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Seroprevalence of *Brucella* Infection among Buffaloes in Gharbyia Governorate

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Abstract: A total 2759,3615 and 2355 serum sample collected from buffaloes from various livestock farms in Gharbyia governorat during period Jan.2010 till Dec.2012 were analysed for brucella antibodies using serological tests including BAPAT,RBPT, Rivanol T,TAT,Modified TAT and finaly ICA. Out of 2759 blood samples collected from Jan to Dec. 2010, the percentage of positive reactors were 1.69%,1.52%, 1.02%, 1.05% and 0.71% using ICA as confirmatory test from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud, respectively. Highest percentage of infection in Tanta then Basyoon,AL Santa finaly Kafer EL Zyaat, Out of 3615 blood samples collected Jan to Dec. 2011. from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud, the percentage of positive reactors using ICA as confirmatory test were 1.53%, 1.92%, 1.18, 0.88% and,0.73% respectively. Highest percentage of infection in Basyoon then Tanta, AL Santa finaly Kafer EL Zyaat, .Out of 2355 blood samples collected Jan to Dec. 2012. from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud, the percentage of positive reactors were 0.42%, 0.76%, 0.40, 0.24% and,0.33% respectively. Highest percentage of infection in Basyoon then Tanta, AL Santa finaly Kafer EL Zyaat,. The lowest percentage of infection in Samanoud.Total percentage of positive reactors in was 36(1.30%),51(1.41%) and 11(0.47%)in 2010, 2011 and 2012 respectively. Prevalence rates in buffaloes, was generally higher in Gharbyia governorate in 2011,2010 than 2012. due to the great improvement in control and mangament occure in 2012 than two previous years,also due to massive vaccination and elimination or control of infection in sheep and goat flocks reduce spread of the disease in buffaloes, results of modified TAT were observed to be more clear and easy to read than routine TAT. It was concluded that modified SAT may be used as an alternate to routine TAT in the diagnosis of brucellosis which may reduce the chances of cross reaction.Out of 14 blood sample 14 *Brucella DNA* were detected using PCR from seropositive buffaloes had molecular weight 731 bp which typed as *Br.melitensis* biovar 3.

Key words: Brucella · Diagnosis · Serology · PCR · Buffaloes

INTRODUCTION

Brucellosis is one of the most common zoonotic diseases worldwide, It was first reported in Egypt in 1939 and is now endemic [1] and as such poses a major threat to human health and animal production [2-4].

It remains a major problem in the Mediterranean region. It can cause appreciable economic losses in the livestock industry because of abortions, decreased milk production, sterility and veterinary care and treatment costs [5]. The Seroprevalence of brucellosis among buffaloes within a village in the Gharbyia governorate was estimated at 1.7% in 2003 [6]. Incidence of positive reactors was 10.2% in buffaloes [7]. The seroprevalence of brucellosis was estimated 0.3 %, among buffalo population [2-8].

In Egypt, the close contact between farmers and their animals considered to be the major risk factors for human infection with *Brucella* spp. El Sherbini *et al.* [6], Glynn and Lynn [9] suggested measures aimed at the occurrence of brucellosis in animals are the most effective means of reducing human infection. Up to 85% of the cows and buffaloes in Egypt are reared as household animals in small herds typically of less than five animals.

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They have frequent contact with sheep and goats, which are kept as household animals in the farmers' houses as reported by Aidaros [10] and Ahmed *et al.* [11].

Brucella isolates recovered in Egypt typed as *B. melitensis* biotype 3. [1,7,12].

Reliable estimates of the frequency of brucellosis in are not available [13].The disease is widespread but more concentrated around major animal markets, the urgent need for an improved control strategy[14].Increased prevalence of brucellosis in buffaloes in Egypt can be attributed to raising mobile sheep and goats with buffaloes in villages as stated by Samaha *et al.* [15]. Elimination or control of infection in sheep and goat flocks can reduce spread of the disease in cattle and buffaloes [2].

Several serological tests that are sensitive but less specific are used for screening purposes and are followed by more specific tests for confirmation [12], Also serological cross reactions with other bacteria [16]. To overcome this problem, the standard TAT was modified and its comparative efficacy with TAT was described by Nasir *et al.* [17]. Seroprevalence of *Brucella* found 3.72 % and 0.6% in female and male buffaloes respectively.These findings suggest an alarming situation of buffaloes brucellosis [18].

Many authers suggested that a project should be financed to establish or develop a feasible regional brucellosis control programme Refai, [19], to survey different animal species and evaluate the applicability of several serological and molecular biological techniques using specific diagnostics prepared by genetic engineering; and to characterize bacteriological and genetic properties of *Brucella* isolates recovered from different animals in various countries of the region and compare them to the standard Elberg strain.

The frequency of buffaloes brucellosis are useful elements for building effective control strategies. So our objectives are to estimate the frequency of buffaloes brucellosis in Gharbyia Governorate and typing of Brucella strain causing infection .

MATERIALS AND METHODS

A study was carried out between January 2010 and Dec. 2012 to estimate the seroprevalence of brucellosis among buffalos in Gharbyia governorate; an area of high density of livestock in the Nile Delta.

Sampling:

Collection of Blood Samples: Two blood samples (with and without EDTA for PCR and serology) were collected from 2759 buffaloes in 2010,3615 buffaloes in 2011 and 2355 buffaloes in 2012 and centrifuged in the laboratory at 2000 rpm for 20 min and sera were separated in a sterile prelabelled eppindorf tube (1.5 ml)., labeled and stored at -20°C until analysed.

Serological Examination for Brucellosis: Seroprevalence of brucellosis was investigated using commonly used serological tests and collected sera examined with : Buffered Acidified Plate Antigen Test [20], Rose Bengal Plate Test (RBPT) [20], Tube Agglutination Test (TAT) [20], Rivanol Test (Riv.T) [20], Modified Tube Agglutination test (Modified) with EDTA [21] and Immunochromatographic assay (ICA) [22]. All the used antigens were kindly supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Molecular Examination of Blood:

Extraction of DNA from Blood Samples for PCR Assay: DNA was extracted from blood using Gene JET™ Genomic DNA Purification Kit, Quick Protocol QP14.

DNA Amplification:

Detection and Identification of PCR Product: PCR amplification for detection of Brucella DNA from blood samples of infected buffaloes as described by Leal-Klevezes *et al.* [23]. PCR products was analyzed by electrophoresis through 1.5 % agarose gel stained with Ethidium bromide solution(0.5mg/ml) and visualized under an ultraviolet transilluminator and photographed. Visible band of appropriate size of 498 bp for B. abortus and 731 bp for B.melitensis were considered positive.

Oligonucleotide Primers: *B. abortus, B. melitensis* and IS711 primers sequence were as described Bricker and Halling [24].

Amplification of *Brucella*-DNA and detection of PCR products. PCR conditions were performed as described by Bricker and Halling [24].

Statistical Analysis: Data were subjected to statistical analysis using t-test according to Snedecor and Cochran [25].

Table 1: Sequences of the oligonucleotide primers used in PCR assay .

Primer	Sequence (5´-3´)
B.abortus-specific primer	GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC
B.melitensis-specific primer.	AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA
IS711-specific primer	TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT

RESULTS AND DISCUSSION

Results of this investigation are presented in Table (1 and 2) which show that out of 2759 blood samples collected from Jan to Dec. 2010, the percentage of positive reactors were 1.69%, 1.52%, 1.02%, 1.05% and,0.71% using ICA as confirmatory test from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud, respectively.Total percentage of positive reactors in Gharbyia governorate in 2010 was 36(1.30%) .Highest percentage of infection in Tanta then Basyoon,AL Santa finaly Kafer EL Zyaat,. The lowest percentage of infection in Samanoud.

Our resultes nearly to that of El Sherbini *et al.* [6] who reported that the seroprevalence within a village in the Gharbyia governorate was estimated at 1.7% in 2003.also less than that of Shafee *et al.* [26] who mentioned that seroprevalence in female buffaloes as 3.72 % and 3.88 % using RBPT and ELISA respectively. prevalence in male was 0.6% using both the tests., also the results were agree with Refai [2] who reported that increased prevalence of brucellosis in buffaloes in Egypt can be attributed to raising sheep and goats with buffaloes in villages. Most sheep or goat herds in Egypt are mobile which can contaminate pastures and spread brucellosis to other animals (e.g., buffaloes). This movement is a major risk factor for failure of brucellosis eradication programs. Elimination or control of infection in sheep and goat flocks can reduce spread of the disease in cattle and buffaloes. The prevalence of brucellosis in buffalo comparatively lower than reported 3.97% using (RBPT) and (TAT) by Faqir [27]. Similarly a much lower prevalence of 8.5 % was recorded in buffaloes in Quetta by Shafee *et al.* [18]. Borrielo *et al.*, [28] who attributed this to the fact that buffaloes are naturally resistant to brucellosis as buffaloes naturally resistant to B. abortus determining a correlation between the BB genotype and resistance to B. abortus infection. Further, these findings are in agreement with Abbas *et al.*, [29] and Iftikhar *et al.*,[30] who reported higher prevalence of brucellosis in cattle (10.5%) than (1.9%) buffaloes,and comparatively lower than that of Samaha *et al.*, [15] who mentioned that was 4.11% by the BAPAT, 3.52% by RBPT, was 4.8% by SAT and 3.37% by the Rivanol test. Also the seroprevalence less than that of Refai, *et al.* [7]who reported that incidence of buffaloes positive reactors by SAT was 10.2% and 7.8% by the Rivanol test and RBPT.Our results less than that of Ghazy *et al.*, [12], 11.9% using RBPT followed by TAT (9.5%),

The incidence of brucellosis in buffaloes in Egypt was found to be lower (0.43-7.5%) than by Ghazi *et al.* [12]. Brucella melitensis biovar-3 was isolated from tissue specimens collected from the brucella-positive seroreactive buffaloes during obligatory slaughter [2].

Results presented in Table (3) show that out of 2759 blood samples collected, the percentage of positive reactors 1/40 or more was 0.98%,doubtful 1/20 was 0.29%, and negative 1/10 % was 98.7% using SAT, While the percentage of positive reactors 1/40 or more was 0.87%,doubtful 1/20 was 0.22%, and negative 1/10 % was 98.9% using Modified SAT from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud. The present findings (Table 3, 6 and 9) similar and in accordance with that conducted by Carin and Trap [31], who found that EDTA added SAT did not result in a decrease in the antibody of infected cattle but it decreased the titre of non specific reactions and was preferable to the SAT. Trap *et al.*, [32] found that EDTA reduced the reaction rate of samples in infected herds from 82.6 to 80.7%, while heating reduced the rate by 10%. Romakhov *et al.*,[33] studied the value of EDTA test for classifying doubtful reactions in comparison to the SAT and confirmed by tests on serum samples from healthy, infected and vaccinated cattle. Corbel [34] reported that the sera in which the agglutinating activity was entirely attributable to EDTA-labile agglutinins, a complete or almost complete loss of titre occurred in the presence of a chelating agent. The present findings are in accordance with those of Otto *et al.*, [16], who reported that non specific reactions with brucella could be reduced by addition of EDTA to the diluent. Macmlillan and Cockrem [35] stated that agglutination reaction was sufficiently affected by the action of EDTA. The results of this study indicate that Modified (EDTA added) SAT may be used as an alternate to routine SAT in the diagnosis of brucellosis, which may reduce the chances of cross reactions [17].

Results in Table (4 and 5) show that out of 3615 blood samples collected from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud, the percentage of positive reactors using ICA as confirmatory test were 1.53%,

Table 1: Seroprevalence of brucellosis among buffaloes from different districts of Gharbyia governorate from Jan to Dec. 2010

		BAPAT		RBPT		Rivanol T.		TAT				Modified SAT				ICA	
	Examined																
City	buffaloes	+ve	%+ve	+ve	%+ve	+ve	%+ve	+ve	± ve	%+ve	-ve	+ve	± ve	%+ve	-ve	+ve	%+ve
Tanta	591	10	1.69	10	1.69	8	1.35	7	2	1.52	582	6	1	1.18	584	10	1.69
Basyoon	919	15	1.63	14	1.52	13	1.41	11	3	1.52	905	10	2	1.30	907	14	1.52
Kafr El-Zyat	588	6	1.02	6	1.02	5	0.85	3	2	0.85	583	3	2	0.85	583	6	1.02
AL Santa	381	4	1.05	4	1.05	4	1.05	4	1	1.31	376	3	1	1.05	377	4	1.05
Samanoud	280	2	0.71	2	0.71	2	0.71	2	0	0.71	278	2	0	0.71	278	2	0.71
Number of examined	2759	37									2724				2729		
Number of Reactors		37		36		32		27	8			24	6			36	
Incidence of reactors	1.34 %	1.34%		1.30%	1.16%	0.98%	0.29%	1.26	98.7%	0.87	0.22	1.09	98.91	1.30			

RBPT: Rose Bengal Plate Test, TAT: Tube Agglutination Test, Modified SAT: Modified Serum Agglutination Test ICA:Immunochromatographic assay. SAT titer : (-ve negative 1:10) (± ve doubtful1:20) or more (+ve positive 1:40)

Table 2: Seroprevalence of brucellosis among buffaloes from different districts of Gharbyia governorate from Jan to Dec. 2010 according to ICA .

	Tanta	Basyoon	Kafer EL Zyat	AL Santa	Samanoud	Total
Number of examined	591	919	588	381	280	2759
Number of Reactors	10	14	6	4	2	36
Incidence of reactors	1.69	1.52	1.02	1.05	0.71	1.30%

Table 3 : comparison of TAT and Modified TAT

		Antibody titer		
Tests	No. of samples	1:10 (negative)	1:20 (doubtful)	1:40 or more (positive)
TAT	2759	2724(98.7 %)	8(0.29 %)	27(0.98 %)
TAT (modified)	2759	2729(98.9%)	6(0.22% %)	24(0.87% %)

Table 4: Seroprevalence of brucellosis among buffaloes from different districts of Gharbyia governorate from Jan to Dec. 2011.

		BAPAT		RBPT		Rivanol T.		TAT				Modified SAT				ICA	
	Examined																
City	buffaloes	+ve	%+ve	+ve	%+ve	+ve	%+ve	+ve	± ve	%+ve	-ve	+ve	± ve	%+ve	-ve	+ve	%+ve
Tanta	715	12	1.68	11	1.53	10	1.39	8	2	1.53	705	7	1	1.12	707	11	1.53
Basyoon	1245	25	2.0	24	1.92	22	1.77	19	3	1.77	1223	16	2	1.45	1227	24	1.92
Kafr El-Zyat	679	8	1.18	8	1.18	6	0.88	4	2	0.88	673	3	2	0.74	674	8	1.18
AL Santa	566	5	0.88	5	0.88	4	0.71	3	2	0.88	561	3	1	0.71	562	5	0.88
Samanoud	410	3	0.73	3	0.73	3	0.73	2	1	0.73	407	2	1	0.73	407	3	0.73
Number of examined	3615										3570				3577		
Number of Reactors		53		51		45		35	10			31	7			51	
Incidence of reactors	1.36 %	1.47%	1.41%	1.24%	0.97%	0.28%	1.24	98.76	0.86%	0.19%	1.05	98.9		1.41			

Table 5: Seroprevalence of brucellosis among buffaloes from different districts of Gharbyia governorate from Jan to Dec. 2011.

	Tanta	Basyoon	Kafer EL Zyat	AL Santa	Samanoud	Total
Number of examined	715	1245	679	566	410	3615
Number of Reactors	11	24	8	5	3	51
Incidence of reactors	1.53%	1.92%	1.18%	0.88%	o.73%	1.41 %

Table 6 : comparison of TAT and Modified TAT

		Antibody titer		
Tests	No. of samples	1:10 (negative)	1:20 (doubtful)	1:40 or more (positive)
TAT	3615	3570(98.76%)	10(0.28 %)	35(0.97%)
Modified TAT	3615	3577(98.9%)	7(0.19%)	31(0.86 %)

Table 7: Seroprevalence of brucellosis among buffaloes from different districts of Gharbyia governorate from Jan to Dec. 2012.

		BAPAT		RBPT		Rivanol T.		TAT				Modified SAT				ICA	
City	Examined buffaloes	+ve	%+ve	+ve	%+ve	+ve	%+ve	+ve	± ve	%+ve	-ve	+ve	± ve	%+ve	-ve	+ve	%+ve
Tanta	472	2	o.42	2	o.42	2	o.42	1	1	o.42	470	1	0	o.21	471	2	o.42
Basyoon	661	6	0.91	5	0.76	4	0.61	3	2	0.76	656	2	2	0.61	657	5	0.76
Kafr El-Zyat	500	2	0.40	2	0.40	2	0.40	1	0	0.20	499	1	0	0.20	499	2	0.40
AL Santa	419	1	0.24	1	0.24	1	0.24	1	0	0.24	418	1	0	0.24	418	1	0.24
Samanoud	303	1	0.33	1	0.33	1	0.33	1	0	0.33	302	1	0	0.33	302	1	0.33
Number of examined	2355	12		11		10		7	3		2345	6	2		2347		
Number of Reactors		12		11		10		10			8			11			
Incidence of reactors		0.51	0.47	0.42	0.42		0.34		0.47								

Table 8: Seroprevalence of brucellosis among buffaloes from different districts of Gharbyia governorate from Jan to Dec. 2012 .

	Tanta	Basyoon	Kafer EL Zyat	AL Santa	Samanoud	Total
Number of examined	472	661	500	419	303	2355
Number of Reactors	2	5	2	1	1	11
Incidence of reactors	0.42	0.76	0.40	0.24	0.33	0.47

Table 9 : comparison of TAT and Modified TAT

		Antibody titer		
Tests	No. of samples	1:10 (negative)	1:20 (doubtful)	1:40 or more (positive)
TAT	2355	2345(99.58%)	3(0.13 %)	7(0.30%)
Modified TAT	2355	2347(99.66%)	2(0.08%)	6(0.25 %)

Table 10: Detection and Identification of Brucella DNA from some blood samples of seropositive obligatory slaughtered buffaloes using PCR

City	NO. of blood samples from seropositive obligatory slaughtered buffaloes	Number and biotyping of Br. biovars detected by PCR	
Tanta	3	3	Br.melitensis biovar 3
Basyoon	5	5	Br. melitensis biovar 3
Kafr El-Zyat	2	2	Br. melitensis biovar 3
AL Santa	2	2	Br. melitensis biovar 3
Samanoud	2	2	Br. melitensis biovar 3
Number of Reactors	14	14	
Incidence		100%	

1.92%, 1.18, 0.88% and,0.73% respectively. Total percentage of positive reactors in Gharbyia governorate was 51(1.41%).Highest percentage of infection in Basyoon then Tanta, AL Santa finaly Kafer EL Zyaat,. The lowest percentage of infection in Samanoud .

Results in Table (6) which show that out of 3615 blood samples collected from Jan to Dec. 2011, the percentage of positive reactors1/40 or more was 0.97%, doubtful 1/20 was 0.28% and negative 1/10 % was 98.76% using SAT, While the percentage of positive reactors 1/40 or more was 0.86%,doubtful 1/20 was 0.19% and negative 1/10 % was 98.9% using Modified SAT from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud .

Results in Table (7 and,8) which show that out of 2355 blood samples collected Jan to Dec. 2012. from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud, the percentage of positive reactors were 0.42%, 0.76%, 0.40, 0.24% and,0.33% respectively.Total percentage of positive reactors in Gharbyia governorate was 11(0.47%). Highest percentage of infection in Basyoon then Tanta, AL Santa finaly Kafer EL Zyaat,. The lowest percentage of infection in Samanoud .

Results in Table (9) which show that out of 2355 blood samples collected from Jan to Dec. 2012, the percentage of positive reactors 1/40 or more was 0.30%, doubtful 1/20 was 0.13% and negative 1/10 % was 99.58% using SAT, While the percentage of positive reactors 1/40 or more was 0.25%,doubtful 1/20 was 0.08% and negative 1/10 % was 99.66% using Modified SAT from Tanta, Basyoon, Kafer EL Zyaat, AL Santa, Samanoud .

Results of this procedure for detection and Identification of Brucella organisms from whole blood sample from seropositive buffaloes by using PCR are presented in Table (10),which proved to be positive by serological tests . Total percentages of detected strains using PCR was 100% (14 out of 14 blood sample) had

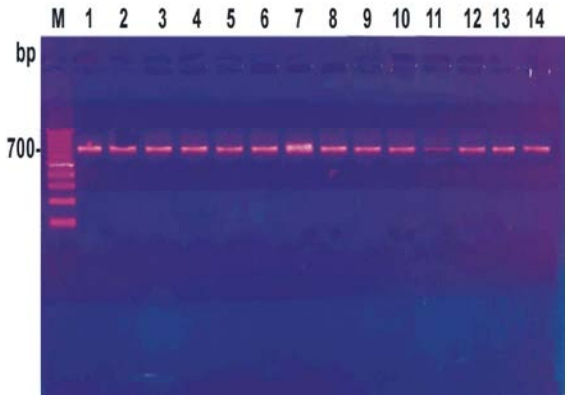

Fig. 1: Ethidium bromide-stained agarose gel electrophoresis of polymerase chain reaction (PCR),Lane M: (Marker) 100-bp DNA ladder, Lane (1-14) whole blood sample from seropositive buffaloes,PCR amplifies, 731 bp. fragment indicated (B.melitensis biovar 3).

molecular weight 731 bp which typed as *Br. melitensis* biovar 3(Fig 1) which agree with that of Montasser *et al.*, [22] and Refai [2] who mentioned that in Egypt, the main isolate in buffaloes is Brucella melitensis biovar 3. Khoudair [36] who reported that *B. abortus* (biovars 1,2 and 4) amplifies a 498-bp product, *B. melitensis* (all biovars) amplifies a 731-bp product and results of bacteriologic isolation from milk and tissues revealed *B. melitensis* biovar 3 [2-15] and agree with that of Bricker and Halling [37] and Bricker and Halling [38], who reported that Br. abortus (biovars 1,2 and 4) amplifies a 498-bp product, *B. melitensis* (all biovars) amplifies a 731-bp product which proved that PCR a rapid and accurate test than culturing methods and might be considered alternative to the traditional culturing methods for *Brucella* diagnosis as screening and confirmatory diagnostic tool for saving cost and time.

Results of this study cleared that infected buffaloes could be screened with a test such as RBPT or BAPAT and confirmed with a more specific test such as Rivanol test and ICA. Similar results obtained by Howard and Smith [39], who reported that RBPT is a good screening test, more efficient, inexpensive and easily performed method in the detection of both early and chronic *Brucella* infection [40].

Our study showed an increase in animals serologically reactive for *Brucella* spp. in Egypt. Prevalence rates in buffaloes, was generally higher in Gharbyia governorate in 2011,2010 than 2012. Variations in infection may be attributed to difference in age, sex, breed, locality, management, stage of infection and immune status of animals [41],environmental factors and stress, which may modulate susceptibility to infection. Its noticed that great improvement in control and mangment occure in 2012 than two last years due to massive vaccination and elimination or control of infection in sheep and goat flocks reduce spread of the disease in buffaloes in 2012 .

In the absence of vaccination and other sanitary measures, this contact structure creates the necessary conditions for sustaining Brucella spp. infection at higher seroprevalence levels than in other regions [2, 42, 43]. So Egypt as having one of the highest rates of human infection worldwide [44].

Finally, this study shows that the finding of higher seroprevalence and to the proximity to the largest animal market in the Nile Delta region in the Gharbyia governorate. By applying different control measures at specific locations it may be possible to maximize public health benefits and to minimize spread of the infection to areas with lower seroprevalence. it could be difficult to implement in Egypt given the intensity of unregulated animal movements [2].

CONCLUSIONS

The results of this study indicate that Modified TAT may be used as an alternative to routine TAT in the diagnosis of buffaloes brucellosis. PCR must be considered alternative to the traditional culturing methods for *Brucella* diagnosis as screening and confirmatory diagnostic tool for saving cost and time. Elimination or control of infection in mobile flocks of sheep and goat ,periodic examination of flocks or newly purchased buffaloes, application of testing and slaughter policies, adoption of vaccination programs and strict quarantine measures. It is easier to sell animals on than to notify the veterinary authorities and wait until they test and slaughter the positive animal. This is also more economical as the compensation received is less than 20% of the market value of the animal and often takes more than a year to receive .Hygienic disposable of placentas and aborted foetuses which disposed by most people into water canals can reduce spread of the disease in buffaloes .

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