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Nair MNB
Center for Ethno-Veterinary Science and Practice, The University of Trans-Disciplinary Health Sciences and Technology (TDU), Jarakabande Kaval, Attur Post, Yelahanka, Bangalore, Karnataka, India

Punniamurthy N
Center for Ethno-Veterinary Science and Practice, The University of Trans-Disciplinary Health Sciences and Technology (TDU), Jarakabande Kaval, Attur Post, Yelahanka, Bangalore, Karnataka, India

Kumar SK
Center for Ethno-Veterinary Science and Practice, The University of Trans-Disciplinary Health Sciences and Technology (TDU), Jarakabande Kaval, Attur Post, Yelahanka, Bangalore, Karnataka, India

Corresponding Author
Nair MNB
Center for Ethno-Veterinary Science and Practice, The University of Trans-Disciplinary Health Sciences and Technology (TDU), Jarakabande Kaval, Attur Post, Yelahanka, Bangalore, Karnataka, India

Reduction of antibiotic residue in milk through the use of cost effective ethno-veterinary practices (EVP) for cattle health

Nair MNB, Punniamurthy N and Kumar SK

Abstract
Antibiotics are extensively used globally to improve animal health, welfare and also to enhance animal productivity. Even though antibiotics play a critical role in livestock production, antibiotic resistant bacteria are a major public health concern. Farm animals are exposed to considerable quantities of antibiotics and act as an important reservoir of antibiotic resistant genes. The use of antibiotics for common cattle diseases, such as mastitis and diarrhoea in calves, is widespread in both in smallholder and large-scale dairy farming. The use of Ethno-veterinary practices (EVP) as alternatives to antibiotics shows significant reduction in the antibiotic residues in the milk from cattle and increased financial saving for the farmers. Adopting the ethno-veterinary science and practices to combat infectious diseases in livestock has been identified and tested as a key game changer in rationalising the use of antibiotics in veterinary health care and reducing antibiotic residue(s) in milk. A progressive reduction in the incidence of mastitis, enteritis, repeat breeding and cowpox were observed from 2016 to 2019 among the cows treated with EVP.

Keywords: Antibiotics residue, antimicrobial resistance, ethno-veterinary practices, herbal formulations, cattle health, dairy farmers, cost effective health care model

1. Introduction
There are many reports of antibiotic residues in milk (Asif *et al*., 2020, Pallavi *et al*., 2019, Kumaraswamy *et al*., 2018, Vishnuraj *et al*., 2016, Gaurav, *et al*., 2014, Dinki and Balcha, 2013) [1-6]. It also causes side effects and allergic reactions on people who consume animal products such as milk and meat (Jeena *et al*., 2020, Priyanka *et al*., 2017, Prajwal *et al*., 2017, Nisha, 2008) [7-10]. It is also predicted that the global consumption of antimicrobials will go up to 67% from 2010 to 2030 (Florence *et al*. 2020, Eili *et al*. 2018, Thomas *et al*. 2015, O'Neill 2016, Shallcross *et al*. 2014) [11-15]. It is predicted that drug-resistant infections will kill an extra 10 million people a year worldwide by 2050 and the total cost of antimicrobial resistance on world Gross Domestic Product (GDP) will be $ 100 trillion (Thomas *et al*.2015, O'Neill, 2016) [13, 14]. One of the immediate challenges to reduce AMR is to limit the use of antibiotics in human and animal health management. As the antibiotic residues finding their way into the food chain, there is an urgent need to focus on reducing the use of antibiotics in veterinary practice. Besides the implementation of regulatory policy on controlling the misuse of antibiotics in dairy sector in India, on farm extension options to use ethno-veterinary practices based on natural plant products is an alternative to control microbial diseases. A high priority need in the livestock sector is therefore to find safe and cost-effective medicinal plant formulations to replace antibiotics. There are reports on the effective use of ethno-veterinary practices from several parts of India (Nair and Unnikrishnan, 2010, Nair 2019, Nair *et al*. 2017a, b, 2021, Nair and Punniamurthy 202, Rana *et al* 2021, Ngwisha *et al*., 2021, Dutta *et al*., 2020, 2022a, b [16- 26].
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* (Punniamurthy *et al.*, 2017a) [27]. Clinical study using traditional formulation for mastitis showed cure of mastitis within 6 days (Nair *et al*., 2017b) [19]. *In silico* approach were used to find the effect of the herbal preparation against the infection. The bioactive compounds were tested for its effect against the target proteins of *S. aureus* using molecular docking studies. Many bioactive components of *Aloe vera* and turmeric interact with target protein.

The pharmacodynamics studies using online server PASS reveal that the compounds in the preparation possess anti-inflammatory and anti-microbial properties (Punniamurthy *et al*., 2017b) [28]. It is anticipated that ethno-veterinary herbal preparations may have major potential as a low cost and efficacious alternative to antibiotics for bovine disease management not only in developing countries like India but also in western countries (Dutta *et al.*, 2020) [24]. In fact, medicinal plant formulations have traditionally been used for centuries for a wide range of veterinary conditions (Satheshkumar and Punniamurthy, 2009, 2010, Satheshkumar *et al*, 2021, Nair *et al*., 2017b, Nair and Punniamurthy, 2021) [29 - 31, 19, 21]. For example, the prevalence of mastitis continues to remain as the most challenging disease and its incidence is increasing with higher production of milk. In the last three decades the prevalence increased by more than 60%. The economic loss due to mastitis alone was Rs. 71655.1 million Indian Rupees (Bansal and Gupta, 2009, Anonymous, 2012) [32, 33]. Ethno-veterinary medicine is suggested as a solution for this condition (Nair, 2019, Nair *et al.*, 2017b [17, 19]. A study on the changes in the microbiome of milk from the cows with clinical mastitis (The mastitis was confirmed with California Mastitis Test) before treatment and after 6 days of treatment with ethno-veterinary herbal formulations indicates that the average abundance of *Staphylococcus* was reduced from 40.59% to 2.03% (20 times), *Streptococcus* from 25.8% to 2.06 (12.52 times) [Figure1], *Pseudomonas*, Pseudomonadaceae family 20.28% to 1.9% (10.67 times), *Klebsiella* from 8.4% to 0.26% (32.31 times) and *Enterobacteriaceae family* from 24% to 1.69% (14.37 times) indicating the cure of mastitis (Hegde *et al.*, 2021) [34]. Ethno-veterinary medicine has also been suggested as alternative to antibiotics (Vishnuraj *et al*., 2016, Prajwal *et al*., 2017, Florence *et al*.2020, Nair, 2019, Nair *et al*, 2021) [4,11, 17, 21].

This study reports change in knowledge, attitude and practice of the ethno-veterinary practices (EVP) among farmers from 10 locations, reduction of the antibiotic residue(s) in the milk after using cost effective ethno-veterinary herbal formulations for prevention and cure of mastitis, foot and mouth disease (FMD), diarrhoea, udder pox, repeat breeding, bloat, indigestion and maggot wounds in cattle.

2. Methods

2.1. Scope of study and Research question

The major focus of this study is on the reduction of antibiotic residue in the milk. In order to achieve this, cost effective herbal alternatives to antibiotics were used to manage cattle health.

Two hundred twenty small farmers were selected from Kerala state, Karnataka and Tamil Nadu (Table 1). One forty farmers were included in the intervention study group and 80 in the control group.

Knowledge, attitude and practice (KAP) survey of Ethno-veterinary practices (EVP) among the selected farmers, were undertaken. The baseline and end line surveys were conducted using a format with 1 to 10 scales and personal interview. Baseline and end line survey of the presence of antibiotic residue were also tested from the milk samples from the 220 selected farmers. To ascertain the presence of antibiotic in the market milk samples thirty five branded market samples in triplicate were collected and tested for the presence of antibiotic residue(s). The antibiotic residue were tested using *Trisensor* (Unisensor, Product code KIT035 Belgium).

Table 1: Show the small scale producer farmers selected as interventions and controls group

No	Location	Farmers selected	Intervention group	Control Group
1	Maneed, Ernakulum Kerala	20	10	10
2	Monippally, Kottayam, Kerala	20	10	10
3	Chakkampuzha, Kottayam Kerala	20	10	10
4	Puthrika Ernakulum Kerala	20	10	10
5	Allappara, Ernakulum Kerala	15	15	0
6	Arakkappadi, Ernakulum Kerala	15	15	0
7	Manikyamangalam, Ernakulum Kerala.	15	15	0
8	Sreemoolanagaram, Ernakulum, Kerala	15	15	0
9	Thirukanurpatti, Thanjavur Tamil Nadu	40	20	20
10	Doddaballapura, Karnataka	40	20	20
	Total	220	140	80

The 140 farmers (intervention group) who consented to use only EVP and not antibiotics, were trained to use validated EVP for Mastitis (table 2), Foot and mouth disease (table 3, 4), Diarrhoea (Table 5), Udder Pox (table 6), repeat breading (table 7) bloat, indigestion (table 8) and Maggot wounds (table 4) repeatedly for one year. Hand books were printed in local languages (Kannada, Malayalam and Tamil) and distributed to the selected farmers for reference. The average cost of treatment, (conventional medicine and EVP) were recorded using a format.

Table 2: Protocol for treatment of mastitis

Sl. No.	Botanical name	Local name	Parts used
1	*Aloe vera*	Aloe	Leaves
2	*Curcuma longa*	Turmeric	Rhizome
3		Slaked lime	Powder
4	*Cissus quadrangularis*	Veldt Grape Winged treebine	stem

Method of preparation and application

Fig 1: Method of preparation and application

Aloe vera leaves are cut into small pieces, add turmeric and calcium hydroxide into it. Grind the ingredients in to a fine paste. Divide this paste into 10 parts for applying 10 times. Wash the udder well with water. Remove the milk completely from the udder. Take one part of the paste and add 200 ml water to dilute it and apply it all over the udder as shown in the figure. After one hour wash the udder with water, remove the milk completely and take one portion of the paste and dilute it and apply as described earlier. Repeat this application for 10 times a day. Continue to apply for 5 days. Cut 2 lemons into two half and feed the cows thrice daily for 5 days. Subclinical, acute and chronic mastitis can be cured using this formulation. Add two pieces of *Cissus quadrangularis* with the above formulation in the case of chronic mastitis and apply at least for three week. Prepare fresh formulation every time.

Table 3: Protocol for treatment of Foot and Mouth Disease (FMD)

Sl. No.	Botanical name	Local name	Parts used
1	*Allium sativum*	Garlic	Bulbs
2	*Cocos nucifera*	Coconut	Fruit
3	*Cuminum cyminum*	Cumin	Seed
4	*Curcuma longa*	Turmeric	Rhizome
5	*Pepper nigrum*	Black pepper	Seed
6	*Trigonella foenum-graecum*	Fenugreek	Seed
7		Jaggery*	

Method of preparation and application

Soak cumin, fenugreek and black pepper in water for half an hour. Grind them well along with turmeric and garlic. Add 120 grams of jaggery and mix them well along with one grated coconut. This is one dose for large animals and 4 doses for goats. Slowly feed the animal little by little the whole preparation. Repeat this three times a day. Prepare fresh formulation every time. This treatment can be continued till the animal is recovered completely

*Jaggery is a concentrated product of cane juice and sometimes of date or palm sap without separation of the molasses and crystals. It is a traditional non-centrifugal cane sugar consumed in the Indian Subcontinent and Southeast Asia

Table 4: Protocol for treatment of foot lesion in FMD

Sl. No	Botanical name	Local names	Parts used
1	*Acalypha indica*	Indian Acalypha	Leaves
2	*Ocimum sanctum*	Sacred basil	Leaves
3	*Lawsonia inermis*	Henna,	Leaves
4	*Curcuma longa*	Turmeric	Rhizome
5	*Azadirachta indica*	Neem	Leaves
6	*Allium sativum*	Garlic	Bulbs
7	*Cocos nucifera*	Coconut	Oil

Methods preparation and application

Grind the ingredients and added it to the oil. Warm the oil and cool it. Clean the hoof with a dry cloth, sprinkle turmeric powder on the wound and apply the oil on the hoof, legs and wherever there is ulceration as many times as possible. Repeat this tills the ulcers are healed. Oil mixed with camphor is applied only for one day to kills the maggots if present in the wound and then the above oil is used for healing the ulcers. This oil also can be used for wounds.

Note: If there is swelling on the leg sesame oil must be used instead of coconut oil

Time of administration: Any time, Quantity: Sufficent, Number of times: As many times as possible, Duration of treatment: Till the condition is cured, Specification: The wound can be dressed with clean cloth if needed. Mode of application: External

Table 5: Protocol for treatment of diarrhoea

No.	Name of the ingredients	Local name	Parts used
1	*Allium cepa* var. *aggregatum*	Shallot	Bulbs
2	*Allium sativum*	Garlic	Bulbs
3	*Cuminum cyminum*	Cumin	Seeds
4	*Curcuma longa*	Turmeric	Rhizome
5	*Ferula assa-foetida.*	Asafoetida	Resin
6	*Papaver somniferum*	Poppy seeds	Seeds
7	*Piper nigrum*	Black pepper	Fruit

8	*Murraya koenigii*	Curry leaves	Leaves
9	*Trigonella foenum-graecum*	Fenugreek	Seeds
10		Jaggery	

Method of preparation and administration

Cumin, asafoetida, poppy seeds, black pepper and fenugreek are fried till they become dark like charcoal. Remove it from the fire, cool and powder them. Grind shallot, Garlic and curry leaves into a paste. Add the prepared powder and Jaggery. Mix them well and make into small balls. Touch the ball on salt (crystal not the free flowing powder) and rub it on the tongue of the cattle or calf. Prepare fresh formulation every time.

Time of administration: No specification.

Quantity: Up to 200 gm., Number of times: 3 times, Duration: 3 days, Mode of administration**:** Oral

Table 6: Protocol for the treatment of udder pox

SL No.	Botanical name	Local name	Parts used
1	*Ocimum basilicum*	Sweet basil	Leaves
2	*Curcuma longa*	Turmeric	Powder/Rhizome
3	*Cuminum cyminum*	Cumin	Seeds
4	*Allium sativum*	Garlic	Bulbs
		Butter	

Method of preparation and application

Soak cumin in water for an hour. Grind all the ingredients into a fine paste and mix well with butter using a spoon. Apply this paste on the affected udder every two hours till the udder pox is cured.

Table 7: Treatment for repeat breeding in cows

Sl. No.	Botanical name	Local name	Parts used
1	*Raphanus sativus*	Raddish	Radish
2	*Alove vera*	Aloe	Leaves
3	*Moringa oleifera*	Drum stick tree	Leaves
4	*Cissus quadrangularis*	Velt grape	Stem
5	*Murraya koenigii*	Curry leaf	Leaves
6	*Curcuma longa*	Turmeric	Rhizome

Method of preparation and administration

In order to remove the uterine infections, feed the cow with one radish per day for 5 days after applying salt on it.

Feed the cow with 4 handful of *Aloe Vera* once a day for 4 days.

Next, feed with 4 handfuls of *Moringa oleifera* leaves once a day for 4 days.

Next, feed with 4 handfuls of *Cissus quadrangularis* once a day for 4 days.

Lastly feed the animal a paste of with 4 handfuls *of Murrya koenigii* leaves made into a paste along with turmeric.

If the cow is not conceived this treatment can be repeated for once again

Table 8: Protocol for treatment of Bloat and indigestion

Sl. No.	*Botanical name*	Local name	Parts used
1	*Allium cepa var. aggregatum*	shallot	Bulbs
2	*Allium sativum*	Garlic	Bulbs
3	*Capsicum annum*	Dry chilli	Fruit
4	*Cissus quadrangularis*	Paranda, Velt grape	Stem
5	*Cuminum cyminum*	Jeera	seeds
6	*Curcuma longa*	Turmeric	Rhizome
7	*Piper betel*	Nagavalli	leaves
8	*Piper nigram*	Black pepper	seed
9	*Zingiber officinale*	Ginger	Rhizome
10		Jaggery	

Method of preparation and administration

Soak black pepper and cumin in water for 30 minutes. Grind all the ingredients into a fine paste. Add jaggery and roll the paste in to small gooseberry size balls and touch the balls on common salt and apply on to the tongue of the affected animal. Repeat this for 3 times for 3 days. Prepare fresh formulation every time.

3. Result

There is significant change in knowledge and attitude about EVP, antibiotic residue in milk and AMR among the farmers from Kerala and Tamil Nadu. The study indicates strongly significant increase in the practices of EVP in this group and no significant change in KAP in EVP, antibiotic residue in the milk and AMR among farmers in Karnataka (Table: 9, 10 and 11**).** The baseline survey indicates that the 95 per cent of farmers were not aware that the milk from the cow have antibiotic residue if treated with antibiotics and 89 per cent had not heard about AMR. They also believed that EVP may take long time to cure the diseases or will not be effective. There were limited skills for preparing the herbal formulations and application. The training had improved their skills for preparation and application of the herbal formulations

Table 9: Percentage change of total score of Baseline and End line scores on Knowledge, attitude and Practice (KAP) among farmers' on Ethno-veterinary Practices (EVP), antibiotic and veterinary drug residue in the milk (Kerala)

% change of pre and post	Intervention Group (n=78)	Control Group (n=63)	Total (n=141)	P value
Percentage Knowledge				
• Decreased/No Change	16(20.5%)	24(38.1%)	40(28.4%)	0.068+
• 1-50	52(66.7%)	37(58.7%)	89(63.1%)	
• 51-75	8(10.3%)	2(3.2%)	10(7.1%)	
• 75-90	1(1.3%)	0(0%)	1(0.7%)	
• >90	1(1.3%)	0(0%)	1(0.7%)	
Percent Change Attitude				
• Decreased/No Change	23(29.5%)	29(46%)	52(36.9%)	0.297
• 1-50	43(55.1%)	28(44.4%)	71(50.4%)	
• 51-75	6(7.7%)	2(3.2%)	8(5.7%)	
• 75-90	2(2.6%)	1(1.6%)	3(2.1%)	
• >90	4(5.1%)	3(4.8%)	7(5%)	
Percent Change practice				
• Decreased/No Change	4(5.1%)	29(46%)	33(23.4%)	<0.001**
• 1-50	39(50%)	24(38.1%)	63(44.7%)	
• 51-75	19(24.4%)	6(9.5%)	25(17.7%)	
• 75-90	5(6.4%)	0(0%)	5(3.5%)	
• >90	11(14.1%)	4(6.3%)	15(10.6%)	

Chi-Square/Fisher Exact Test

Table 10: Percentage change of total score of Baseline and End line scores on Knowledge, attitude and Practice (KAP) among farmer's on Ethno-veterinary Practices (EVP), antibiotic and veterinary drug residue in the milk (Karnataka)

% change of pre and post	Intervention Group (n=20)	Control Group (n=18)	Total (n=38)	P value
Percentage Knowledge				
• Decreased/No Change	9(45%)	12(66.7%)	21(55.3%)	0.395
• 1-50	10(50%)	5(27.8%)	15(39.5%)	
• 51-75	0(0%)	0(0%)	0(0%)	
• 75-90	0(0%)	0(0%)	0(0%)	
• >90	1(5%)	1(5.6%)	2(5.3%)	
Per cent Change Attitude				
• Decreased/No Change	10(50%)	10(55.6%)	20(52.6%)	0.374
• 1-50	7(35%)	4(22.2%)	11(28.9%)	
• 51-75	1(5%)	4(22.2%)	5(13.2%)	
• 75-90	1(5%)	0(0%)	1(2.6%)	
• >90	1(5%)	0(0%)	1(2.6%)	
Per cent Change practice				
• Decreased/No Change	9(45%)	7(38.9%)	16(42.1%)	0.917
• 1-50	6(30%)	8(44.4%)	14(36.8%)	
• 51-75	2(10%)	1(5.6%)	3(7.9%)	
• 75-90	1(5%)	1(5.6%)	2(5.3%)	
• >90	1(5%)	1(5.6%)	2(5.3%)	

Chi-Square/Fisher Exact Test

Table 11: Percentage change of total score of Baseline AND End line scores on Knowledge, attitude and Practice (KAP) among farmer's on Ethno-veterinary Practices (EVP), antibiotic and veterinary drug residue in the Milk (Tamil Nadu)

% change of pre and post	Intervention Grou (n=23)	Control Group (n=5)	Total (n=28)	P value
Per cent Change Knowledge				
• Decreased/No Change	6(26.1%)	3(60%)	9(32.1%)	0.290
• 1-50	17(73.9%)	2(40%)	19(67.9%)	
• 51-75	0(0%)	0(0%)	0(0%)	
• 75-90	0(0%)	0(0%)	0(0%)	
• >90	0(0%)	0(0%)	0(0%)	
Per cent Change Attitude				
• Decreased/No Change	7(30.4%)	4(80%)	11(39.3%)	0.062+
• 1-50	16(69.6%)	1(20%)	17(60.7%)	
• 51-75	0(0%)	0(0%)	0(0%)	
• 75-90	0(0%)	0(0%)	0(0%)	
• >90	0(0%)	0(0%)	0(0%)	
Per cent Change practice				

• Decreased/No Change	3(13%)	4(80%)	7(25%)	
• 1-50	20(87%)	1(20%)	21(75%)	
• 51-75	0(0%)	0(0%)	0(0%)	0.008**
• 75-90	0(0%)	0(0%)	0(0%)	
• >90	0(0%)	0(0%)	0(0%)	

Chi-Square/Fisher Exact Test
Significant figures
+ Suggestive significance (P value: 0.05<*P*<0.10), * moderately significant (P value: 0.01<*P*≤0.05), ** strongly significant (P value: *P*≤0.01)

3. 1. Antibiotic residue analysis of the Market samples

Thirty five brands of milk sold in the Market were tested for presence of antibiotic residue. Twenty two had Beta lactams and Sulphonamides, 10 had only Sulphonamide. One brand had 4 antibiotics (Beta lactams, Sulphonamides, Gentamicin and Tetracycline) residues and 2 did not have any antibiotic residue (Table 12).

Table 12: Antibiotic residues in the Market Samples

Market sample	Beta lactam & Sulphonamide.	Sulphonamide	Gentamycin	Tetracycline
35 brands	22	10	1	1
Codex Alimentarius International food standard (MRL)	0-3 PPB is negative 4-10 PPB is positive	0-10 PPB is negative 15 PPB > Positive	0-50 PPB is negative 50-75 PPB Low Positive 100-200 PPB positive	0-80PPB Negative 80-100 PPB Low Positive 100-120 PPB Positive

2 brands were without any antibiotic residues

3.2. Baseline

The milk sample from 11 locations had Quinolones, Beta lactams, Tetracycline and Sulphonamides as residues (Table 13). Gentamicin, Streptomycin, Neomycin and Chloramphenicol were absent.

Table 13: Antibiotics in the pooled Samples from the milk 11 locations (L= Low positive)

Location	Quinolones	Beta lactams	Tetracycline	Sulfonamides
Maneed, Ernakulam Kerala		Positive (L)		
Monippally, Kottayam, Kerala		Present		Positive
Kottayam Kerala		Positive		
Puthrika Ernakulam Kerala	Positive	Positive		
Allappara, Ernakulam Kerala	Positive (L)	Positive		
Arakkappadi, Ernakulam Kerala		Positive		
Manikyamangalam, Ernakulam		Positive		
Sreemoolanagaram, Ernakulam,		Positive	Positive	
Thirukanurpatti, Tamil Nadu		Positive (L)		
Doddaballapura, Karnataka	Positive	positive	Positive	

Quinolones, Beta lactams, Gentamicin, Sulphonamides were present as residues in the milk samples from farmers selected for the study except the milk from farmers from Tamil Nadu (Table 14). Tetracycline, Streptomycin, Neomycin, Chloramphenicol were absent.

Table 14: Antibiotics residue(s) in the milk of Pooled samples from the selected farmers' baseline (L= Low positive)

Location	Quinolones	Beta lactams	Gentamicin	Sulfonamides
Maneed, Ernakulum Kerala		Positive (L)		
Monippally, Kottayam, Kerala		Positive		Positive
Chakkampuzha, Kottayam Kerala		Positive		
Puthrika Ernakulum Kerala	Positive	Positive		
Allappara, Ernakulum Kerala		Positive (L)		
Arakkappadi, Ernakulum Kerala		Positive		
Manikyamangalam, Ernakulum	Positive	Positive		
Sreemoolanagaram, Ernakulum,		Positive		
Thirukanurpatti, Tamil Nadu		Negative		
Doddaballapura, Karnataka	Positive	Positive (L)	Positive	

3.3. The end line survey

The end line survey indicated that the milk samples from 123 farmers out of 140 (87.86%) were without any detectable antibiotic residue, 11 (7.85%) samples showed low positive of Beta lactams or sulphonamides and 6 (4.29%) showed positive to antibiotic residue(s) of Beta lactams and or sulphonamides (Table 15). These 17 farmers used antibiotics along with EVP

Table 15: Antibiotic residue in the milk: Farmer's samples one year after intervention

Location	Number of farmers	Antimicrobial Residue Negative	residue Low Positive	Residue Positive
Maneed, Ernakulum Kerala	15	12	2	1
Monippally, Kottayam, Kerala	15	11	2	2
Chakkampuzha, Kottayam Kerala	10	10	0	0
Puthrika Ernakulum Kerala	10	7	3	0
Allappara, Ernakulum Kerala	15	12	2	1
Arakkappadi, Ernakulum	10	6	2	2
Manikyamangalam, Ernakulum.	10	10	0	0
Sreemoolanagaram, Ernakulum,	15	15	0	0
Thirukanurpatti, Tamil Nadu	20	20	0	0
Doddaballapura, Karnataka	20	20	0	0
	140	123	11	6
Per cent		87.86%	7.85%	4.29%

3.4. Reduction of incidence of disease conditions in cattle in the area selected for the studies when EVP is used

The incidence of mastitis was 66 in 2016, reduced to 37 in 2018 and to 11 in 2019 among the cows of farmers selected for the study. The overall reduction of mastitis from 2016 to 2019 is 83.3%. There is also reduction in the incidence of enteritis (63.6%), repeat breeding (96%) and cowpox (100%) from 2016 to 2019 (Table 16).

Table 16: Reduction of the incidence of Mastitis, Enteritis, repeat breeding and cow pox from 2016 to 2019 among the cows of the farmers selected for the study

Disease	Mastitis			Enteritis			Repeat breeding			Cowpox		
Year	2016	2018	2019	2016	2018	2019	2016	2018	2019	2016	2018	2019
Average	66	37	11	11	7	4	9	2.5	0.38	2.38	2.13	0
Per cent reduction		44	83.3		36.4	63.6		72.2	95.8		11	100

The milk samples from control group had Beta lactams and/or sulphonamide (Table 17). Tetracycline, Streptomycin, Neomycin, Chloramphenicol Gentamicin were absent.

Table 17: Antibiotics residue in the milk of Pooled samples from the control group after one year (L= Low positive)

Location	Beta lactams	Sulfonamides
Maneed, Ernakulum Kerala	Positive	
Monippally, Kottayam, Kerala	Positive	Positive
Chakkampuzha, .Kottayam Kerala	Positive	
Puthrika, Ernakulum, Kerala	Positive	
Allappara, Ernakulum Kerala	Positive (L)	
Arakkappadi, Ernakulum, Kerala	Positive	
Manikyamangalam, Ernakulum, Kerala.	Positive (L)	Positive
Sreemoolanagaram, Ernakulum, Kerala	Positive	
Thirukanurpatti, Thanjavur ,Tamil Nadu	Negative	
Doddaballapura, Karnataka	Positive	

3.5. Economic benefit of using Herbal formulation

Average expenditure for treatment of mastitis with conventional medicine was Rs. 3000; maggot wound Rs.963, boat & indigestion Rs.719, repeat breeding Rs.3061, cow pox Rs.583, foot and mouth disease (FMD) Rs. 3165, and diarrhoea Rs.500 per episode (Table 18). It is indicated that there is reduction of expenditure for the management of mastitis (Rs.3000 to 120), maggot wound (Rs. 963 to 60), bloat and indigestion (Rs.719 to 224), repeat breeding (Rs. 3061 to 430), cow pox (Rs. 583 to 335), FMD (Rs. 3165 to 1640) and diarrhoea (Rs. 500 to 166) with EVP.

Table 18: shows Average expenditure in Rupees for the treatment of various diseases in cattle using conventional drugs (allopathic) and EVP (one episode) and the saving (1 USD = Rs.73.52 on 01/12/ 2020)

No	Disease conditions	N	Western Drug Treatment	EVP treatment	Amount saved
1.	Mastitis	35	3000	120	2880
2.	Maggot wound	28	962.5	60	881.7
3.	Bloat& Indigestion	34	719.4	224	495.4
4.	Repeat breeding	23	3060.7	430	2630.9
5.	Cow pox	18	583.3	335	250
6.	Foot and Mouth Disease (FMD)	22	3165	1640	1525
7.	Diarrhoea	3	500	166	334

4. Discussion

This study shows that veterinarians, farmers and para-vets are using antibiotics and there is antibiotic residue in the milk. This indicates that farmers/veterinarians do not practice health ministry's withdrawal time. The rule states that the antibiotics used for therapeutic purpose in animals should be labeled

with the withdrawal periods i.e. “milk and eggs should be kept out of human food minimum for one week, poultry and meat products 28 days, fish and other marine products 500 degree days” (Health ministry: withdrawal time. the amendment in rule 97 of the drug and cosmetic rules 1947**)**. The baseline survey of the farmers indicates that the farmers were not aware about this rule and their cows’ milk could have antibiotic residue. They were also ignorant about the AMR. All 200 milk samples collected from 11 locations before the intervention tested positive for one or the other antibiotic residue. The farmers / Veterinarians from Thirukanurpatti also rarely use antibiotics as these farmers were trained earlier to use EVP by Prof. Punniamurthy.

4.1. The intervention

The herbal formulations used for Mastitis, Foot and mouth disease (FMD), Diarrhoea, Udder Pox, Repeat Breeding, Bloat, Indigestion and Maggot wound, not only cure these clinical conditions but also prevent these diseases. This is evident from the substantial reduction of incidence of mastitis (83.8%), enteritis (63.6%) repeat breeding (95.8%) and udder pox (100%). The reduction of antibiotic residue is significant (87.86%). There is limited number of such studies done on the role of EVP (herbal formulation) in animal health conditions (Nair *et al*., 2017b, Punniamurthy *et al*.,2017a,b, Suresh *et al*.,2018, Subhasree Pradhan and Suryakanta Mishra, 2018) [18, 27, 28, 35, 36]. The residue in the milk from the control group after one year is largely Beta lactams and sometimes Sulphonamides indicating the widespread use of these groups of antibiotics. This intervention also has increased the knowledge, aptitude and practice among the farmers and awareness about the antimicrobial residue in the animal products and associated antimicrobial resistance.

4.2. Economic benefit

Misuse of the drug, non-adherence to withdrawal period, economic reasons, ignorance, lack of medication records are the major reasons of appearance of veterinary drug residues in the animal products (Halasa, 2007) [37]. The combination of improved management practices with the use of herbal formulation in animal health care evidently is cost effective. It also minimised the production loss and improved quality of dairy milk. This work also indicates the reduction of use of antibiotics for management of cattle health and their residue in the milk. This is a step towards renewed interest in ethno-veterinary practices (herbal alternatives) among the national dairy development of India initiatives and enterprises, which face the problems related to antibiotic residue in the dairy milk due to misuse of antibiotics.

5. Conclusion

The use of herbal preparation as alternatives to antibiotics shows significant reduction in the antibiotic residues in the milk from cattle and increased financial saving for the farmers. Adopting the Ethno-veterinary Science and Practices to combat infectious diseases in livestock has been identified and tested as a key game changer in rationalising the use of antibiotics in veterinary health care and reducing antibiotic residue(s) in milk.

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