Type of the Paper (Article, Review, Communication, etc.)

Reduction of Antibiotic residue in milk through the use of cost effective

Ethno-Veterinary Practices (EVP) for cattle health management

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Abstract: This study demonstrated that antibiotic residue in milk can be reduced when dairy farmers use Ethno-veterinary Practices (EVP) based on herbal alternatives to prevent and cure common clinical conditions in cattle instead of antibiotics. Of the 220 farmers selected for the study, 140 were trained and motivated to use validated herbal formulations, 80 were kept as control. Milk samples from the selected farmers (except Thirukanurpatti milk society) tested positive for antibiotic residue in the baseline survey. One year after interventions, the milk from 123 (87.86%) farmers out of 140, were without any detectable antibiotic residue, while samples from 11 farmers (7.85%) were low positive for either Beta-lactams or sulphonamides and 6 (4.29%) were positive for Beta lactams and/or sulphonamides. These 17 (11 + 6) farmers had used antibiotics along with herbal formulations. The milk samples from the control groups were positive for beta lactam and sulphonamide. There was suggestive significance of change in knowledge, attitude and practice of EVP among the farmers from Kerala and Tamil Nadu. 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. Use of herbal alternative also resulted in a significant reduction in health care expenditure of cattle.

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 [1-12]. It also causes side effects and allergic reactions on people who consume animal products such as milk and meat [13-18]. In many countries AMR is a threat to public health and the world is looking for safer alternatives through herbs. It is also predicted that the global consumption of antimicrobials will go up to 67 % from 2010 to 2030 [16, 18, and 20]. Tackling a Crisis for the Health and Wealth of Nations was published on the request of the UK Prime Minister to address the growing global problem of 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 [17, 21]. 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. With limited or no strategic implementation of regulatory policy on controlling the

misuse of antibiotics in dairy sector in India, the focus needs to be shifted to work with farmers, animal keepers and veterinarians in identifying approaches and options to use ethno-veterinary practices based on natural plant products to control microbial diseases. There are reports on the documentation of ethno-veterinary practices from several parts of India [22-44]. Ethno-veterinary medicine has also been suggested as alternative to antibiotics [14, 15, and 20]

The University of Trans-disciplinary Health Sciences and Technology (TDU) along with Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) have documented Ethno-veterinary practices from 24 locations in 10 states of India and rapidly assessed them using Ayurveda and established that 353 formulations out of 441 are safe and efficacious [39] There are reports of validation of herbal formulations for Mastitis [40-43] and reduction of retention of placenta (ROP) [44]. This study reports change in knowledge, attitude and practice of the ethno-veterinary practices (EVP) among farmers for selected animal health conditions and significant reduction of the antibiotic residue(s) in the milk after using cost effective ethno-veterinary medicine for prevention and cure of mastitis, foot and mouth disease (FMD), diarrhoea, udder pox, repeat breeding, bloat, indigestion and maggot wounds in cattle.

2. Results

2.1. KAP analysis

There is suggestive significance of 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: 1, 2 and 3**). During the baseline many of the farmers were not aware that the milk from their cow could have antibiotic residue(s) and had no idea of AMR. They also believed that EVP may take long time to cure or will not be effective. There were limited skills for preparing the herbal formulations. The training had improved their skills for preparation and application of the herbal formulations

Table 1: 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%)	
• 1-50	52(66.7%)	37(58.7%)	89(63.1%)	
• 51-75	8(10.3%)	2(3.2%)	10(7.1%)	0.068+
• 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%)	
• 1-50	43(55.1%)	28(44.4%)	71(50.4%)	
• 51-75	6(7.7%)	2(3.2%)	8(5.7%)	0.297
• 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%)	
• 1-50	39(50%)	24(38.1%)	63(44.7%)	
• 51-75	19(24.4%)	6(9.5%)	25(17.7%)	<0.001**
• 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 2: 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 Contra hange of pre and post Group Group $(n=20)$ $(n=18)$		Total (n=38)	P value	
Percentage Knowledge					
Decreased/No Change	9(45%)	12(66.7%)	21(55.3%)		
• 1-50	10(50%)	5(27.8%)	15(39.5%)		
• 51-75	0(0%)	0(0%)	0(0%)	0.395	
• 75-90	0(0%)	0(0%)	0(0%)		
• >90	1(5%)	1(5.6%)	2(5.3%)		
Percent Change Attitude					
Decreased/No Change	10(50%)	10(55.6%)	20(52.6%)		
• 1-50	7(35%)	4(22.2%)	11(28.9%)		
• 51-75	1(5%)	4(22.2%)	5(13.2%)	0.374	
• 75-90	1(5%)	0(0%)	1(2.6%)		
• >90	1(5%)	0(0%)	1(2.6%)		

Percent Change practice				
Decreased/No Change	9(45%)	7(38.9%)	16(42.1%)	
• 1-50	6(30%)	8(44.4%)	14(36.8%)	
• 51-75	2(10%)	1(5.6%)	3(7.9%)	0.917
• 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 3: 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	Group Group			
Percent Change				
Knowledge				
Decreased/No Change	6(26.1%)	3(60%)	9(32.1%)	
• 1-50	17(73.9%)	2(40%)	19(67.9%)	
• 51-75	0(0%)	0(0%)	0(0%)	0.290
• 75-90	0(0%)	0(0%)	0(0%)	
• >90	0(0%)	0(0%)	0(0%)	
Percent Change Attitude				
Decreased/No Change	7(30.4%)	4(80%)	11(39.3%)	
• 1-50	16(69.6%)	1(20%)	17(60.7%)	
• 51-75	0(0%)	0(0%)	0(0%)	0.062+
• 75-90	0(0%)	0(0%)	0(0%)	
• >90	0(0%)	0(0%)	0(0%)	
Percent Change practice				
Decreased/No Change	3(13%)	4(80%)	7(25%)	
• 1-50	20(87%)	1(20%)	21(75%)	0.008**
• 51-75	0(0%)	0(0%)	0(0%)	

• 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: $0.01 < P \le 0.05$), ** strongly significant (P value: $P \le 0.01$)

2.2. 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 4**).

Table 4.Antibiotic residues in the Market Samples

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

2 brands were without any antibiotic residues

2.3. Baseline

The milk sample from 11 milk Union's collection centres had Quinolones, Beta lactams, Tetracycline and Sulphonamides as residues (**Table 5**). Gentamicin, Streptomycin, Neomycin and Chloramphenicol were absent. Quinolones, Beta lactams, Gentamicin, Sulphonamides were present as residues in the milk samples from farmers selected for the study except the milk from Thirukanurpatti, (Table 6). Tetracycline, Streptomycin, Neomycin, Chloramphenicol were absent.

Table 5.Antibiotics in the pooled Samples from the milk Union's collection centres (L= Low positive)

MILK Union	Quinolones	Beta lactams	Tetracycline	Sulfonamides
Allapra		Positive (L)		
Arakkapady		Present		Positive
Chakkampuzha		Positive		
Maneed	Positive	Positive		
Manikyamangalam	Positive (L)	Positive		

Monippally		Positive	
Puthrika		Positive	
Sreemoolanagaram		Positive	Positive
Thirukanurpatti		Positive (L)	
Aralumallige	P0sitive	Positive (L)	Positive
Ekashipura		positive	

Table 6: Antibiotics residue(s) in the milk of Pooled samples from the selected farmers' before intervention

·	·		Sulfonamides
	Positive (L)		
	Positive		Positive
	Positive		
Positive	Positive		
	Positive (L)		
	Positive		
Positive	Positive		
	Positive		
	Negative		
Positive	Negative	Positive	
	Positive (L)		
	Positive	Positive Positive Positive Positive Positive (L) Positive Positive Positive Positive Positive Negative Positive Negative	Positive Positive Positive Positive (L) Positive Positive Positive Positive Positive Positive Positive Negative Positive Positive Positive Negative Positive

⁽L= Low positive)

2.4. 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(s), 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 (Fig 1, **table 7**).

Fig 1.Antibiotic residue: Farmer Sample After one year intervention

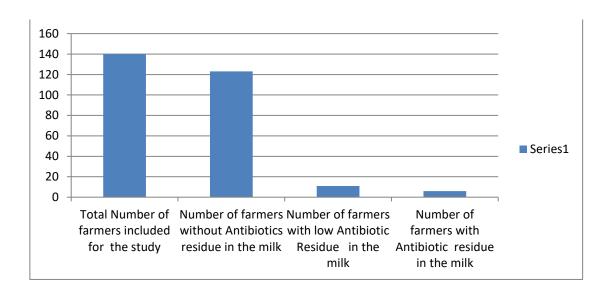


Table 7. Antibiotic residue: Farmer samples one year after intervention

MILK Union	Number	of	Antimicrobial	residue	Residue
	farmers		Negative	Low	Positive
				Positive	
Allapra	15		12	2	1
Arakkapady	15		11	2	2
Chakkampuzha	10		10	0	0
Maneed	10		7	3	0
Manikyamangalam	15		12	2	1
Monippally	10		6	2	2
Puthrika	10		10	0	0
Sreemoolanagaram	15		15	0	0
Thirukanurpatti (TN)	20		20	0	0
Aralumallige (Karn)	20		20	0	0
	140		123	11	6
Per cent			87.86%	7.85%	4.29%

2.5. 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 8**)

Table 8. 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	I	Mastiti	s	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		44	83.3		36.4	63.6		72.2	95.8		11	100
reduction												

2.6. The control group

The milk samples from control group had Beta lactams and/or sulphonamide (**Table 9**). Tetracycline, Streptomycin, Neomycin, Chloramphenicol Gentamicin were absent

Table 9: Antibiotics residue in the milk of Pooled samples from the control group after one year (L= Low positive)

MILK Union	Beta lactams	Sulfonamides	
Allapra	Positive		
Arakkapady	Positive	Positive	
Chakkampuzha	Positive		
Maneed	Positive		
Manikyamangalam	Positive (L)		
Monippally	Positive		
Puthrika	Positive (L)	Positive	
Sreemoolanagaram	Positive		
Thirukanurpatti	Negative		
Aralumallige	Positive (L)		
Ekashipura	Positive		

2.7. Economic benefit of using Herbal formulation

Average expenditure for treatment of mastitis with western 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 10**). 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 10: shows Average expenditure in Rupees for the treatment of various diseases in cattle using Western drugs (allopathic) and EVP (one episode) and the saving (1 USD = Rs.73.52 on 01/12/2020)

No	Disease conditions	n	western	EVP	Amount
			drug	treatment	saved
			treatment		
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.	Diarrhea	3	500	166	334

2.8. Production loss

The average milk production loss was 15 to 22.2 litres for 6 days when treated with western medicines. The details are given in the table 11. However, the loss is only 0.2 to 0.5 litres when treated with EVP (Table 12). In the case of FMD there is no loss but sometimes there is an increase of production by 0.7 litres

Table 11. Shows the loss of milk in litters when treated with western (allopathic) drugs (1 USD = Rs.73.52 on 01/12/2020)

Disease conditions		Before	After	Loss of milk		Financial
		treatment	treatment	In liters /	days in	loss in Rs
				day		
Mastitis	35	12.8	10.5	2.5	15.0	390
Maggot wound	28	13.1	10.6	2.5	15.0	390
Bloat& Indigestion	34	14.0	10.5	3.5	21.0	546
Repeat breeding	23	16.0	13.2	2.8	16.8	437
Cow pox	18	14.9	11.8	3.1	18.6	484
FMD	22	9.71	06.0	3.7	22.2	577
(Rs 26 per litre of milk - procurement cost)						

Table 12 shows the average loss of milk in litres and financial loss before and after treatment of diseases in cattle with EVP (1 USD = Rs.73.52 on 01/12/2020)

No	Disease conditions	n	Before treatment	After treatment	Loss of milk in 6 days in	Financial loss in Rs	
					liters		
1	Mastitis	35	14.4	14	0.4	10	
2	Maggot wound	28	13.7	13.3	0.4	10	
3	Bloat& Indigestion	34	14	14	Nil	Nil	
4	Repeat breeding	23	15	14.8	0.2	5	
5	Cow pox	18	15.6	15.1	0.5	13	
6	FMD	22	10.3	11.	- 0.7	+18	
	Total 5.5 Liters gain, average. (Rs 26 per liter)						

3. Discussion

This study shows that veterinarians, farmers and para-vets are using antibiotics and there is antibiotic residue in the milk [1-12]. This indicates that farmers/veterinarians do not practice health ministry's withdrawal time [2, 11, 13, 14, 15 and 20]. 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(s). They were also ignorant about the AMR. All 200 milk samples collected from collection centres before the intervention tested positive for one or the other antibiotic residue. The farmers / Veterinarians from Thirukanurpatti also use antibiotic however, during the period when the milk samples were collected, probably there were no clinical conditions needing antibiotic treatment.

3.1. 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. [14, 15, 39-44]. 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.

3.2. 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 [45-47]. 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 antimicrobials 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 antimicrobial residue in the dairy milk due to misuse of antibiotics.

4. Material and Methods

Two hundred twenty farmers were selected from eight milk societies from Kerala state, two societies in Karnataka and one in Tamil Nadu. The names of the selected milk societies and number of farmers selected for the study are given in **table 13.** One forty farmers were included in the intervention study group and 80

in the control group.

Table 13 Show the name of the milk society, number of farmers selected as interventions and controls group

No	Name of the society	Number	Intervention	Control
		Farmers	group	Group
		selected		
1	Maneed KheeraUlpadaka Society	20	10	10
	Ernakulam Kerala			
2	Monippally KheeraUlpadaka	20	10	10
	Society. Kottayam, Kerala			
3	ChakkampuzhaKheeraUlpadaka	20	10	10
	Society Kottayam Kerala			
4	PuthrikaKheeraUlpadaka Society	20	10	10
	Ernakulan Kerala			
5	Allappara KheeraUlpadaka Society	15	15	0
	Ernakulan Kerala			
6	Arakkappadi KheeraUlpadaka	15	15	0
	Society, Ernakulan Kerala			
7	Manikyamangalam Kheera	15	15	0
	Ulpadaka Society Ernakulan Kerala.			
8	Sreemoolanagaram Kheera	15	15	0
	Ulpadaka Society Ernakulan, Kerala			
9	Thirukanurpatti, Thanjavur Tamil	40	20	20
	Nadu			
10	Aralumallige, Doddaballapura,	20	20	0
	Karnataka			
11	Ekashipura, Doddaballapura,	20	0	20
	Karnataka			
	Total	220	140	80

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.

Thirty five market samples in triplicate were collected and tested for the presence of antibiotic residue(s). The pooled milk samples from 11 milk union collection centres and 220 farmers before and after intervention were tested for the presence of antibiotic residue(s) using *Unisensor* (Belgium).

The 140 farmers from 11 unions (intervention group) who consented to use only EVP and not antibiotics, were trained to use validated EVP for Mastitis, Foot and mouth disease (FMD), Diarrhoea, Udder Pox, Repeat Breeding, Bloat, Indigestion and Maggot wounds repeatedly for one year. The treatment protocols by

farmers were monitored randomly. The field support was given whenever it was necessary. Hand books were printed in local languages (Kannada, Malayalam and Tamil) and distributed to the selected farmers for reference.

5. Conclusion

The use of herbal preparation as alternatives to antimicrobials 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.

6. Acknowledgement

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7. Author Contributions

Field study at Kerala conducted by M N B Nair, Tamil Nadu by N Punniamurthy and Karnataka by Kumar SK and all authors approved the final version of the manuscript.

8. Author Biography

- **8.1. DR. Balakrishnan Mannoor Narayanan Nair** is working as Professor Emeritus and Head of Centre for Ethno-veterinary sciences and Practice at the University of trans-disciplinary health sciences and technology (TDU). He has BSc and MSc in Botany, and Ph. D. in developmental anatomy. Nair has 39 years of teaching and research experience, over 45 publications and contributed to different books. His Research interests are trans-disciplinary research, one health, documentation of ethno-veterinary practices, validation and promotion of them through mainstreaming in veterinary curriculum, training of vets, farmers and Dairy professionals to reduce antimicrobial use in dairy production and associated residues in the animal products. He has expertise in wood structure and natural gum and gum-resin secretion, their sustainable tapping and uses. He is a life member of Indian Botanical society, Tree scientist, International Association of wood Anatomists, Executive Board member of Natural Livestock Faming, Netherlands and Director NLF India.
- **8.2. Dr Natesan Punniamurthy** is currently Professor-Emeritus of TDU Bangalore, India, had BVSC, MVSc PhD from Madras Veterinary College, India. As professor of Pharmacology and Toxicology he taught UG, PG veterinary-students for 12 years. Has 40 years' experience in teaching, research, extension in TANUVAS/TDU. He has worked in neuropharmacology of feeding behaviour. He has 20 years research

experience in documentation and validating veterinary clinical-herbal-medicine; currently train veterinarians across India and mainstreaming EVP in veterinary curriculum. Gold medal in PhD 1995, Life Member "Indian Veterinary Association", Achiever award IVA 2019. Fellow "National society for Ethno pharmacology" 2017, Fellow "Indian Society for Veterinary pharmacology and Toxicology" 2016, Lifetime Achievement Award for promoting Siddha medicine, Government Siddha Medical College, Tamil Nadu 2014, Tamil Nadu Scientist award by State Council for Science and Technology for decade of research accomplishments in EVM, 2013, Achievement award for EVM recipes dissemination, Thamizhar Vazhviyal Iyakkam, 2005 from Nammazhlwar.

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9. Conflicts of Interest

The authors declare that they have no Conflicts of Interest.

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