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Study the effect of health care practices on antimicrobial residue in milk of indigenous and cross breed cattle

Jeetender Jaipal, Vijay Kumar, Mohan Lal Choudhary and Vikramjit Singh

Abstract
In the present study a total of 200 milk samples were collected 100 from each indigenous cattle and Cross Breed cattle from dairy farms of the Bikaner and adjoining area. Each milk sample, was screened for the presence of antimicrobial drugs using charm EZ Rosa technique and the questionnaire prepared to assess the effect of health care practices over antibiotic residue in milk. The result reveals that the all samples examined, 7 per cent indigenous cow milk samples and 21 per cent cross breed milk samples were found to be positive ($p<0.01$). Statistically data show that there is highly significant difference between indigenous and cross breed cattle for antibiotic residue in milk. For beta lactam antibiotic 0 percent and 6 per cent ($p<0.05$), Tetracycline 4 per cent ($p>0.05$) and 4 per cent, Quinolone 3 per cent and 10 per cent ($p<0.05$) and sulpha drug 0 per cent and 1 per cent ($p>0.05$) samples were found positive in indigenous and cross breed cattle milk sample. In the present study questionnaire data reveals that the various health care practices such as vaccination, record keeping, withdrawal period of antibiotic, separate milking, veterinarian advice play significant role in antimicrobial residue in milk of dairy farms.

Keywords: Charm EZ ROSA, indigenous cattle, cross breed cattle, antimicrobial residue

Introduction
The frequent and inadequate use of antibiotics in clinical practice lead to passing antimicrobial drug residue in various food products of animal origin including milk, egg, and meat. Existence of antimicrobial residues in various food products above the maximum residue limit [MRL] has been considered worldwide by various public authorities (Kempe and Verachtert, 2000) [1]. These residues of antimicrobials are one of the foremost serious issues of the twenty-first century. The farm animal business is quickly gathering at the side of increasing administration of antibiotics in food-producing animals to prevent and treat disease (Khachatourians, 1998) [2]. The foremost used antimicrobial groups in food manufacturing animals are β-lactams, tetracyclines, quinolones, aminoglycosides, macrolides, lincosamides, sulphonamides etc. (Lee *et al*., 2001) [3]. As we all know that India has highest livestock population in the world with 192.49total cattle population in total cattle population the indigenous/non-descriptive cattle population is 142.11 million and exotic/Cross breed cattle population in country is 50.42 million. (20th Livestock Census, DAHD-2019) (fig.1) (DAHD, 2019)[4] and the milk yield of Exotic/Cross breed cows reported 7.95 kgs per cow per day whereas milk yield in Indigenous/Non descriptive cow was 3.01 95 kgs per cow per day during the year 2018-19 (fig. 2) and the total milk production in Rajasthan is around 23,668 Thousand of ton. (BAHS 2018-19) [5]. Farmers within the Michigan study thought that the foremost important management factors resulting in drug residues in milk were insufficient knowledge regarding withdrawal periods, errors due to hired help, deficient records of treatment, and identification of animals (Kaneene and Ahl, 1987) [6]. In the present study questionnaire data reveals that the various health care practices such as vaccination, record keeping, withdrawal period of antibiotic, separate milking, veterinarian advice play significant role in antimicrobial residue in milk of dairy farms Managemental factors like record-keeping, vaccination, clean milk production, hygiene and sanitation of farm as well as animal, veterinarian/farmer interaction and correct monitoring are indirectly associated with the antibiotic residue in milk. Due to the poor managemental practices at farm risk of occurrence of disease in an animal is considerably high. In this paper, we describe associations amongthe occurrence of antibiotic residues in the milk of cattle and their relation with various farm management practices which are followed by farmers at their dairy farm.

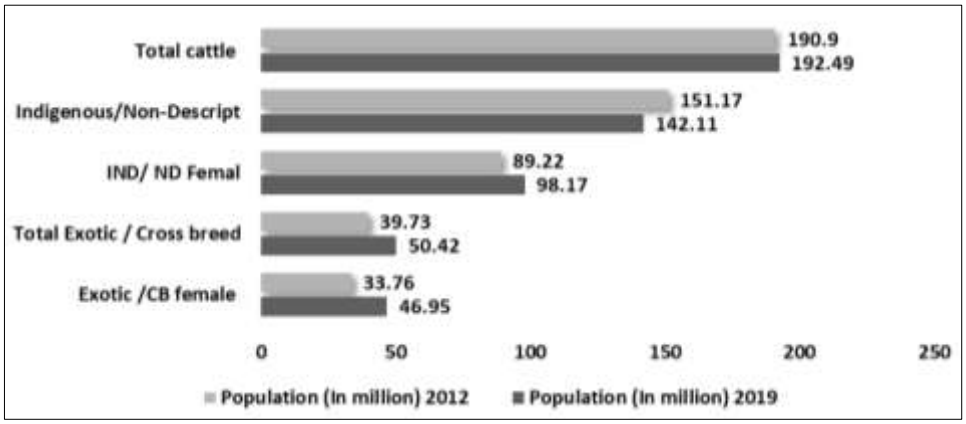

Fig 1: cattle population- breed group-wise population

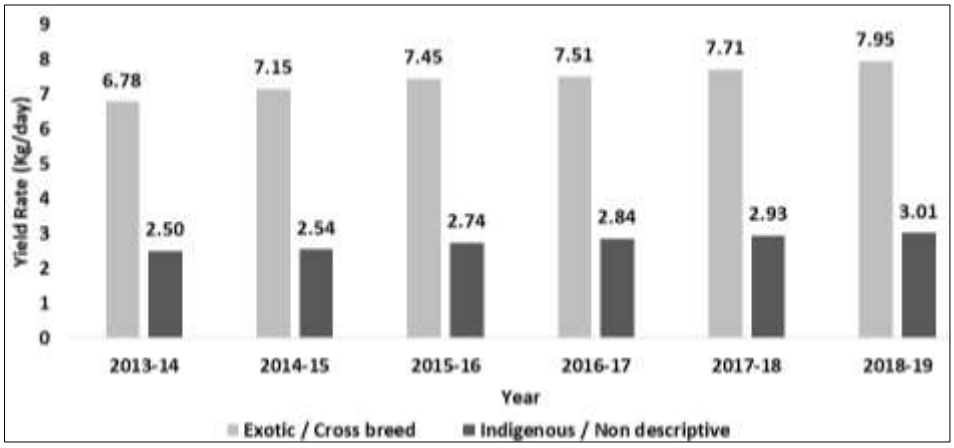

Fig 2: Average milk yield per cow (All India)

Materials and Methods

A total of 200 milk samples 100 from each indigenous and cross breed cattle were collected from dairy farmers of the Bikaner and adjoining area. Approximately, 50 ml of milk sample were collected in properly labelled sterilized tubes. All the samples were transported to the laboratory on the same day of collection under chilled conditions and stored at -20°c. The experimental work was carried out in the laboratory of milk adulteration, antibiotic residue and pesticide residue detection "centre for organic animal products technology" Rajasthan University of Veterinary and Animal Sciences, Bikaner (RAJUVAS) by Charm EZ ROSA test for antibiotic residue detection (CSTP, 2007) [7]. The semi structured questionnaire was designed (containing closed and open-ended questions) to assess the knowledge and health care practices of dairy farmers towards antibiotic usage. The verbal consent was obtained from all the farmers who was agreed to participate in the study.

The data collected during the present investigation were subjected to statistical analysis by adopting appropriate methods described by (Snedecor and Cochran, 1994) [11]. Chi square test was performed by using SPSS computer package (IBM SPSS version 20.0, SPSS Inc. Chicago, USA).

Results and Discussion

The results obtained after screening 200samples reveals the presence of antimicrobial residue in the milk samples collected from indigenous and cross breed cattle of different locations Udasar, Raisar, Pemasar. In all 200 milk samples 100 from each indigenous and cross breed cattle were collected out of 100 milk sample of indigenous 7 per cent sample show presence of antibiotic and in cross breed milk sample 21 per cent milk sample found positive (Table 1). The findings highlighted that the percentage of positive samples were higher in milk samples of cross breed cattle as compared to indigenous cattle. Also, the statistical analysis highlighted highly significant difference in antibiotic residues detected in milk of cross breed cattle as compared to indigenous cattle. ($p<0.05$).

The residues of beta lactam were detected in indigenous and cross breed cattle milk samples, In all 200 milk sample 3 per cent samples showed the presence of antibiotic in which 100 milk sample of indigenous and 100 sample of cross breed were taken out of 100 milk sample of indigenous none of the sample show presence of antibiotic and in cross breed milk sample 6 per cent milk sample found positive (Table 2). The statistical analysis showed significant difference in beta lactam antibiotic residues detected in milk of cross breed cattle as compared to indigenous cattle. ($p<0.05$). The response to the question is not corroborate with the observations of Kumar, P. (2010) studied on the antimicrobial drug residue in milk of cattle and buffaloes. It might be due to the species variation (Kumar, 2010) [8].

Table 1: Distribution of antimicrobial residues based on breed sampled

S.No	Breed	Sample	Positive	Negative	p Value	Chi square
1.	Indigenous	100	7	93	0.004	8.140**
2.	Crossbreed	100	21	79		
	Total	200	28	172		

Table 2: Breed-wise residues of Beta lactam in milk of indigenous and cross breed Cattle

S.No	Breed	Sample	Positive	Negative	p Value	Chi square
1.	Indigenous	100	0	100	0.013	6.186*
2.	Crossbreed	100	6	94		
	Total	200	6	194		

The residues of Tetracycline (TC) were detected in indigenous and cross breed cattle milk samples, In all 200 milk sample 8.4 per cent samples showed the presence of antibiotic in which 100 milk sample of indigenous and 100 sample of cross breed were taken out of 100 milk sample of indigenous 4 per cent sample show presence of antibiotic and in cross breed milk sample 4 per cent milk sample found positive (Table 3). The statistical analysis showed there was no any significant difference in TC antibiotic residues detected in milk of cross breed cattle as compared to indigenous cattle. (p>0.05). The response to the question is not corroborate with the observations of Kumar, P. (2010) studied on the antimicrobial drug residue in milk of cattle and buffaloes. Species variation may be the possible reason of disparity in both researches (Kumar, 2010) [8].

Table 3: Breed-wise residues of Tetracycline in milk of indigenous and cross breed

S. No	Breed	Sample	Positive	Negative	p Value	Chi square
1.	Indigenous	100	4	96	1.00	-
2.	Crossbreed	100	4	96		
	Total	200	8	192		

The residues of Quinolone were detected in indigenous and cross breed cattle milk samples, In all 200 milk sample 6.5 per cent samples showed the presence of antibiotic in which 100 milk sample of indigenous and 100 sample of cross breed were taken out of 100 milk sample of indigenous3 per cent sample show presence of antibiotic and in cross breed milk sample 10 per cent milk sample found positive (Table 4). The statistical analysis showed significant difference in beta quinolone antibiotic residues detected in milk of cross breed cattle as compared to indigenous cattle (p<0.05). Similarly, v Analysis Quinolones Residues in Milk using High Performance Liquid Chromatography found total of 8 samples out of 100 were positive for quinolone antimicrobial residues in milk (Priyanka *et al*., 2019) [9]. The residues of sulpha drugs were detected in indigenous and cross breed cattle milk samples, in all 200 milk sample only 0.5 per cent samples showed the presence of antibiotic in 100 milk sample of indigenous none of the sample found positive for sulpha drugs residue which whereas 1 per cent sample found positive out of 100 milk sample of cross breed cattle (Table 5). The statistical analysis showed there was no any significant difference in sulpha drugs residues detected in milk of cross breed cattle as compared to indigenous cattle. (p>0.05). Similar study found by (Chung *et al*., 2009) [10] monitored sulfonamides residues milk samples using HPL. In HPLC analysis, 3 samples were detected positive among 269 samples (Chung *et al*., 2009) [10]. Location-wise TC residues were detected for which 200 milk sample ware taken 65 from Udasar, 67 from Raisar and 68 From Pemasar, Out of these sample 7 (10.76 per cent), 10 (14.92 per cent), 11(16 per cent) were pound positive for antibiotic residue in milk sample of indigenous and cross breed cattle (Table 6). There is no any significant difference (p>0.05) in antimicrobial drugs residue in milk taken from different location in Bikaner.

Table 4: Breed- wise residues of Quinolone in milk of indigenous and cross breed Cows

S.No	Breed	Sample	Positive	Negative	p Value	Chi square
1.	Indigenous	100	3	97	0.045*	4.031
2.	Crossbreed	100	10	90		
	Total	200	13	187		

Table 5: Breed-wise residues of Sulpha drugs in milk of indigenous and cross breed Cows

S.No	Breed	Sample	Positive	Negative	p Value	Chi square
1.	Indigenous	100	0	100	0.316	1.005
2.	Crossbreed	100	1	99		
	Total	200	1	199		

Table 6: Distribution of antimicrobial residues based on breed sampled

S.No	Breed	Sample	Positive	Negative	p Value	Chi square
1.	Udasar	65	7	58	0.644	0.879
2.	Raisar	67	10	57		
3.	Pemasar	68	11	57		
	Total	200	28	172		

Statistical data shows that the antibiotic residue is much higher in milk of cross breed cattle possible reason may be the environment condition and disease resistance. The cross-breed cattle have lower disease resistance in compare to indigenous cattle on that account occurrence of disease is higher in cross breed cattle hence the antibiotic are more frequently used in cross breed cattle that may lead to comparatively higher antimicrobial residue higher in milk of cross breed cattle.

Effect of health care practices on antimicrobial drugsre side in milk of indigenous and cross breed cattle

The cross-sectional study to assess the Response of dairy farmers to health care practices which are using on their dairy farm was conducted in 80 local dairy farmers 25 from Udasar 26 from Raisar and 29 from Pemasar. A structured questionnaire consisting of series of questions was used to interview the farmers.

Various health care practices such as judicial uses and knowledge of antibiotic, vaccination, record keeping, follow withdrawal period of antibiotic, separate milking of treated and untreated cow, clean milk productionand veterinarian advice etc. are play major and important role in antimicrobial residue in milk. in present study data reveals (Table 7) that in Pemasar most of the farmers not follow these health care practices in compare to Udasar and Raisar. This may lead to higher antimicrobial drug in milk sample of Pemasar. Possible reason behind that may be the lower knowledge, lower literacy level, lack of extension contacts, mass media exposer and scientific orientation and limited resources of farmers of Pemasar. These health care practices play significant role in antimicrobial residue in milk of dairy farms. If the farmers follow these practices on their dairy farm that will help to reduce antimicrobial residue in milk which is safe for animal and human being.

Table 7: Response of dairy farmers towards health care practices and their effect on antimicrobial drugs residue in milk of indigenous and cross breed cattle.

S. No	Particular		Response									Chi square	Positive Samples (%)		
			Udasar			Raisar			Pemasar						
			Never	Sometime	Always	Never	Sometime	Always	Never	Sometime	Always		Udasar	Raisar	Pemasar
1.	medicated feed		20 (80)	5 (20)	0 (0)	19 (73.07)	7 (26.92)	0 (0)	19 (65.51)	10 (34.48)	0 (0)	1.419	10.76	14.92	15.94
2.	Type of drugs uses in treating farm animal	Allopathy	0 (0)	5 (24)	19 (76)	0 (0)	5 (19.23)	21 (80.76)	0 (0)	4 (13.79)	25 (86.20)	0.51	10.76	14.92	15.94
		Ayurvedic	0 (0)	19 (76)	6 (24)	0 (0)	21 (80.76)	5 (19.23)	0 (0)	24 (82.75)	5 (17.24)	0.40	10.76	14.92	15.94
		Homeopathic	25 (100)	0 (0)	0 (0)	26 (100)	0 (0)	0 (0)	29 (100)	0 (0)	0 (0)	-	10.76	14.92	15.94
3.	vaccination for cattle to prevent from disease		17 (68)	8 (32)	0 (0)	20 (76.92)	6 (23.07)	0 (0)	25 (86.20)	4 (13.79)	0 (0)	2.560	10.76	14.92	15.94
4.	interval between two antibiotics dose		17 (68)	8 (32)	0 (0)	21 (80.76)	5 (19.23)	0 (0)	26 (89.65)	3 (10.34)	0 (0)	3.949	10.76	14.92	15.94
5.	Veterinarian's advice		3 (12)	5 (20)	17 (68)	6 (23.07)	7 (26.92)	13 (50)	9 (31.03)	11 (37.93)	9 (31.03)	7.489	10.76	14.92	15.94
6.	Farm record keeping		20 (80)	5 (20)	0 (0)	22 (84.61)	4 (15.38)	0 (0)	26 (89.65)	3 (10.34)	0 (0)	0.99	10.76	14.92	15.94
7.	antibiotic withdrawal period		22 (88)	3 (12)	0 (0)	24 (92.30)	2 (7.69)	0 (0)	28 (96.55)	1 (3.44)	0 (0)	6.428	10.76	14.92	15.94
8.	physically separation of Treated cows from untreated one		21 (84)	4 (16)	0 (0)	23 (88.46)	3 (11.54)	0 (0)	27 (93.10)	5 (6.89)	0 (0)	0.26	10.76	14.92	15.94
9.	Separate milking of Treated and untreated cow		20 (80)	5 (20)	0 (0)	22 (84.61)	6 (23.07)	0 (0)	25 (86.20)	4 (13.79)	0 (0)	0.63	10.76	14.92	15.94
10.	Antimicrobial residue testing		25 (100)	0 (0)	0 (0)	26 (100)	0 (0)	0 (0)	29 (100)	0 (0)	0 (0)	-	10.76	14.92	15.94
11.	Use of antibiotic treated cow milk (throwing it away)		20 (80)	6 (20)	0 (0)	22 (84.61)	4 (15.38)	0 (0)	25 (86.20)	4 (13.79)	0 (0)	0.92	10.76	14.92	15.94
12.	mixing of treated and untreated cow milk		3 (20)	4 (16)	18 (72)	1 (3.84)	4 (15.38)	21 (80.76)	1 (3.44)	3 (10.34)	25 (86.20)	2.66	10.76	14.92	15.94
13.	Washing of equipment with disinfectant		21 (84)	3 (12)	1 (4)	22 (84.61)	4 (15.38)	0 (0)	26 (89.65)	3 (10.34)	0 (0)	2.55	10.76	14.92	15.94

*=significant ($p<0.05$), **=highly significant ($p<0.01$), () = percentage

Conclusions

It can be concluded that the antimicrobial residue was significantly higher in milk of cross breed cattle in compare of indigenous cattle due to the uses of antibiotic for treating of various disease which higher in cross breed cattle. Health care Practices such as proper vaccination, record keeping, follow withdrawal period of antibiotic, separate milking of treated and untreated cow, follow proper time interval between two antibiotic doses, and veterinarian advice for the antibiotic uses in animal that all play direct and indirect role to prevent antimicrobial residue in milk. If the farmers follow these practices chances of antimicrobial residue will reduce which is safe for animal and human being also the study highlighted a need to generate awareness among local dairy farmer towards judicious antibiotic uses of antibiotic and health care practices to prevent occurrence of antibiotic residues in milk and thus preventing unacceptable health risks to consumers**.**

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Conflict of Interest

We declare that we have no conflict of interest.

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