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Monoclonal Antibody based Blocking ELISA for Diagnosis of Brucellosis

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ABSTRACT

Background: Porcine brucellosis is a contagious and emerging zoonosis but neglected in most of the endemic countries including India. The disease in pigs is rarely reported due to non-availability of diagnostics or major focus is on bovine brucellosis. Hence, the necessity was felt to diagnose porcine brucellosis by Monoclonal antibody based ELISA, RBPT and STATS for the detection of anti-Brucella antibodies and to record spatial seroprevalence of porcine brucellosis in the country.

Methods: The Rose Bengal Plate Test (RBPT), Standard tube agglutination test (STAT) and Bru Alert monoclonal antibody based c-ELISA was employed to screen 148 sera samples for the presence of Brucella antibody.

Result: A total 148 samples were collected and tested, the overall prevalence of Brucella infection in pig was 43.2% per cent (64/148) by C-ELISA followed by RBPT 40% (60/148) and STAT 25% (40/148) in the study area comparatively high seropositivity was found in female animals. The agreement between the two test was excellent (Kappa = 0.167) and also, chisquare test indicated an evidence of strong diagnostic weapon for detection of swine brucellosis.

Key words: Antibody detections tests, Brucellosis, *C-ELISA*, Porcine, RBPT, STATS.

INTRODUCTION

Porcine brucellosis is a contagious complaint with higher zoonotic disease characterized by infertility and birth of dead or weak piglets in sows, orchitis and infection of secondary coitus organs in boars and lameness and palsy in both relations (WHO, 2009; Woldemeskel, 2013: Thirlwall *et al.,* 2008). The complaint is generally transmitted during coition and by consumption of feed polluted by birth and/or abortion material and uterine discharges (Shimshony, 2009; Ames, 2009). The ingrain of infection substantially occurs in organized swine herds where creatures from different areas are brought in indiscriminately for breeding or fattening purpose without proper illness checks or quarantine. Hence routine screen at the event of every reproductive failure or before intro of new pigs into the grange is authentically substantial. Confirmative conclusion of brucellosis requires segregation of the causal agent but isolation is largely jeopridizing (Ilhan *et al*., 2008). PCR-predicted analysis is not competent for common diagnosis (Yu *et al*., 2010), rose bengal plate test (RBPT) is compounded with false positive results and complement fixation test isn't considered suitable, as swine complement interact with guinea pig complement. The primary binding assays for discovery of anti-Brucella antibodies have been regularized elsewhere (Abdoel *et al.,* 2008) and needs to be imported to the country. The MAb grounded blocking ELISA detected Brucella specific antibodies in vaccinated pigs as early as 5 days post vaccination. The indirect ELISA detected only at 10 days post vaccination. Brucella MAb grounded blocking ELISA has developed sensitiveness (100%) and specificity (99%) over indirect ELISA in detecting brucella antibodies. This technology is cost effective, compared to similar imported kits. This test detects, both IgM and IgG antibodies.

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Hence, present prospective study is aimed to regularize MAb grounded blocking ELISA to ease corroboration of spatial prevalence of porcine brucellosis in Chengalpattu district Tamil Nadu.

MATERIALS AND METHODS

The experiment was conducted during June 2021 to February 2022 at Veterinary University training and Research centre, Melmaruvathur. In this study, swine sera samples were collected, randomly from organized farms and unorganized farms around Chennai and Veterinary University training and Research centre, Melmaruvathur, Tamil Nadu India. Most of the samples were collected, randomly from apparently healthy animals of different age, sex and breed (Pigs). In a few of the animals, serum samples were collected based on history or clinical evidence of

Table 1: Result of swine Brucellosis in organized farms.

Farms	RBPT			C-ELISA			STAT		
	No. of serum samples	Positive	% of positivity	No. of serum samples	Positive	% of positivity	No. of serum samples	Positive	% of positivity
Farm-1	50	28	56	50	30	60	50	20	40
Farm-2	40	20	50	40	20	50	40	13	32.5
Farm-3	20	7	35	20	8	40	20	4	30
Farm-4	20	3	15	20	6	30	20	3	15
Farm-5	18	2	11	18	0	0	18	0	0

Table 2: Result of swine brucellosis in male and female animal.

RBPT		C-ELISA		STAT	
Male	Female	Male	Female	Male	Female
28 (18.9%)	32 (21.6%)	28 (18.9%)	36 (24.3)	16 (10.8%)	15

brucellosis, like abortion, orchitis from 15 pigs. Blood samples (3 ml) were collected from 148 animals by ear vein puncture in sterile vaccutainertubes (5 ml) and were allowed to clot and then centrifuged at 2000 rpm for 15 minutes. Sera were separated and stored at- 20°C until further use.

Serological tests

Rose bengal plate test (RBPT)

The coloured antigen claimed for RBPT was attained from the Division of Biological products, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh and the trial was carried out as per the common protocol of agglutination test (OIE, 2008). Compactly, a droplet of serum (30 µl) was positioned on unstained grease free glass slide and an equal amount of coloured antigen was added and mixed thoroughly with the assist of inoculation circle. The admixture was observed for agglutination/ clumping for one min. and the results were recorded as clumping (+) and no clumping (-).

Monoclonal based blocking ELISA

Monoclonal grounded blocking ELISA kit (Bru Alert) for resolution of brucellosis in bovine was acquired from TRPVB, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India and used for sampling the sera samples. All reagents were permitted to achieve room temperature (22-25°C) before application. All reagents were normalized by inversion. The protocol bestowed by the manufacturer was succeeded to accomplish C-ELISA. Illustration of C-ELISA For each sample, the probability Inhibition (PI) was reckoned as follows applying the sampling and controller values.

PI = 100 - { (Test sample OD/ Negative control)

In disposal to assimilate disparate characteristic experiments and calculate percentage, Chi- squared test and kappa statistics were calculated using MS office 2007 Excel spread sheet, coded and analyzed by SPSS version 20.

RESULTS AND DISCUSSION

Out of 148 samples tested, the prevalence of Brucella infection in pig was 43.2% per cent (64/148) by C-ELISA followed by RBPT 40% (60/148) and STAT 25% (40/148) in the study area. In this study, the per cent positive by STAT is lower than the RBPT. The seroprevalence of brucellosis (43.2%) by competitive ELISA observed in the present study was similar to/ close to that described by previous studies by Shome *et al*. (2018). However, low seropositivty were reported by (Koppel *et al*., 2007; Leuenberger *et al*., 2007) with a seropositivity of 11.3% and 9.5%, respectively. The prevalence is higher in the study area may be due the animal were purchased from highly infected farm. Exotic germplasm of the crossbred animals make them more susceptible under stress conditions (Aulakh *et al*., 2008). Vaccination against swine brucellosis is not practiced in this region and hence continued surveillance and removal of infected pigs should be strictly adopted by using user friendly screening test to make the farmers to eliminate the infected pigs in infected premises.On the basis our finding, there is slight variation in seropositivity between RBPT and C-ELISA, hence RBPT could be a screening test and C-ELISA for confirmatory test for elimination infected animal.

On sex wise distribution high positivity was seen in females. It is observed that 32 (21.6%) 36 (24.3) among pigs by RBPT and c-ELISA respectively.Erythritol content of the placenta influence the multiplication of Brucella in gravid uterus, hence it's predispose female high susceptible to the Brucella infection. Other studies of this aspect also indicated higher infection level in female than male animals. The possibility of venereal transmission being rare and

Table 3: Chi-square tests.

Tests	Value	df	Asymp. Sig. (2-sided)
Pearson chi-square	8.000[a]	8	.433
Likelihood ratio	11.090	8	.197
Linear-by-linear association	.013	1	.909
N of valid cases	10		

a. 18 cells (100.0%) have expected count less than 5. The minimum expected count is .50.

Table 4: RBPT * Elisa cross tabulation.

			Elisa					Total
			.00	6.00	8.00	20.00	30.00	
RBPT	2.00	Count	1	0	0	0	0	1
		% within RBPT	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
		% within Elisa	100.0%	0.0%	0.0%	0.0%	0.0%	20.0%
	3.00	Count	0	1	0	0	0	1
		% within RBPT	0.0%	100.0%	0.0%	0.0%	0.0%	100.0%
		% within Elisa	0.0%	100.0%	0.0%	0.0%	0.0%	20.0%
	7.00	Count	0	0	1	0	0	1
		% within RBPT	0.0%	0.0%	100.0%	0.0%	0.0%	100.0%
		% within Elisa	0.0%	0.0%	100.0%	0.0%	0.0%	20.0%
	20.00	Count	0	0	0	1	0	1
		% within RBPT	0.0%	0.0%	0.0%	100.0%	0.0%	100.0%
		% within Elisa	0.0%	0.0%	0.0%	100.0%	0.0%	20.0%
	28.00	Count	0	0	0	0	1	1
		% within RBPT	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%
		% within Elisa	0.0%	0.0%	0.0%	0.0%	100.0%	20.0%
Total		Count	1	1	1	1	1	5
		% within RBPT	20.0%	20.0%	20.0%	20.0%	20.0%	100.0%
		% within Elisa	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 5: Symmetric measures for detection of kappa values.

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.167	.124	2.236	.025
N of Valid Cases		5			P<0.001

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

hence limits the spread of infection, even when prevalence in females is high (McDermott *et al*., 2002).

Comparison was formed between RBPT and c-ELISA applied for serological opinion of brucellosis in this current study. Advantageous grade of agreement was plant between two tests (Table 1-5) Albeit, the two essays were exposed degree of agreement, still the variation in frequency by the two tests could be due to false positive. Veritably often RBPT is used as a rattling screen test for opinion of brucellosis in swine. c-ELISA is a confirmational test for multinational trade but none of conventional serological tests including RBPT has been promised to be entirely dependable for routine opinion in individual gormandizers (OIE, 2012; Shome *et al*., 2016). RBPT is sensitive test but not specific while c-ELISA is both specific and sensitive test (Perrett *et al*., 2010; Praud *et al*., 2012) and can exclude cross-reaction due to the *Y. enterocolitica* serotype O 9 and *E. coli* or distinct cross-reacting antibodies by, similar as IgM. For a better appraisal of the status of brucellosis in pigs, it's consigned to use c-ELISA over RBPT (Erdenebaatar *et al*., 2004) to exclude false positive results amongst positive sera (Chand and Sharma, 2004). Grounded on our study, we suggest RBPT could be successfully used in foremost webbing of brucellosis in swine population and c-ELISA as a conformational test to exclude false positive results amongst positive sera. A study from India has revealed the seroprevalence (3.25%) of Brucella among pig farmers and pig slaughterhouse workers in Punjab (Jindal *et al*., 2016). Therefore the zoonotic potential of Breucellosis in pigs should not be neglected because the occupational risk among pig farmers and handlers are high. So the seroprevalence in this region must be seriously looked because ingrain of live animals for meat purpose from other parts may facilitate transmission of the brucellosis within no time.

CONCLUSION

This study reported the seroprevalence among the swine herds by C-ELISA and RBPT. The blood samples revealed 50 per cent positive brucellosis. The essence of the present study suggests that continued surveillance and removal of infected pigs should be strictly followed in organized farms as cattle farms to control and eradicate the disease in swine herds, as vaccination against swine brucellosis is not in practice in India.

Conflict of interest: None.

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