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Investigation on the Seroprevalence and Pollution Severity to *Brucella abortus*and *Brucella melitensis* Bacteria in Cows and Sheeps Livingin the Villager Region of Toyserkan City, Hamedan, Iran

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Abstract: Brucella are very small, gram-negative coccobacilli that cause a zoonosis called brucellosis. These bacteria enter the body through mucous membranes. The blood carries Brucella to organs such as the liver, spleen, bone marrow and kidneys where they cause lesions. Symptoms of brucellosis include fluctuating fever, chills, sweating, headache, muscle pain and weight loss. One species of Brucella called *B. abortus* infects the placenta and fetus of gestating cows and causes the fetus to abort. When humans are infected by this organism they develop a severe fever. Cow and sheep production in this region is an important livelihood source. In this study, 300 milk samples were collected from villages of Toyserkan City in seasons autumn 2009 were collected randomly from cows and sheeps of different ages This milk samples were examined for detecting *Brucella abortus* and *Brucella melitensis* with Milk Ring Test (MRT). From 300 milk samples which collected from milk samples about *B. abortus* 21 (14%) and *B. melitensis* 18 (12%) showed positive reaction with Milk Ring Test.

Key words: *B. abortus*, *B. melitensis*, Milk Ring Test (MRT), sheep, cows, village

INTRODUCTION

Brucella, a Gram-negative bacterium belonging to the a2-proteobacteriacae is a zoonotic pathogen that induces abortion and sterility in domestic mammals and a chronic fever in humans known as Malta fever (Smith and Ficht, 1990). Brucellosis continues to be a problem for animal and humans throughout the world (Bricker, 2002).

Brucellosis in cattle is usually caused by biovars of *Brucella abortus*. In some countries, particularly in Southern Europe and Western Asia where cattle are kept in close association with sheep and goats, infection can also be caused by *B. melitensis*.

This picture will be a significant zoonotic problem for cattle and human health. Refai (2002) has reported that only *B. melitensis* biovar 2 was responsible of brucellosis abortion cases which were taken from sheep, goat, cattle and camel in Saudi Arabia in 10 years period.

Some investigators reported that *B. melitensis* was regularly isolated from cattle in Southern Europe and it was considered that *B. melitensis* has replaced of *B. abortus* in cattle (Godfroid and Kasbohrer, 2002). The nature of infection and the immune response in cattle against *B. melitensis* is still unknown.

The transmission of the infection to human beings occurs through breaks in the skin following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas (FAO, 2003). Until recently, only the Milk Ring Test (MRT) with a sensitivity of about 89% (Hunter and Allen, 1972) was available for detection of brucella antibodies in fresh milk. Cattles production in Toyserkan City is an important livelihood source. Problems effecting cattles health is affecting their owners economically. It is hoped that this investigation with its inspection of spreading rate among the region's cattles and presentation of the needed statistic data would help to improve milk's quality as well as the region's economy.

MATERIALS AND METHODS

Sources of samples: Sampling for detecting *Brucella abortus* and *Brucella melitensis* in Toyserkan cattles were taken in autumn 2009. In autumn, 300 milk samples from 30 villages are collected randomly from cows of different ages. This study was done at laboratory of Biology, Payam Noor University of Toyserkan, Iran at 2009.

Collection of samples: The teat were disinfected with alcohol and then allowed to dry. The first streak of milk was discharged into sterile tubes. These tubes were kept in ice and then transferred to laboratory.

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Milk Ring Test (MRT): A modification of the procedure described by Alton *et al*. (1988) was used in the MRT. The test was performed in sterile tubes and the milk samples were tested within 2 h after collection. Fresh milk samples from the individual sheep and cow were thoroughly shaken and 1 mL of the milk was transferred into to a fresh tube and 30 μL of MRT antigen was added.

The tubes were mixed thoroughly and incubated at 37°C for 3 h. When the antigen precipitated in the bottom of tubes and/or the purple band occurred at the top of milk, these samples were then regarded as positive (Turutoglu *et al*., 2003).

Statistical analysis: The data were analyzed by one-way Analysis of Variance (ANOVA) and Tukey test. Values of $p<0.05$ were considered statistically significant (Steel *et al*., 1997).

RESULTS AND DISCUSSION

From 150 milk samples which collected about *B. abortus* 21 (14%) showed positive reaction and From 150 milk samples collected about *B. melitensis* 18 (12%) showed positive reaction.

About *B. abortus* 129 (86%) and *B. melitensis* 132 (88%) showed negative reaction with Milk Ring Test. As a total, from 300 milk samples, 39 (13%) showed positive reaction and 261 (87%) showed negative reaction. Statistical analysis revealed that there are significant statistical differences between two groups ($p<0.05$). In Table 1-5 where results of statistical analysis are presented values at each column for groups. All Fig. 1 and 2 were drawed using the Statistical Software Package (SPSS Version 16).

Brucellosis is endemic in Toyserkan and as elsewhere causes severe economic losses to livestock farmers and ranchers and is a serious risk to human health. Studies in various parts of the region indicate that the disease is widespread among cattle populations particularly in ranches, livestock breeding centres and dairy farms. Cattle, sheep and goats are the principal farm animals in Toyserkan.

Milk is an important source of nutrient to man and animals. Milk meant for human consumption must be free from pathogenic organisms. Brucellosis in animals causes abortion infertility, neonatal mortality and hygroma and is spread by materials contaminated by body fluids including milk. The prevention of brucellosis in human is the basis for the advocacy of milk pasteurization worldwide. Despite the existence of regulations that require milk pasteurization most (over 75%) of milk marketed in many developing countries is sold raw through local informal pathways (Staal *et al*., 2000).

Although, the disease prevalence in cattle has been reported to be quite high in Iran, its serological presence in cattle of Toyserkan is reported to be low in comparison. In the present study the seroprevalence of brucellosis in cattles were found to be 39 (13%).

Table 1: The group comparisons with each other by One way Anova

Species	Sum of squares	df	Mean square	F	Sig.
Between groups	9.759	2	4.879	22.212	0.000
Within groups	65.241	297	0.220	-	-
Total	75.000	299	-	-	-

Table 2: The evaluate statistically significant (multiple comparisons)

Species (Tukey HSD)					95% Confidence interval	
(I) Septic	(J) Septic	Mean difference (I-J)	SE	Sig.	Lower bound	Upper bound
Non septic	*Brucella melitensis*	0.49425*	0.11422	0.000	0.2252	0.7633
	Brucella abortus	-0.50575*	0.10631	0.000	-0.7562	-0.2553
Brucella melitensis	Non septic	-0.49425*	0.11422	0.000	-0.7633	-0.2252
	Brucella abortus	-1.00000*	0.15055	0.000	-1.3546	-0.6454
Brucella abortus	Non septic	0.50575*	0.10631	0.000	0.2553	0.7562
	Brucella melitensis	1.00000*	0.15055	0.000	0.6454	1.3546

*The mean difference is significant at the 0.05 level

Table 3: Means for groups in homogeneous subsets are displayed

		Subset for alpha = 0.05		
Tukey HSD septic	N	1	2	3
Brucella melitensis	18	1.0000	-	-
Non septic	261	-	1.4943	-
Brucella abortus	21	-	-	2.0000
Sig.	-	1.0000	1.0000	1.0000

Table 4: Report species

Septic	Mean	N	SD
Non septic	1.4943	261	0.50093
Brucella melitensis	1.0000	18	0.00000
Brucella abortus	2.0000	21	0.00000
Total	1.5000	300	0.50084

Table 5: Anova

Species	Sum of squares	df	Mean square	F	Sig.
*Septic between groups (Combined)	9.759	2	4.879	22.212	0.000
Linearity	1.600	1	1.600	7.284	0.007
Deviation from linearity	8.159	1	8.159	37.141	0.000
Within groups	65.241	297	0.220	-	-
Total	75.000	299	-	-	-

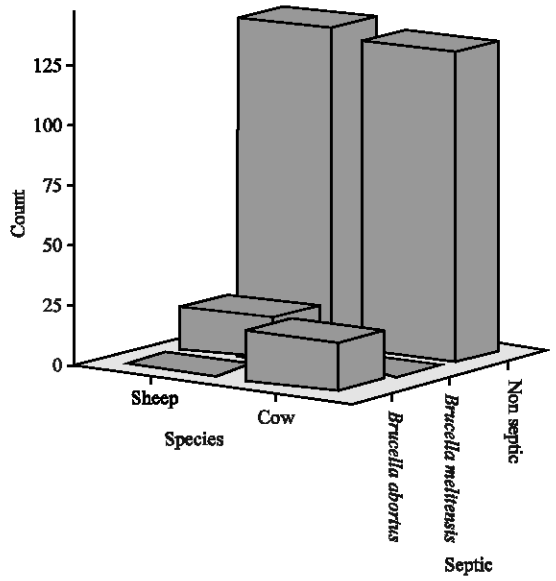

Fig. 1: Multiple comparisons

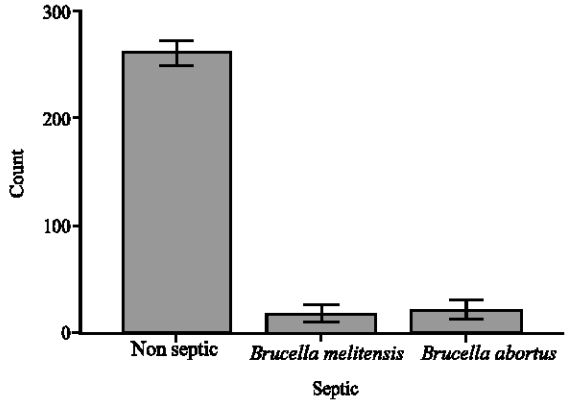

Fig. 2: Number of contaminated cattles to *B. abortus* and *B. melitensis*

CONCLUSION

Seroprevalence of the disease causing abort in cattles were found to be high in this region. Therefore, to eradicate such disease and to reduce economical looses more detailed studies should be made in this region to make proper and effective challenge.

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