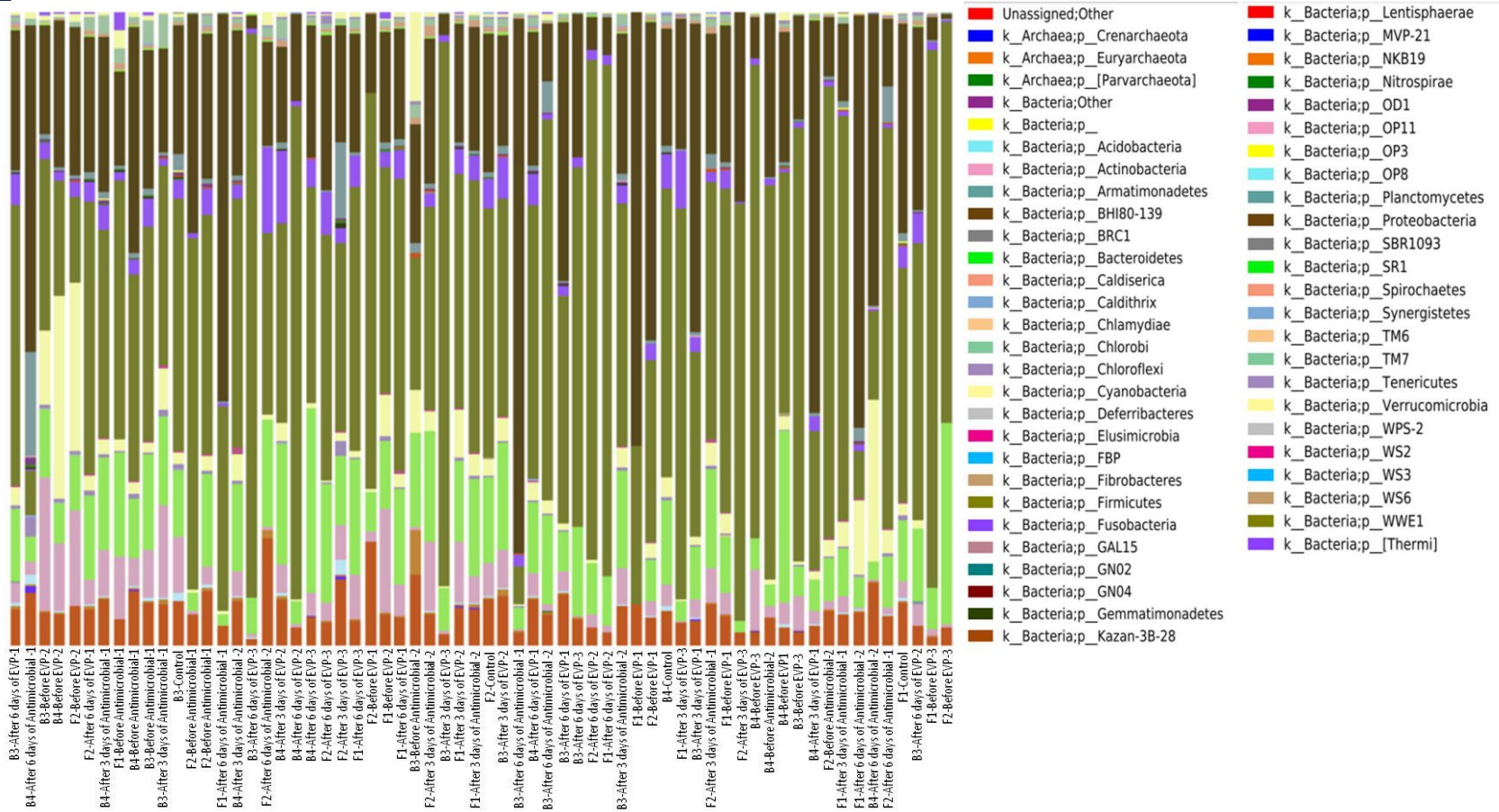
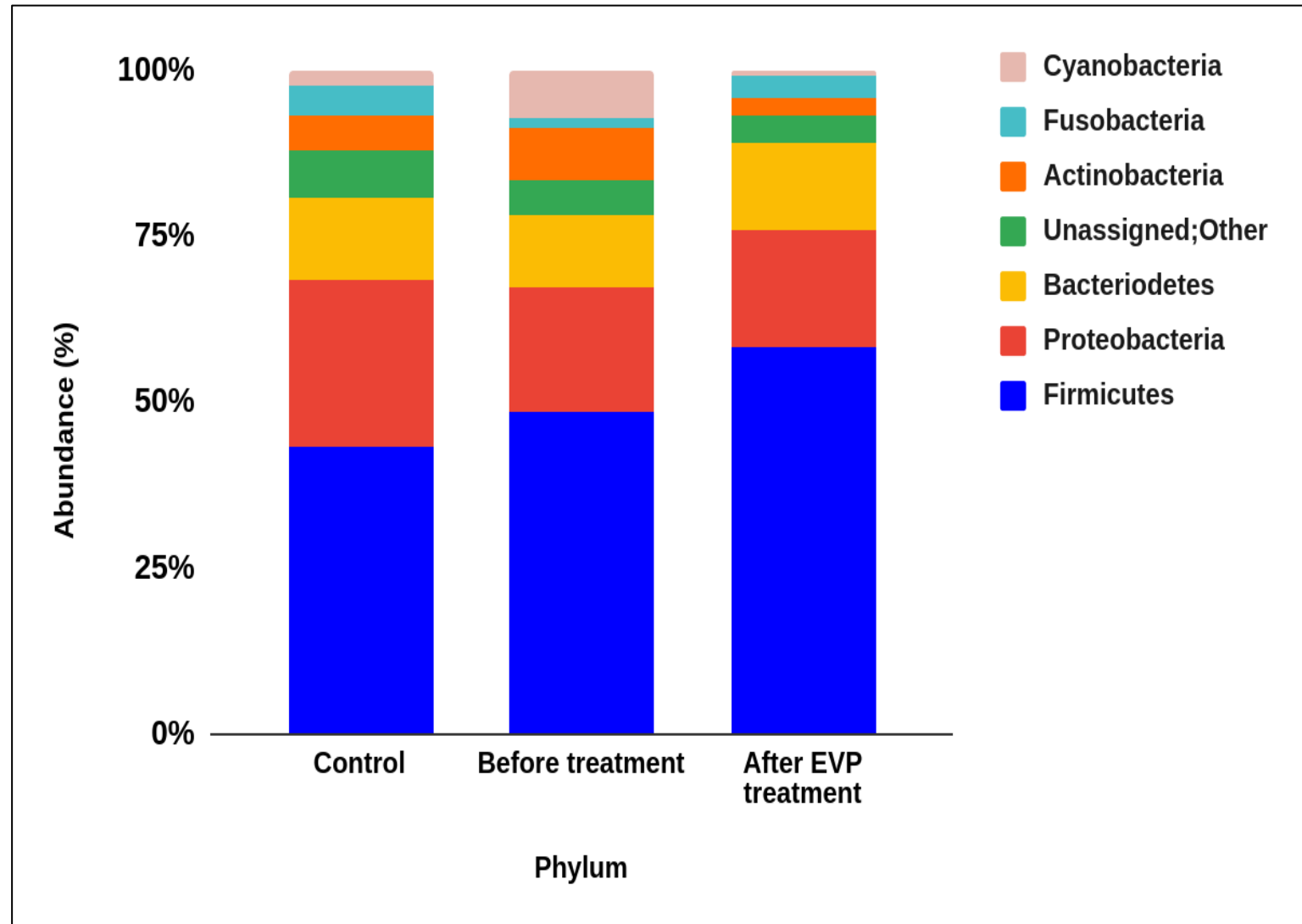
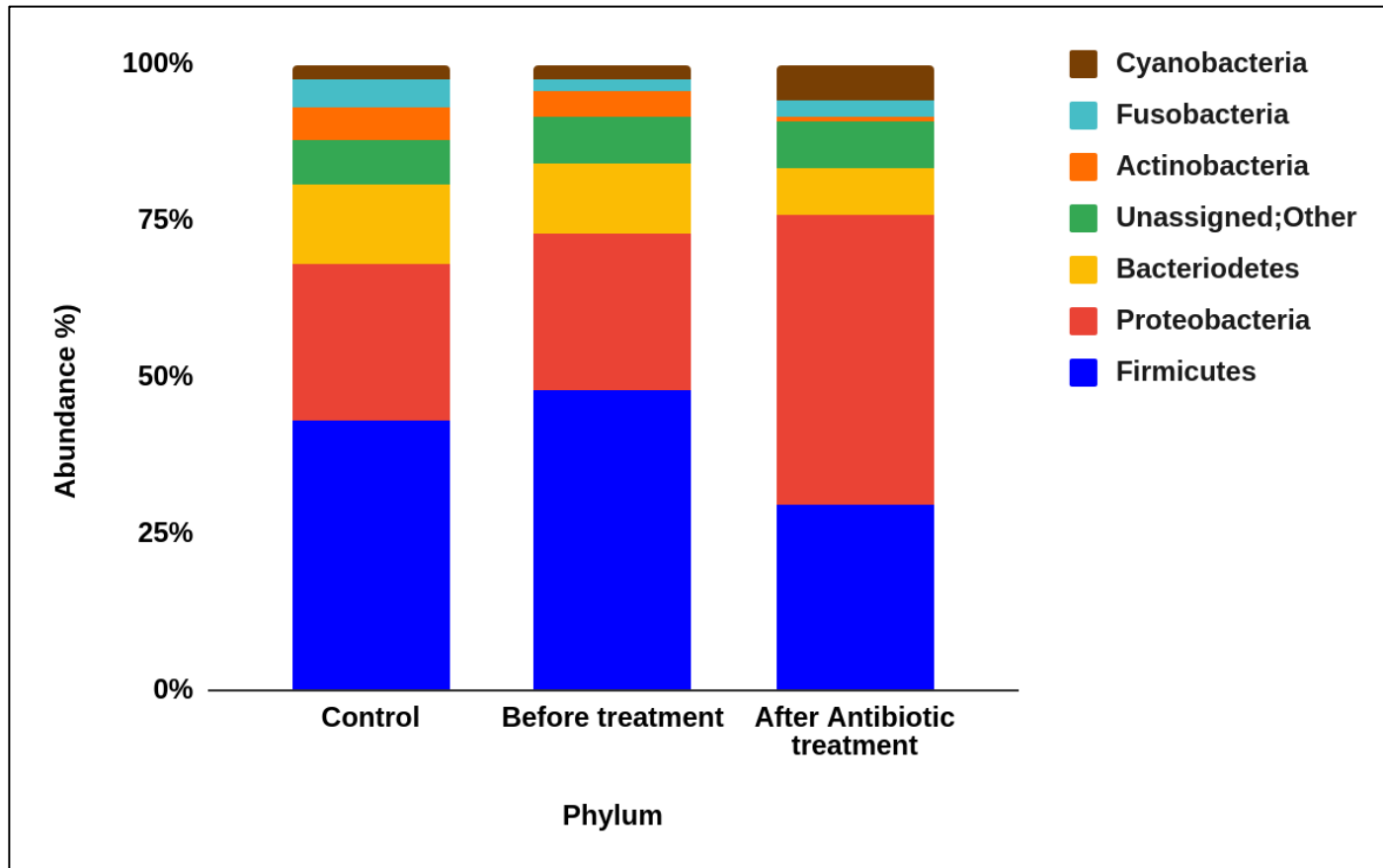


Figure: Bar plot of phylum level bacterial abundance of all 64 milk samples

Taxonomic profile at Phylum level bacterial abundance in milk samples of control compared with mastitis affected cows before and after treatment of Ethno-veterinary formulation (EVP)

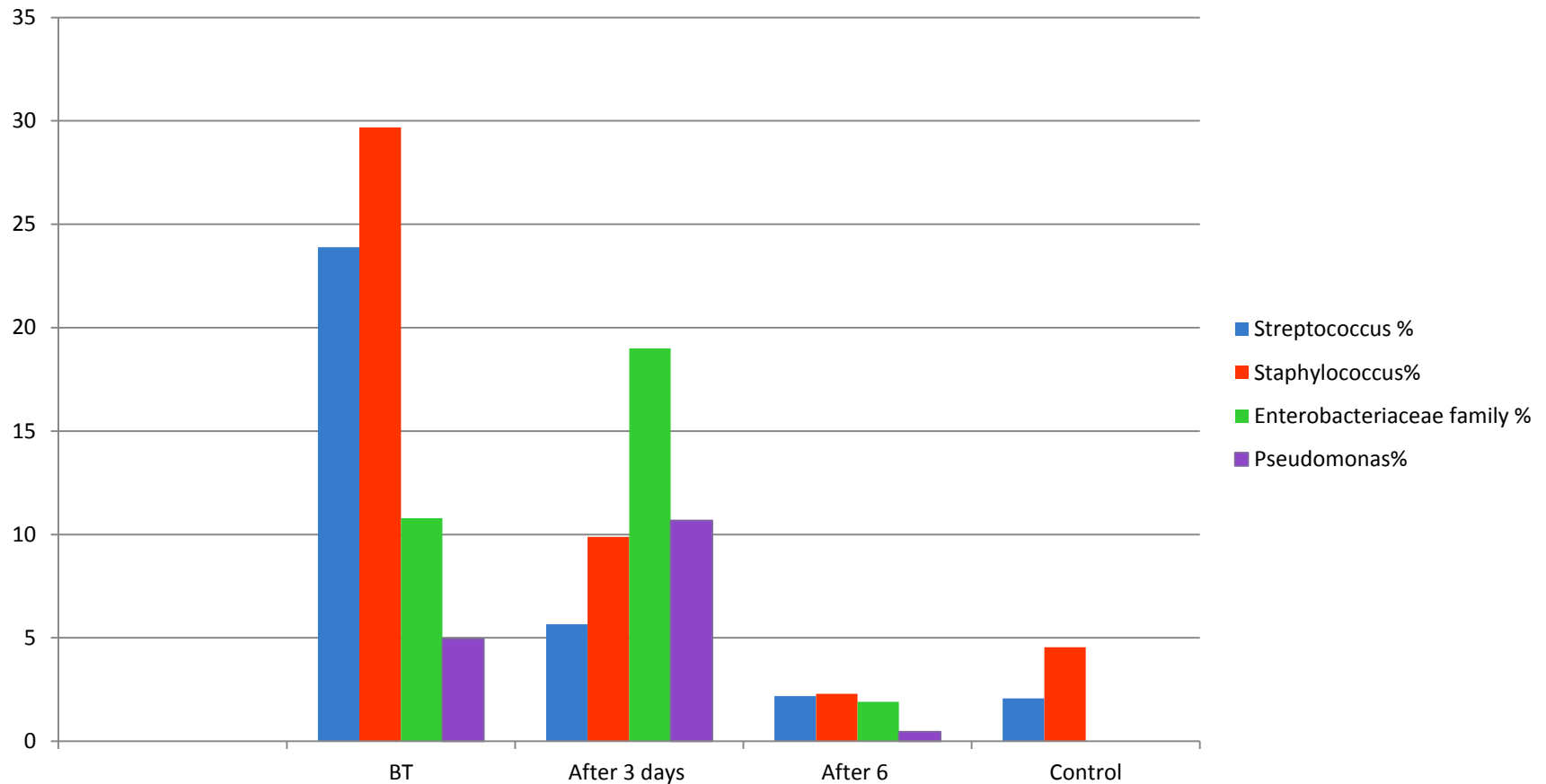


Taxonomic profile at Phylum level bacterial abundance in milk samples of control compared with mastitis affected cows before and after treatment of antimicrobials



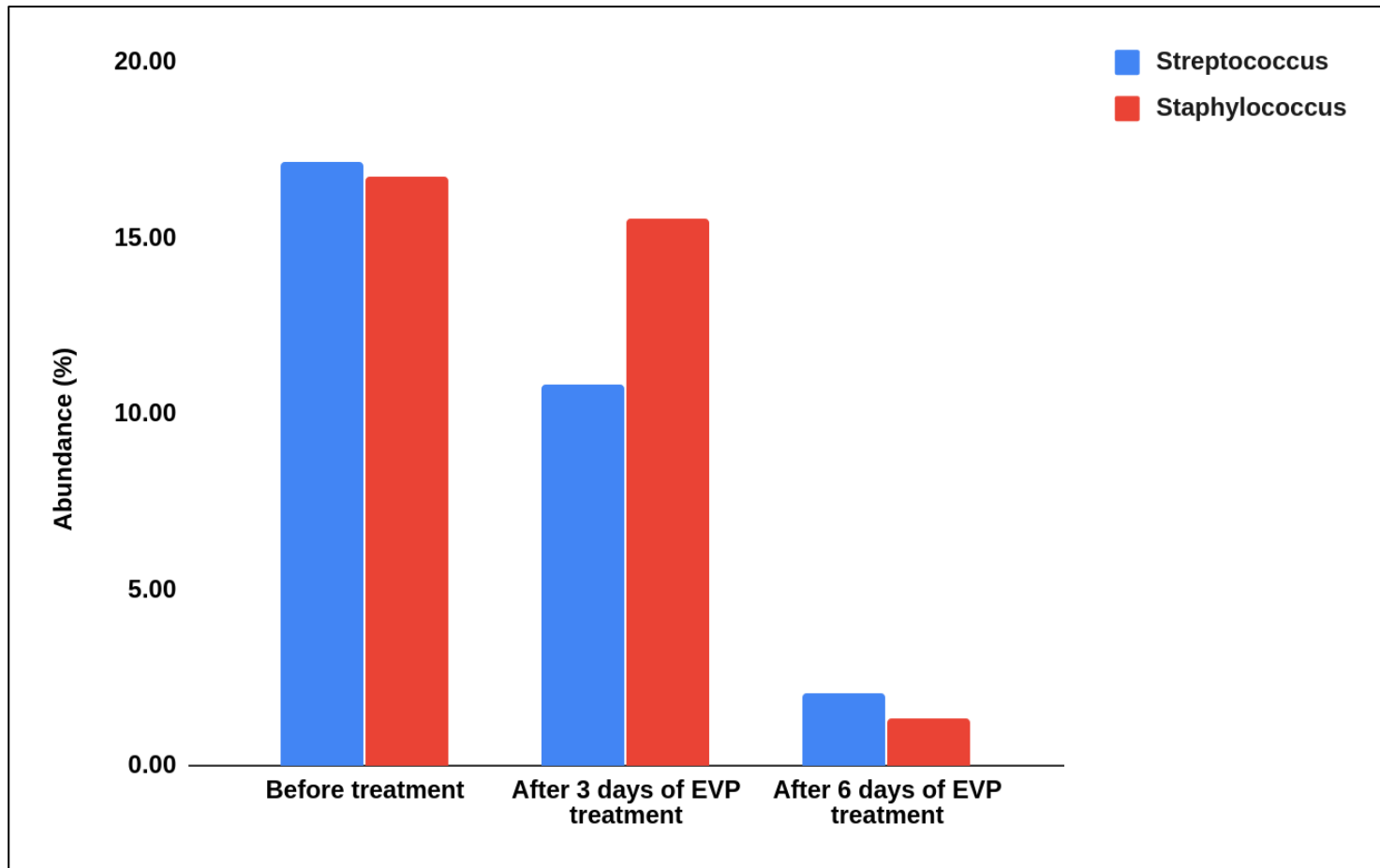
Treated with Doecf + citriaxone +Sulbactum 3 gr + Melpol (Meloxium paracetamol) 15 ml + Avil 10 ML (IM). Treated for 5 days

Abundance of *Streptococcus*, *Staphylococcus*, *Enterobacteriaceae* family and *Pseudomonas* in the milk samples of control compared with Mastitis affected cows before after EVP treatment.

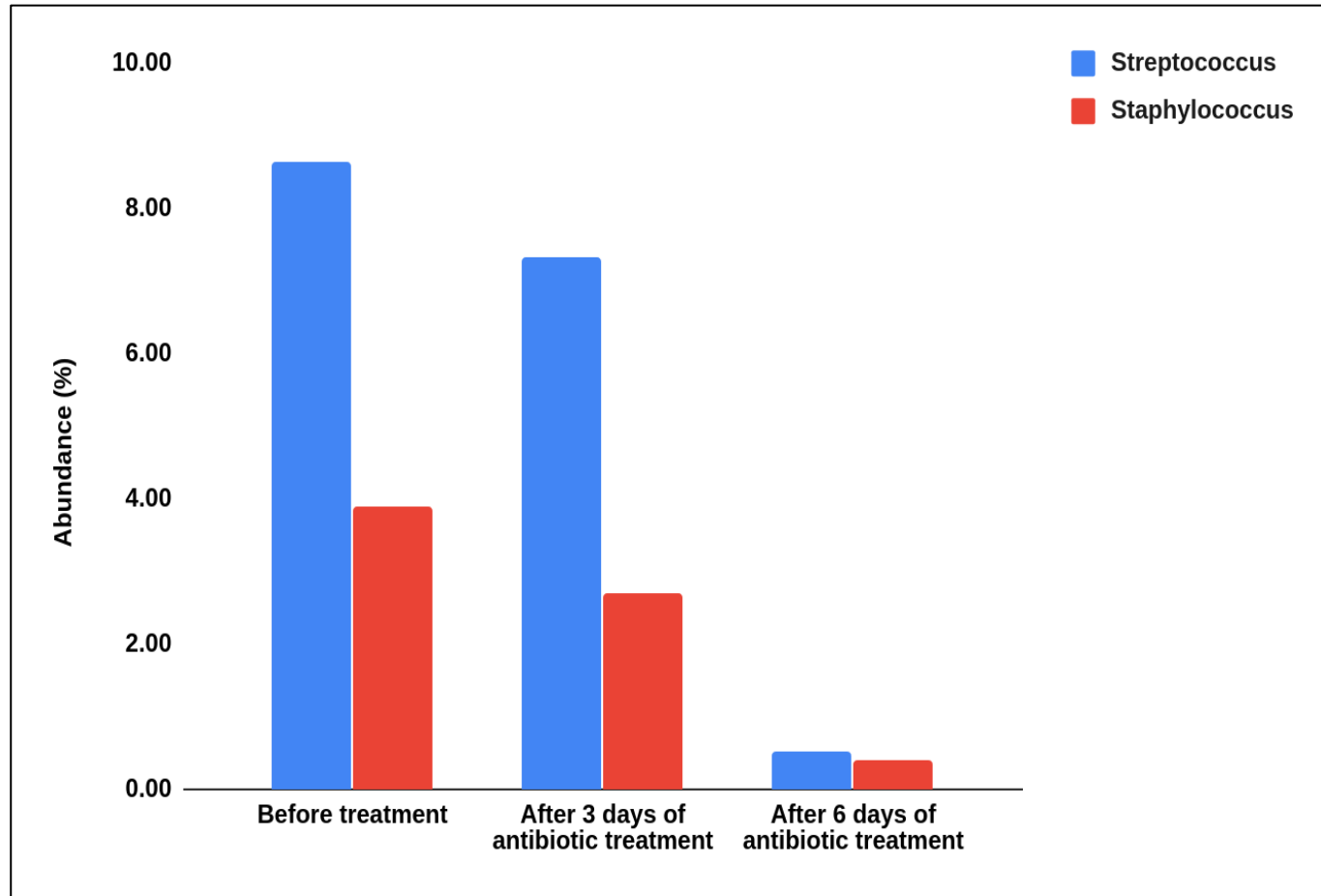


BT-Before treatment, Alter 3 days treatment , after 6 days treatment and control

Abundance of *Streptococcus* and *Staphylococcus* in the milk samples of Mastitis affected cows before and after treatment with EVP



Abundance of *Streptococcus* and *Staphylococcus* in the milk samples of Mastitis affected cows before and after treatment with antibiotics



Treated with Doecf + citriaxone + Sulbactum 3 gr + Melpol (Meloxium paracetamol) 15 ml + Avil 10 ML (IM). Treated for 5 days

PCoA indicates Taxonomic profile at phylum level of microbe in the milk of EVP treated cows is almost similar to that of the control after 6 days. However, in the milk from the antibiotic treated cows varied substantially from the milk of the control animal (EVP- **Red - before treatment, Blue - after 3 days, orange - after 6 days, green - control/** Antibiotic treatment – **Red – control, Blue - before treatment, Orange - after 3 days, Green after 6 days**

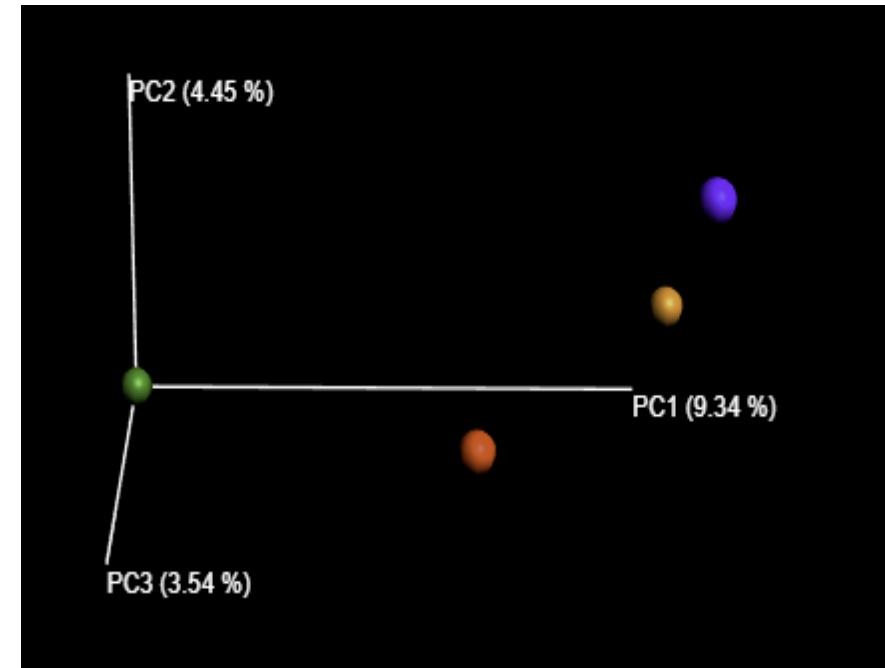
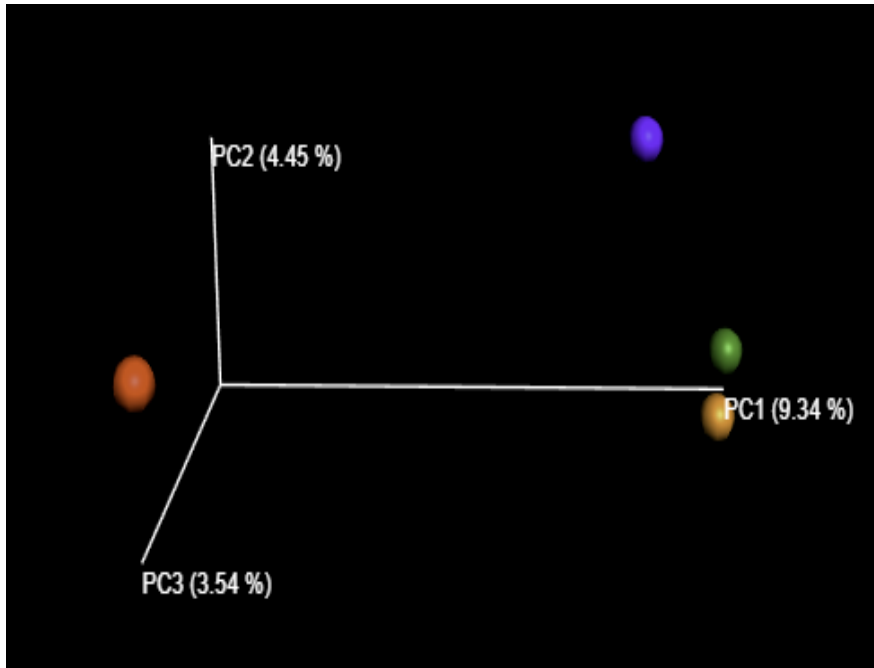


Figure 5: A) Principal coordinates analysis (PCoA) derived from one of the EVP treated sample at different stages. before EVP, after 3 days of EVP, after 6 days of EVP and control samples of F2 udder. Colored dots are representative of the 4 samples. Red – F1 before EVP; Blue – after 3 days of EVP; Orange – after 6 days of EVP; Green – control. b) Principal coordinates analysis (PCoA) derived from one of the antimicrobial treated sample at different stages; before antimicrobial treatment, after 3 days of antimicrobial treatment, after 6 days of antimicrobial treatment and control) samples. Colored dots are representative of the 4 samples. Red – Control; Blue – F1 before Antimicrobial treatment; Orange – after 3 days of antimicrobial treatment; Green – after 6 days of antimicrobial treatment

Feedback from various milk societies from NDDB through INAPH *, Abbott and TDU on the Efficacy of EVP for Mastitis in cattle from 2017-18 to 2021-22

S. No	Mastitis	Total treated cases	Total clinical recovery	% clinical recovery
1	Acute Mastitis	104475	82878	79.3
2	Chronic mastitis	52791	41502	78.6
3	Sub-clinical Mastitis	23986	19780	82.5
4	Mastitis (Abbott)	1692	1563	92.38
5	Mastitis (TDU)	1561	1432	91.7
	Total	184, 505	147, 155	84.9

The recovery is 84.9% which is a reasonably high outcome by any standard from the clinical point of view.

Reduction of incidence of Mastitis from the year 2016 to 2019

Disease	Mastitis		
	2016	2018	2019
Year			
Average incidence per unions	65.63	36.5	10.6
Per cent reduction		44.4	83.8

Cost in Rs. for the treatment of Mastitis in cattle using veterinary drugs and herbal formulations

Disease condition	Number of animal treated	Average expenditure for vet service.	Average expenditure for EVP is used Rs	Amount saved in Rs
Mastitis	35	Rs.3000	Rs.120	2880

Cost impact (Production loss) when Western medicine and herbal formulations is used

S. No	Mastitis	N	Loss of milk/ day L	Loss for 6 days L	Financial loss
1	Allopathic treatment	35	2.5	15	390
2	EVP teatment	35	0.4	2.4	62.4
	(Rs 26 per liter)				

Mastitis Spray development

R and D	Remark
Lab study done	Herbal formulation against mastitis had inhibitory activity against <i>E. coli</i> and <i>S. aureus</i> .
Testing & Validation, Field study	Field study Phase 1 - 19 cases treated (only clinical mastitis) 90% cure Phase 2 - 20 cases treated (only clinical mastitis) 89% cure Phase 3 - target is 50 cases, this phase is on going
Efficacy study : Microbiome study before and after treatment	After 6 days of treatment with herbal formulations, the average abundance of <i>Staphylococcus</i> was reduced from 40.59% to 2.03% (20 times), <i>Streptococcus</i> from 25.8% to 2.06 (12.52 times), <i>Pseudomonas</i> , 20.28% to 1.9% (10.67 times), <i>Klebsiella</i> from 8.4% to 0.26% (32.31 times) and <i>Enterobacteriaceae</i> family from 24% to 1.69 % (14.37 times) indicating cure of mastitis
Toxicological study	Done -Non toxic
Priority patent application	Patent application submitted
shelf- life study	Good stability, 1 .5 year
Phytochemical study	Will have to do
Clinical trial	Will have to do
Scale up manufacture and sale	January 2023





Thank You