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Comparative efficacy of different therapeutic regimens against brucellosis in cows

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Abstract
Bovine brucellosis has become a serious problem in Indian dairy herds because of certain religious, social and animal husbandry practices. Despite extensive studies on different aspect of disease in dairy cattle in past 50 years data on 'optimum antibiotic treatment' for therapeutic management of brucellosis is either not available or is still disputed. In the present study 24 cows (*Brucella* positive) were randomly divided into 4 group *viz*. T_1, T_2, T_3 and T_4, each group comprising of 6 cows and treatment was given to them according to regimen. Combination of long acting Oxytetracyclin, Dihydrostreptomycin, Rifampicin and Isoniazid was found to be most effective followed by long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid combinations. Combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid was reported to be least effective.

Keywords: Brucellosis, Oxytetracyclin, Dihydrostreptomycin, Rifampicin, Marbofloxacin

Introduction
Brucellosis is one of the most serious diseases in developing countries. The rate of infection varies greatly from one country to another and between regions within the country, with highest prevalence in dairy cattle. In general, risk factors such as unrestricted trade, movements of animals, use of local cattle yards or fairs for trading, sending dry animals back to villages for maintenance, use of semen from unscreened bulls for artificial insemination and poor farm hygiene probably attribute to the spread and transmission of the infection.
Despite the advances made in the diagnosis and therapy, brucellosis is still wide spread and prevalent in many developing countries. Bovine brucellosis has become a serious problem in Indian dairy herds because of certain religious, social and animal husbandry practices (Singh *et al*., 2014) [8]. Despite extensive studies on different aspect of disease in dairy cattle in past 50 years data on 'optimum antibiotic treatment' for therapeutic management of brucellosis is either not available or is still disputed. This may be due to intracellular localization of *Brucella* and it's ability to adapt to the environmental condition encountered in it's 'replicative niche' e.g. macrophage, Treatment failure and relapse rates are high and depend on the drug combination and high cost of treatment. Streptomycin has been replaced by newer amino-glycosides and their effects on brucellosis have not been further reported (Skalsky *et al*., 2008) [9]. Finally, the advantage of combination therapy over mono-therapy has not been quantified.
Keeping the above facts in view, the present study was under taken with the objective to compare the different therapeutic regimens against brucellosis in cows.

Material and Methods
Therapeutic regimen
Selection of the animals for the therapeutic regimen was done on the basis of serological tests. The 24 positive cases of brucellosis were randomly divided in to four groups having six animals in each group. However, six healthy animals were used as negative control.

Testing of samples
The samples from suspected animals were processed using MRT, RBPT, STAT for identification of brucella positive animals.

Standard tube agglutination test (STAT)
The antigen was procured from biological products division, Indian Veterinary Research Institute Izzatnagar (U.P.)

Table 1: Therapeutic regimen in different treatment groups

Group	No. of animal	Drugs
T_1	6	Inj. Oxytetracycline (Long acting) @20 mg/kg b.wt I/m every 3rd day (7 dose)
T_2	6	Inj. Enrofloxacin (Long acting) @10mg/kg b.wt I/m every 3rd day (7 dose)
T_3	6	Inj. Marbofloxacin(Long acting) @ 10 mg/kg b.wt I/m every 3rd day (7 dose)
T_4 (Positive control)	6	No treatment
T_5 (Negative control)	6	No treatment

Note: Common treatment was given to the groups T1, T2 and T3
Inj. Dihydrostreptomycin @ 20 mg/kg b.wt sid i/m for 15 days,
Tab. Rifampicin and Isoniazid (600mg+300mg) @ 6 tab sid for 21 days and
Hepato protective liquid (Liv 52 @100ml) was given to each animal under treatment.

Antigen
Brucella SAT antigen is a suspension of a pure smooth culture of *Brucella abortus* strain 99 in phenol saline.

Methodology
The standard tube agglutination test was performed according to Weybridge technique (Alton *et al*., 1975) [1]. All the serum samples were tested up to minimum of nine dilutions. For high titred sera, more dilutions were prepared in order to achieve end point titre. In brief, eleven agglutination tubes were placed in a rack. Further, 0.8 ml of 0.5 per cent phenol saline was taken in a first tube and 0.5 ml in rest of the tubes. 0.2 ml of serum was added in the first tube, mixed well and 0.5 ml of diluted serum transferred to the second tube. The process was continued up to the ninth tube and 10th tube was kept for control tube, 0.5 ml was discarded from the last tube after mixing. Then 0.5 ml *B.abortus* plain antigen was added to each tube and mixed thoroughly. This provided a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160 and so on. Considering the special significance of 50 per cent end point, a control tube was set up to simulate 50 per cent clearing by mixing 0.25 ml antigen with 0.75 ml of 0.5 per cent phenol saline in an agglutination tube. All the tubes were incubated at 37ºC for 24 hour

Table 2: Procedure for standard tube agglutination test

Tube No.	1	2	3	4	5	6	7	8	9	10	11
a. 0.5%Phenol saline	0.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
b. Test Serum	0.2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5
Mixed thoroughly and transferred until tube no. 9 discarded 0.5 ml from tube no. 11 i.e. discard tube.											
c. Brucella abortus plain antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
d. Final dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560		

Interpretation
The results were read by comparing with the control. The highest dilution of the serum which showed 50 per cent agglutination was taken as end titre. The titre so obtained was expressed in unit system by doubling of the serum titre as International Unit (IU) per ml of serum. The antibody titre of 1:80 (160 IU / ml) and above was taken as positive for brucella.

Therapeutic response study
Therapeutic response of the treatment was assessed on the basis of decrease in the antibody titre of all the animals under the treatment were measured using STAT on day 0 (pre-treatment) and days 15, 30 and 45 (post treatment).

Statistical analysis
To know the effect of treatment, the data were analyzed by the t-test and analysis of variance with one way classification and Duncan's Multiple Range Test (Snedecor and Cochran, 1994) [10].

Results
Group T_1
Treatment with long acting Oxytetracyclline, Dihydrostreptomycin, Rifampicin and Isoniazid combination resulted in decrease in serotitre of group T_1 cows when compared with zero day (pre-treatment). However, the mean serotitre on different treatment intervals was significantly decreased (i.e. 136.66±100.72 IU, 56.66±20.92 IU and 33.33 ±4.22 IU on days 15th, 30th and 45th). During different observation period, decrease in serotitre ranged 20-40 IU on 15th day in all cows except one cow (640 IU). On 45th day of treatment serotitre of two cows were found normal (20 IU) and another four cows were found (40 IU). On the basis of serotitre on days 15th and 30th the recovery was 83.33% (5/6) and on day 45th post treatment, there was 100% recovery in the *Brucella* infected cows.

Group T_2
Treatment with long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid combination resulted in decrease in serotitre of group T_2 cows when compared with zero day (pre-treatment). The mean serotitre on days 15th (80.00±48.17 IU) and 30th (56.66±20.92 IU) were significantly lower in comparison to day Zero. However, on day 45th post treatment, serotitre was slightly increased in comparison to days 15th and 30th although the serotitre was significantly lower in comparison to day Zero. During the observation period, decrease in serotitre ranged 20-40 IU on 15th day in all cows except one cow (320 IU). On day 30th the serotitre of that one cow was decreased 160 IU. Moreover, the serotitre on day 45th of treatment, that one cow again showed marked increased (i.e. 640 IU) in serotitre. Although the serotitre of two cows were found normal (20 IU) and another three cows had (40 IU). On the basis of serotitre, 83.33% (5/6) recovery was obtained during entire period of treatment.

Group T_3
Treatment with long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid combination resulted in decrease in serotitre of group T_3 cows when compared with zero day (pre-treatment). After treatment the mean serotitre value was significantly lower i.e. 146.66±53.30 IU on day 15th when compared with zero day (pre-treatment).

However the mean serotitre on day 30th (200.00±97.98 IU) and on day 45th (306.66±112.03 IU) was slightly higher in comparison to day 15th post treatment. During the observation period, decrease in serotitre ranged 40-320 IU on day 15th. On day 45th of the treatment the serotitre of all the cows (ranged 80-640 IU) was increased when compared to the serotitre on day 15th. On the basis of serotitre, only two cows (33.33%) showed the cure on days 15th and 30th. However, on day 45, all cows of this group had serotitre above normal limit (1:40 IU).

Group T_4

On other hand, on day zero the serotitre of group T_4 (untreated) cows on STAT ranged 320-1280 IU. The serotitre of three cows showed increasing trends and three rest remain unchanged on 15th day, while on 30th day four out of six cows showed escalating trend in the serotitre and two rest remain unchanged in comparision to zero day. On 45th day serotitre ranged 1280-2560 IU showed the strong serotitre.

Response to therapy was evaluated on the basis of results of STAT on pre and post treatment (days 0, 15th, 30th and 45th). The cows of group T_1 (received combination of long acting Oxytetracyclline, Dihydrostreptomycin, Rifampicin and Isoniazid) showed better recovery on day 15th post treatment except one cow. Although better recovery (100%) in all the cows reported on day 45th. The cows of group T_2 (received combination of long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid) showed recovery on day 15th except one cow and that remain infective during the entire period of treatment rather the serotitre was increased on day 45th of treatment and overall 83.33% (5/6) recovery was noticed. The cows of group T_3 (received combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid) showed decreasing trend in serotitres (2/6) on 15th day of treatment however, the five cows showed increased serotitre on day 45th when compared with serotitre of cows on days 15th and 30th post treatment.

Therefore, combination of long acting Oxytetracyclline, Dihydrostreptomycin, Rifampicin and Isoniazid found to be most effective followed by combination of long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid and least being combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid.

Table 3: Pre and post treatment sero-titre (IU) of brucella infected cows

Group	Interval (days)	Sero-titre of brucellosis in individual cow					
		1	2	3	4	5	6
T_1 Oxytetracycline (LA) + Dihydrostreptomycin + Rifampicin+Isoniazid	0	640	1280	12 80	1280	1280	1280
	15	40	640	20	40	40	40
	30	40	160	40	20	40	40
	45	20	40	40	40	40	20
T_2 Marbofloxacin (LA) + Dihydrostreptomycin+ Rifampicin+Isoniazid	0	1280	1280	1280	1280	1280	640
	15	40	40	20	40	20	320
	30	40	40	40	20	40	160
	45	40	20	40	20	40	640
T_3 Enrofloxacin (LA) + Dihydrostreptomycin+ Rifampicin+Isoniazid	0	1280	1280	1280	1280	640	1280
	15	320	80	40	320	80	40
	30	320	80	40	640	40	80
	45	640	80	80	640	80	320
T_4 Positive Control	0	1280	1280	1280	320	320	640
	15	1280	1280	1280	640	1280	1280
	30	1280	1280	2560	640	1280	1280
	45	1280	1280	2560	1280	1280	1280
T_5 Negative Control	0	Nil	Nil	Nil	Nil	Nil	Nil
	15	Nil	Nil	Nil	Nil	Nil	Nil
	30	Nil	Nil	Nil	Nil	Nil	Nil
	45	Nil	Nil	Nil	Nil	Nil	Nil

Table 4: Mean values of serum antibody titre in *Brucella* infected cows in different treatment groups

Group	Observation period (in days)			
	Pre-treatment	Post treatment		
	0	15th	30th	45th
T_1	$1173.33^{aA} \pm 106.67$	$136.66^{bB} \pm 100.72$	$56.66^{bB} \pm 20.92$	$33.33^{cB} \pm 4.22$
T_2	$1173.33^{aA} \pm 106.67$	$80.00^{bB} \pm 48.17$	$56.66^{bB} \pm 20.92$	$133.33^{bcB} \pm 101.41$
T_3	$1173.33^{aA} \pm 106.67$	$146.66^{bB} \pm 53.30$	$200.00^{bB} \pm 97.98$	$306.66^{bB} \pm 112.03$
T_4 Positive Control	$853.33^{bC} \pm 196.68$	$1173.33^{aB} \pm 106.67$	$1386.66^{aAB} \pm 256.89$	$1493.33^{aA} \pm 213.33$
T_5 Negative Control	0.00	0.00	0.00	0.00

Mean values with different superscript lowercase (between treatment), uppercase (between interval) vary significantly ($p \leq 0.05$)

Discussion

Present findings are similar to the observations made by Milward *et al.* (1984), Barman (1991), Mahato and Sharma (2002), Kumar *et al.* (2005), Nitu *et al.* (2013) and Singh *et al.* (2014) [2, 3, 4, 5, 7, 8]. Moreover, these scientists documented the variable pattern of the recovery using broad spectrum antibiotics, singly or in combination with various schedules. However, these schedules had limited success in complete cure of infection. Several chemo-therapeutic agents have been employed in recent decades for the treatments of *Brucella abortus* infection in cows; however none of these has been entirely successful (Singh *et al.*, 2014) [8].

Dose and duration of application of selected antibiotics in the present study indicate that the long term therapy and the drug combinations used, yielded better results than several other regimens. The oxytetracycline was used it is capable of penetrating intracellularly and inhibits bacterial protein synthesis at the level of ribosomes. The long acting oxytetracycline was selected to save the time and effort and to provide long lasting oxtetracycline concentration in blood plasma, as one injection gave and effective concentration of 0.6µg/ml for 3 days (Nicoletti *et al.,* 1989) [6]. Streptomycin was also used, because it is known to inhibit protien synthesis in gram negative bacteria. Furthermore, streptomycin acts synergistically with oxytetracycline to inhibit growth of *Brucella abortus* within bovine cell culture *in vitro.* Isoniazid and rifampicin combination is commonly used as anti tubercular drug for the treatment of tuberculosis in human being at therapeutic levels. Isoniazid is bacteriocidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms. Rifampicin have good intracellular diffusion and *in vitro* bactericidal activity on *Brucella*, and is effective in experimental brucellosis in mice. It was therefore legitimate to use rifampicin in cows.

Conclusion

In conclusion, when treatment protocols were strictly observed, the combination of long acting Oxytetracyclline, Dihydrostreptomycin, Rifampicin and Isoniazid found to be most effective. On the basis of serum antibody titre by STAT in brucella infected cows, the recovery was 100% on day 45th of treatment. However, this recovery may be for limited period. To insure the 100% recovery, there must be observation on antibody serotitre of the same cows after next calving and the antibody sero-titre by STAT should be less than 1:80 IU. Consequently, at the present time this treatment would probably be of limited value in breeding animals. However, further research may yield a regimen which could be useful for the treatment of brucellosis. Developing countries like India where test and slaughter policy cannot be implemented strictly due to various social, emotional, mythological and economical reasons. We must do some Intensive efforts towards therapeutics to save the livestock.

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